Journal of World's Poultry Research

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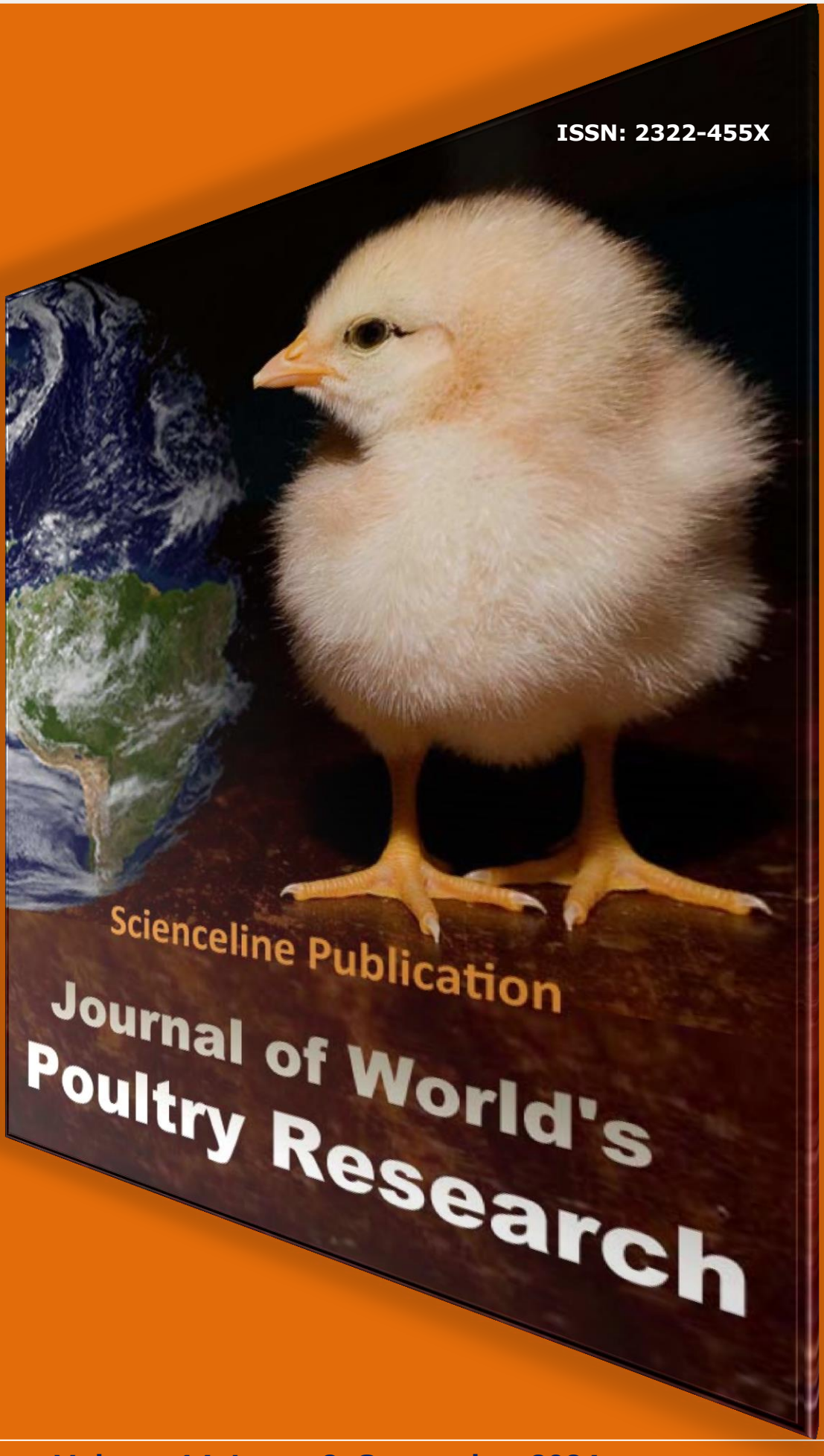

Volume 14, Issue 3, September 2024

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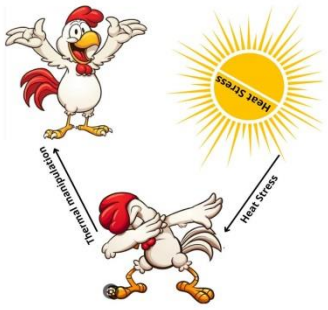
Research Paper

Effects of Thermal Manipulation During the Second Half of Incubation on Embryo Physiology, Hatching Parameters, and Quality of Broiler Chickens in Tropical Climate of Togo

Tankouano RA, Meteyake H, Oke OE, Lawson-Evi P, and Tona K.

J. World Poult. Res. 14(3): 264-272, 2024; pii: S2322455X2400027-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.27

ABSTRACT: Chickens are sensitive to environmental challenges caused by temperature. The current study aimed to determine the effects of heat manipulation during embryonic development on the physiological responses of Goliath chickens. A total of 2000 hatching eggs from 48-week-old breeders were weighed, numbered, and randomly distributed equally into 4 incubators. Each incubator received 500 eggs (4 replicates of 125 eggs each). Eggs in two of the incubators were rotated hourly at a 45° angle and maintained at 37.8°C and 60% relative humidity (T0 groups). Between embryonic days (ED) 10 and 18 of incubation, the eggs from the other two incubators were heated to 38.5°C for 6 hours per day (T1 groups). The eggs were reweighed and candled, and viable eggs were moved to the hatching baskets at ED 18 of incubation. Hatching eggs were examined individually for hatching events every three hours during the final three days of incubation. On day 21, blood samples were collected from 12 chicks per group for hormonal and biochemical analyses. The evaluated blood parameters included Triiodothyronine (T3), T4 (thyroxine), cortisol, uric acid, lactate dehydrogenase, and total protein. At hatch, chicks were weighed and their quality (survival after hatching and performance standards) was evaluated. Data were collected on embryonic development, hatching window, hatching events, biochemical parameters, and hormonal concentrations. Results indicated that hatchability, chick's weight, Tri-iodothyronine, and corticosterone were higher in the T1 group, compared to the control group. At hatch on day 21, the pipping muscle of chicks in the treated group (T1) was significantly heavier than that of the control group, while the embryonic mortality rate was significantly higher in the T0 group. In conclusion, applying heat treatment for 6 hours at 38.5°C from ED10-ED18 of embryogenesis increased significantly the hatching rate, the pipping muscle, and the chick's weight in this study.
Keywords: Embryonic development, Physiology, Slow-growing broiler, Thermal manipulation, Tropical climate



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[Full text-PDF] [Crossref Metadata]

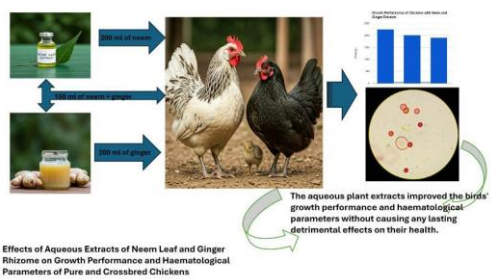
Research Paper

Effects of Aqueous Extracts of Neem Leaf and Ginger Rhizome on Growth Performance and Haematological Parameters of Pure and Crossbred Chickens

Anizoba NW, Ugwu SO, Ndofor-Foleng HM, Onyimonyi AE, Ikeh NE, Ezenwosu C, Amaefule BC, Ugwu CM, Nwoga CC, Udeh FU, Ugwuoke JI, Madu PO, Damian-Ozoke R, Chukwudi P, Onuorah SI, and Machebe NS.

J. World Poult. Res. 14(3): 273-281, 2024; pii: S2322455X2400028-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.28

ABSTRACT: Neem leaf and ginger rhizome contain numerous chemical components that are biologically active and are widely utilized in medications to treat various illnesses. The purpose of the current study was to assess the effect of aqueous neem leaf and ginger rhizome extracts on the growth performance and hematological parameters in the three breeds of chicken. A total of 360 one-day-old chicks from 3 genetic groups consisting of 120 Noiler chicks, 120 Heavy Ecotype chicks, and 120 main cross chicks were considered for this study. Each breed of chickens was randomly distributed into four groups, with three replications per group. Each replication consisted of eight females and two males, raised in a deep litter system. A 3×4 factorial arrangement



Effects of Aqueous Extracts of Neem Leaf and Ginger Rhizome on Growth Performance and Haematological Parameters of Pure and Crossbred Chickens

was employed, involving four levels of plant extracts: a control group receiving the basal diet without any extract, a group receiving 200 ml of neem extract (NE200), a group receiving 200 ml of ginger extract (GE200), and a group receiving 100 ml of neem + 100 ml of ginger extract (NE100+GE100). The chickens were evaluated for growth parameters such as initial weight (IW), final weight (FW), average daily gain (ADG), average feed intake (AFI), feed conversion ratio (FCR) as well as some haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC), platelet (P), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Noiler chickens receiving NE100+GE100 and GE200 showed the highest final body weight and daily weight gain. The results of the haematological indices revealed that the interaction effect of genotype and plant extracts on all the treatment groups were significantly different for haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), and platelet (P). Some haematological indices such as Hb, PCV, WBC, and platelet were better for chickens receiving GE200 and NE100+GE100 compared to NE200 and control groups. In conclusion, the interaction of GE200 and NE100+GE100 with Noiler and main cross chickens was beneficial, with no adverse effects on the physiological traits and health status of the chickens 16 weeks of age.
Keywords: Haematology, Heavy ecotype, Heterosis, Noiler, Performance, Plant extracts

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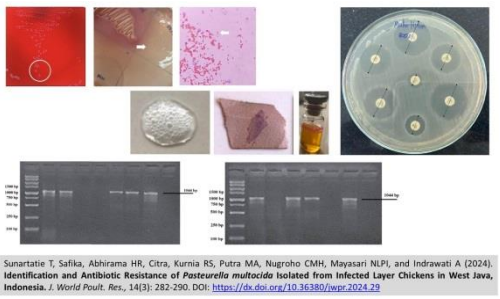
Research Paper

Identification and Antibiotic Resistance of Pasteurella multocida Isolated from Infected Layer Chickens in West Java, Indonesia

Sunartatie T, Safika, Abhirama HR, Citra, Kurnia RS, Putra MA, Nugroho CMH, Mayasari NLPI, and Indrawati A.

J. World Poult. Res. 14(3): 282-290, 2024; pii: S2322455X2400029-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.29

ABSTRACT: Bacterial infections, such as those caused by *Pasteurella multocida* serotype A, pose significant threats to poultry farming. The use of antibiotics to treat these infections can lead to antibiotic resistance. The present study aimed to identify *Pasteurella multocida* from 14 Hisex Brown layer chicken hen farms, with chikens aged 25-55 weeks, in West Java, Indonesia, and to evaluate their resistance to various antibiotics. Three samples from each farm were collected from dead chickens having symptoms of fowl cholera. Initially, the study involved isolating and identifying isolates from liver, heart, and lung organs via polymerase chain reaction. The colony was then tested for antibiotic resistance using the disk diffusion method. The results showed that 13 samples were *Pasteurella multocida* and nine were serotype A. The test results also indicated that all isolates were resistant to colistin (10 µg) and sensitive to tetracycline (30 µg), amoxicillin (25 µg), enrofloxacin (5 µg), sulfamethoxazole (25 µg), lincomycin (109 µg), and ciprofloxacin (5 µg). The study concluded that none of the *Pasteurella multocida* type A isolates were any longer sensitive to colistin, with some isolates still sensitive to tetracycline, amoxicillin, enrofloxacin, sulfamethoxazole, lincomycin, and ciprofloxacin, and two isolates showing multidrug resistance patterns.
Keywords: Antibiotic, Fowl cholera, *Pasteurella multocida*, Layer chicken


Sunartatie T, Safika, Abhirama HR, Citra, Kurnia RS, Putra MA, Nugroho CMH, Mayasari NLPI, and Indrawati A (2024). **Identification and Antibiotic Resistance of *Pasteurella multocida* Isolated from Infected Layer Chickens in West Java, Indonesia.** *J. World Poult. Res.*, 14(3): 282-290. DOI: https://dx.doi.org/10.36380/jwpr.2024.29

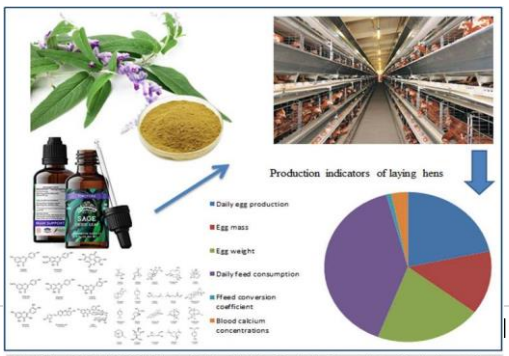
[Full text-PDF] [Crossref Metadata]

Research Paper

Effects of Salvia officinalis on Production Characteristics of Laying Hens

Al Hadi RA and Al Fadel F.
J. World Poult. Res. 14(3): 291-296, 2024; pii: S2322455X2400030-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.30

ABSTRACT: Due to the extreme importance of the poultry industry in securing animal proteins for humans, it is necessary to expand the research related to increasing egg production without resorting to antibiotics, which pose significant drawbacks. This study explored the impact of sage plant extracts, known for their bioactive compounds, on the production indicators of laying hens. Thirty chickens were randomly assigned to three groups, including a control group and two experimental groups (T1 and T2) receiving sage plant aqueous extract at 0.1% and 0.2% in their diets, respectively. The egg production percentage, egg weight percentage, percentage of daily feed consumption, feed conversion coefficient, and blood calcium concentrations were measured. The results indicated that supplementation of sage extract in the diet of



Al Hadi RA and Al Fadel F (2024). **Effects of *Salvia officinalis* on Production Characteristics of Laying Hens.** *J. World Poult. Res.*, 14(3): 291-296. DOI: https://dx.doi.org/10.36380/jwpr.2024.30

the laying hens under study increased daily egg production percentage and daily egg yield significantly in group T2 (87.63%, 59.7 eggs/day) and improved average egg weight (68.23 grams) in group T1. Moreover, there was no significant difference in daily feed consumption among the tested hens. A notable reduction was also observed in the feed conversion ratio to 2.09 in group T2.
Keywords: Feed additive, Laying hen, Plant extract, Productivity, Sage

[Full text-PDF] [Crossref Metadata]

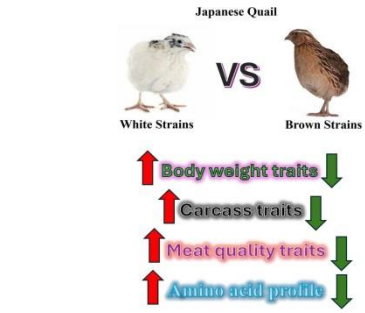
Research Paper

Heritability and Genetic Correlations of Carcass and Meat Quality Traits in White and Brown Strains of Japanese Quail

El-Attrouny MM, Iraqi MM, and Nassar FS.

J. World Poult. Res. 14(3): 297-307, 2024; pii: S2322455X2400031-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.31

ABSTRACT: Successful breeding programs for Japanese quails rely on accurately estimating genetic parameters linked to economically important traits such as body weight, carcass characteristics, and meat quality. The objective of the present study was to evaluate body weight (BW) characteristics, carcass attributes, and their genetic correlations with select meat quality traits in two strains of Japanese quail (white and brown). A total of 530 quail chicks, with 265 from each strain, were included in the analysis. At six weeks of age, the quails were slaughtered, and carcass traits as well as amino acid profiles were measured. For BW traits, the heritability (h^2) estimates ranged from 0.27 at d 1 to 0.36 at d 42. The h^2 estimated for carcass traits ranged from 0.19 for liver weight, to 0.42 for carcass yield (CY). The h^2 estimated for drip loss (DL) of meat quality was 0.21, and the h^2 estimate was 0.35 for the meat's ultimate Ph (Phu). White quail quails recorded the heaviest weight of all carcass traits. Also, white quails had the highest water-holding capacity (WHC), yellowness (b*), and lightness (L*) with the lowest level of DL, cooking losses (CL), and redness (a*) in muscles compared with brown quails. A high genetic correlation of 0.32 was noted between CW carcass weight (CW) and b*. For the pHU, a negative correlation of -0.11 was exhibited with BW. In contrast, L* appeared to have a positive but smaller relationship with CW and CY. High negative correlations were noted for b* with BW and CY -0.24 and -0.27, respectively. The CW showed a moderate relationship (0.19) with CL. In conclusion, the current study revealed that the white quail strain had high BW, as well as the finest carcass traits and meat quality. Therefore, white plumage Japanese quail might be preferred as a meat-producing strain.
Keywords: Amino acid, Carcass, Genetic correlation, Meat quality, Heritability, Quail



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[Full text-PDF] [Crossref Metadata]

Research Paper

Carcass Characteristics and Blood Biochemical Parameters of Cobb-500 and Hubbard Chicken Strains Fed on Commercial and Farm-Formulated Diets

Negari B, Yusuf Y, Hundie D, Ameha N, Kebede K, Abrar K, and Diba D.

J. World Poult. Res. 14(3): 308-323, 2024; pii: S2322455X2400032-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.32

ABSTRACT: The limits of commercial diets, their quality, and their rising costs are some of the major challenges to broiler production in Ethiopia. The purpose of this investigation was to evaluate carcass yield characteristics and blood biochemical parameters of Cobb-500 and Hubbard chicken strains fed on farm-formulated diets (T1) and three different commercial diets (T2, T3, and T4). A total of 384 mixed-sex day-old chicks (192 per strain) were randomly assigned to four dietary treatments with four replicates, each consisting of 12 broilers. The experiment was set up as a 2 × 4 factorial design, providing each strain with four diets in a completely randomized design. After 42 days of the experiment, one male and one female of each strain from each pen (eight birds per treatment) were slaughtered for carcass yield and hematological analysis. Although diets had a significant impact on live body weight, feed conversion ratio, and feed consumption among the study treatments, they had no significant effect on the mortality rate of the broilers as a whole. There was a


Negari B, Yusuf Y, Hundie D, Ameha N, Kebede K, Abrar K, and Diba D (2024). **The Effect of Artemisia on Immune Response and Productive Performance Against Newcastle Disease in Broiler Chickens.** *J. World Poult. Res.*, 14(3): 308-323. DOI: https://dx.doi.org/10.36380/jwpr.2024.32

significant effect of strains on the weight of eviscerate, dress, thigh, drumstick, breast, neck, back, and eviscerate yield percentage, with Cobb 500 showing higher values than Hubbard broilers. The farm-formulated diet (T1) significantly increased the weight of non-edible offal compared to the commercial diets, except for the weight of crops and lungs, which were similar to those in commercial diet group T4. The Hubbard strain showed a higher least square mean for packed cell volume than the Cobb-500 strain. Sex was found to have no significant impact on the hematological parameters. The farm-formulated diet (T1) also resulted in a higher marginal return rate than that of the commercial diet (T3) in the Cobb-500 strain. These findings suggest that locally sourced farm-formulated diets could be a viable alternative to commercial diets for broiler chickens in the study area.
Keywords: Broiler chicken, biochemical parameter, Carcass trait, Farm-made diet, Haematology, Profitable

[Full text-PDF] [Crossref Metadata]

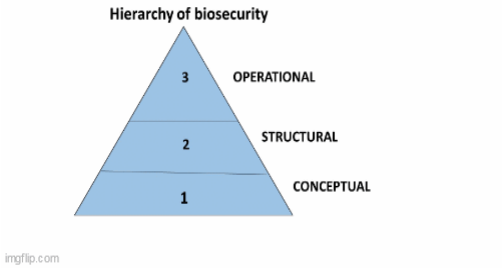
Research Paper

Biosecurity Compliance and Its Applications in Poultry Production Sectors

Mohammed AN.

J. World Poult. Res. 14(3): 324-330, 2024; pii: S2322455X2400033-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.33

ABSTRACT: Poultry farming has been recognized as one of the most vital sectors for the economy and revenue generation in many countries. For the production of high-quality freshly hatched chicks, effective cleaning and sanitation of the hatchery environment and hatching eggs were crucial components of proper management and hygiene in chicken hatcheries. The current review aimed to assess the efficient ways of mitigating the risk of disease introduction (external biosecurity) and its subsequent dissemination (internal biosecurity) within and between poultry farms and hatcheries. In addition to identifying the variety of risk categories that are applied to various biosecurity industries, this article clarified the equivalent tools, including checklists and/or questionnaires, that can be used to assess biosecurity compliance. The checklist was aimed to evaluate numerous biosecurity protocol categories, including the farm's infrastructure, employees, their education and training, access control mechanisms, cleaning and disinfection procedures, handling of litter and waste, chick control, registrations, and pest management. In conclusion, external biosecurity was critical to preventing infections from entering hatcheries and poultry farms. Questionnaires or checklists were effective instruments for gathering information on biosecurity and evaluating compliance in poultry farms.



Keywords: Biosecurity compliance, Checklist, Hazard, Poultry sector

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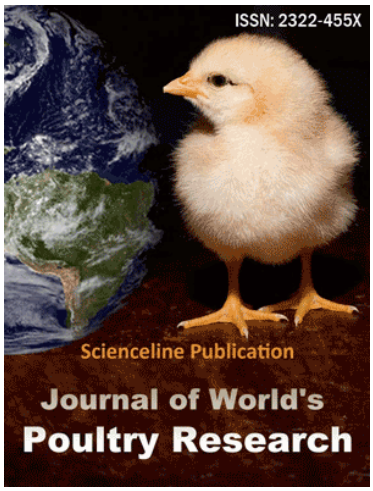

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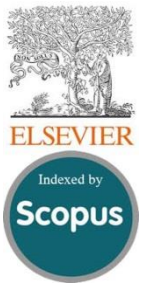

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Effects of Thermal Manipulation During the Second Half of Incubation on Embryo Physiology, Hatching Parameters, and Quality of Broiler Chickens in Tropical Climate of Togo

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[2]*Physiopathology, Bioactive Substances and Safety Research Unit (PSBI Unit), Faculty of Science, University of Lomé, Togo, P.O. Box 1515, Togo*

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ABSTRACT

Chickens are sensitive to environmental challenges caused by temperature. The current study aimed to determine the effects of heat manipulation during embryonic development on the physiological responses of Goliath chickens. A total of 2000 hatching eggs from 48-week-old breeders were weighed, numbered, and randomly distributed equally into 4 incubators. Each incubator received 500 eggs (4 replicates of 125 eggs each). Eggs in two of the incubators were rotated hourly at a 45° angle and maintained at 37.8°C and 60% relative humidity (T0 groups). Between embryonic days (ED) 10 and 18 of incubation, the eggs from the other two incubators were heated to 38.5°C for 6 hours per day (T1 groups). The eggs were reweighed and candled, and viable eggs were moved to the hatching baskets at ED 18 of incubation. Hatching eggs were examined individually for hatching events every three hours during the final three days of incubation. On day 21, blood samples were collected from 12 chicks per group for hormonal and biochemical analyses. The evaluated blood parameters included Triiodothyronine (T3), T4 (thyroxine), cortisol, uric acid, lactate dehydrogenase, and total protein. At hatch, chicks were weighed and their quality (survival after hatching and performance standards) was evaluated. Data were collected on embryonic development, hatching window, hatching events, biochemical parameters, and hormonal concentrations. Results indicated that hatchability, chick's weight, Tri-iodothyronine, and corticosterone were higher in the T1 group, compared to the control group. At hatch on day 21, the pipping muscle of chicks in the treated group (T1) was significantly heavier than that of the control group, while the embryonic mortality rate was significantly higher in the T0 group. In conclusion, applying heat treatment for 6 hours at 38.5°C from ED10-ED18 of embryogenesis increased significantly the hatching rate, the pipping muscle, and the chick's weight in this study.

Keywords: Embryonic development, Physiology, Slow-growing broiler, Thermal manipulation, Tropical climate

INTRODUCTION

Poultry farming is one of the fastest-growing livestock industries in tropical nations. This expansion is caused by the prominent position that poultry products play on household menus, the absence of religious restrictions, their high nutritious value, and the ease of production (Jaovelo, 2007). Poultry meat is particularly popular since it is low in fat, an excellent source of protein, and unlike red meat, it does not raise the risk of certain diseases like metabolic or cardiovascular disorders (Pan et al., 2011; Jilo and Hasan, 2022; Connolly and Campbell, 2023).

Stress is the collection of responses to any external demand or challenge that causes the flock of hens to adjust to an unusual occurrence (Khan and Liu, 2012; Oke et al., 2022; Onagbesan et al., 2023). Providing ideal environmental conditions for chicken development, growth, and production is a prerequisite for poultry farming to operate at its peak efficiency (Muchacka et al., 2012; Oke et al., 2021). Heat stress occurs when an animal generates more internal heat than it can dissipate externally (Elizabeth et al., 2023). Chickens are more sensitive to environmental challenges posed by

temperature, particularly heat stress (Nawab et al., 2018). Heat stress is a significant factor contributing to financial losses in the poultry sector (Lin et al., 2006; Lu et al., 2007). It increases the mortality rate and reduces growth performance (Kumar et al., 2021; Belhadj et al., 2016). Compared to domestic chickens, broilers are more vulnerable to high temperatures (Gous and Morris, 2005), although the reaction to heat differs from one chicken to another according to their genetic upbringing (Altan et al., 2003; Star et al., 2008; Felver-Gant et al., 2012). In addition to the fast-growing strains, heat stress negatively affects the slow-growing strains (Tan et al., 2010; Soleimani et al., 2011; Rimoldi et al., 2015).

During the hottest months, the appropriate microclimatic parameters are often exceeded, disrupting the homeostasis of the chickens' internal environment. Consequently, the management of poultry and the equipment used in hot weather must be reevaluated to reduce heat stress (Akşit et al., 2006; Kpomasse et al., 2023).

Perinatal or postnatal acclimatization through thermal manipulation is one way to help chickens adjust to climate change and enhance their growth performance (Collin et al., 2007; Yalçin et al., 2008; Meteyake et al., 2020). Growth performances, metabolic rate physiological response, and hatching of poikilothermic embryos can be affected by variations of temperature from the standard incubation temperatures range of 37 to 37.5°C, (Tazawa et al., 2004; Black and Burggren, 2004). Lowering the incubation temperature increases incubation time and inhibits embryo growth (Black and Burggren, 2004), while elevated temperatures accelerate embryo growth and development (Willemsen et al., 2010; Narinç et al., 2016). Embryo weights were lower on embryonic day (ED) 18 when the eggs were exposed to a temperature of 39.6°C for 6 hours daily from ED10 to ED18 of incubation, even though the weights were similar to the control (Yalçin et al., 2005) or a bit lower than the control group (Yalçin et al., 2005). Because epigenetic adaptation to elevated or low post-hatch environmental temperatures is induced during the pre-hatch period, lower or higher incubation temperatures affect post-hatch thermoregulation systems (Nichelmann and Tzschentke, 2002; Al Amaz et al., 2024; Iraqi et al., 2024). Several studies have been conducted on the acclimatization of fast-growing broilers, but fewer studies have been carried out on slow-growing broilers, especially on Goliath chicken embryos which are also known to be slow-growing strains (Madougou, 2023). Hence, this study aimed to assess the physiological reactions of Goliath chicken embryos subjected to embryonic thermal manipulations from day 10 of embryogenesis to day 18 under tropical climate conditions.

MATERIALS AND METHODS

Ethical approval

The current study was performed with strict adherence to the University of Lome/Togo's Guide for the Care and Use of Experimental Animals (008/2021/BC-BPA/FDS-UL).

Experimental design

This experiment was carried out at the Regional Centre of Excellence for Poultry Science (CERSA) experimental unit at the University of Lomé.

A total of 2000 Goliath hatching eggs from 48-week-old breeders stored for 7 days were used. The eggs were purchased from a production farm in the Republic of Benin. These eggs were weighed, numbered, and incubated until day 10 of incubation in the same incubator (© Petersime Incubator, Belgium) at the appropriate temperatures and humidity conditions (37.8°C, 60%). On day 10 of incubation, the eggs were divided randomly into four groups (500 eggs each) and incubated in four different incubators of the same model (PasReform, Zeddam, SmartProCombi model, Netherlands). Each incubator had 4 replicates of 125 eggs. From ED10 to ED18, the eggs from two incubators (T1 groups) were subjected to 38.5°C and relative humidity (RH) of 60% for six hours daily, whereas the eggs from the other two incubators (T0 groups) were maintained at standard conditions. Eggs from all treated groups were incubated in complete darkness. On day 18 of incubation, the eggs were candled, and the fertile ones were weighed and conveyed in the hatcher for three days of hatching (until day 21 of incubation; Yalçin et al., 2008)

Egg and embryo weights

Before the setting of eggs and at ED18, egg weight (EWT) was recorded. These weights were used to determine the egg weight loss (EWTL) at ED18 of incubation using Formula 1.

$$\text{Egg weight loss (\%)} = \frac{EWT(ED0) - EWT(ED18)}{ED0} \text{ x } 100$$

(Formula 1)

Where ED 0 indicates the day the eggs were placed in the incubator.

At ED10, ED14, and ED18 12 eggs/treatment were broken at each embryonic day to measure embryo weights.

Hatching event, embryonic mortality, hatchability, and chick quality

Every three hours starting on day 19 of incubation, the time of internal pipping (IP), external pipping (EP), and hatching for each egg was recorded. The number of chicks hatched was counted. To determine the early and late embryonic mortalities, the unhatched eggs were broken and examined macroscopically at the end of incubation. Deaths before the 18th day of incubation were classified as early death. Deaths that happened at IP, during IP and EP, or when the embryo was positioned incorrectly were considered late embryonic mortality. The data collected were used to determine the spread of hatch according to various treatments, the entire incubation period (between setting and hatching), the hatchability (Formula 2), and the embryonic mortalities (Formula 3). The quality of the chicks at the hatch was evaluated using the Tona scoring system (Tona et al., 2004). The major objective of this method was to score physical attributes, such as response, appearance, down and eyes, legs conformation, navel area, yolk sac, and remaining membranes and yolk.

The total of the ratings given to each quality parameter was used to create the chicks' quality score :

$$\text{Hatchability (\%)} = \frac{\text{Total number of Hatched eggs}}{\text{Total number of Fertile Eggs}} \text{ x } 100$$

(Formula 2)

Organs, day-old chick body weights, and cloaca temperature at hatch

On day 21 after hatching, the weights of the liver, heart, and pipping muscles were calculated by cervical dislocation on a sample of 12 chicks per treatment. These data were used to determine body weights and the absolute weights of the heart and liver. An electronic thermometer inserted about 3 cm into the colon was used to record the cloaca temperatures of the same chicks at hatch.

$$\text{Mortality (\%)} = \frac{\text{Total number of dead embryos}}{\text{Total number of Fertile Eggs}} \text{ x } 100$$

(Formula 3)

Blood biochemical traits, hematology, and hormonal analysis

At hatch (day 21), blood samples were collected from 12 chicks via the wing vein using insulin syringes (1CC), to collect blood samples (1ml) into anti-coagulant-free tubes. These samples were used to evaluate uric acid, Lactate Dehydrogenase (LDH), total proteins, triiodothyronine (T3), thyroxine (T4), and corticosterone. In preparation for analysis, serum samples (obtained from centrifuged blood (15000g for 15 min) were frozen and kept at -20°C. Using the Biolabo kit (France), a spectrophotometer was used to quantify proteins, uric acid, and LDH. ELFA equipment and the Vidas kit were used to measure the serum T3 and T4 concentrations. Utilizing Cobas equipment and the Eclia technique, corticosterone concentration was determined (Repetto et al., 2017). The same chickens' blood was also drawn into heparinized tubes, where blood cells (Lymphocytes and Heterophils) were identified.

Statistical analysis

Data were analyzed using R software (R Core Team Development, 2023; Version 4.3.1). Descriptive statistics, including the Shapiro-Wilk normality test, means and standard errors, were calculated for the main quantitative variables. For variables with a normal distribution, the Student's parametric test was applied to compare the means between the treatment groups. On the other hand, the non-parametric Wilcoxon test was employed for variables that did not have a normal distribution. To compare the proportions between the various groups, the Chi-square test was also performed. The results were presented as the mean ± the Standard Deviation (SD). The significance rate was 5%.

RESULTS AND DISCUSSION

Embryonic development

Figure 1 shows the impact of thermal manipulation on embryonic development from day 10 to day 18 of incubation. The heat treatment did not affect the development of embryos ($p > 0.05$). These results confirm those reported by Al-Zghoul et al. (2019) but contradict those reported by Horowitz (1986), indicating that heat treatments had an instantaneous impact on the development of embryos, resulting in slowed growth by day 14. The heat treatment, which in their case reached 39.6°C, may have contributed to this outcome.

Hatching window

The spread of the hatch in relation to various heat treatments is depicted in Figure 2. Chicks in the T1 group began hatching three hours earlier than those in the T0 group. The first chicks in the T1 group were observed at 451 hours (day 19 of incubation), with the peak hatch occurring at 472 hours (day 20 of incubation). In contrast, chicks in the T0 group started hatching at 454 hours (day 19 of incubation), reaching their peak at 478 hours (day 20 of incubation). The T0 group exhibited a shorter hatching window compared to the T1 group.

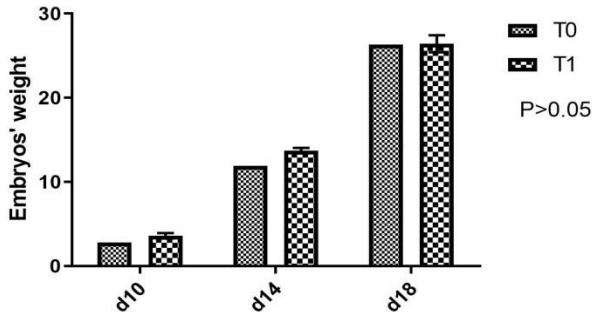

Figure 1. Effects of thermal manipulation on embryonic development (gr) of Goliath chickens from day 10 to day 18 of embryogenesis for 6 hours at 38.5°C. T0: Control group, T1: Thermal manipulated group

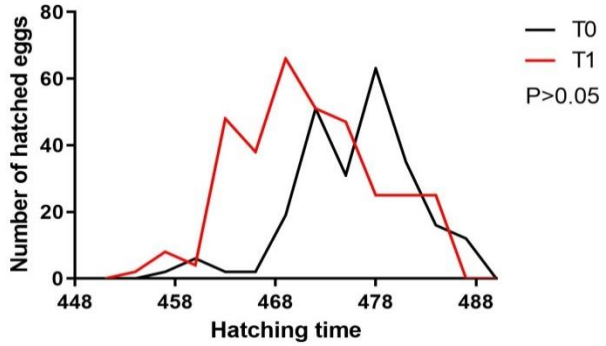

Figure 2. Effect of thermal manipulation on the hatching window of Goliath chickens from day 10 to day 18 of embryogenesis for 6 hours at 38.5°C. T0: Control group, T1: Thermal manipulated group

Internal pipping, external pipping, hatching durations, and cloacal temperature

Table 1 shows the effect of heat treatment on IP, EP, and hatching durations. Raising the temperature to 38.5 for 6 hours from ED10 to ED18 affected the duration of IP ($p < 0.05$), EP ($p < 0.05$), and cloaca temperature ($p < 0.001$). The difference was not significant between the two groups for the duration of hatching ($p > 0.05$). Embryos from the treated batch started the IP, EP, and hatching earlier than those in the control group (T0). The quality of chicks (surviving hatching, and performance standards) at hatch was similar in T0 and T1 groups ($p > 0.05$). The significant difference in the duration of IP and EP between the two treatment groups might be due to the fact that embryos use more oxygen when the temperature is higher. Because of that increased demand, the embryos must switch to pulmonary respiration in order to meet their oxygen requirements. This rise in oxygen demand may encourage the embryos to pip and hatch earlier (Molenaar et al., 2010). This result confirms those reported by Piestun et al. (2013) but contradicts those reported by Willemsen et al. (2010) who found that high heat treatment delayed the hatching process (IP, EP, and hatch) in the treated group. Willemsen et al. (2010) applied a heat treatment of 40.6°C from day 16 of incubation to day 18.5 of incubation. This incubation period is very critical for the development of embryos (Kpodo and Proszkowiec-Weglarz, 2023) and could explain why the results are contradictory. In his study, the thermal manipulation was applied during the late embryonic development. The higher cloaca temperature in the T1 group ($p < 0.05$) may be due to increased thermal manipulation induced by the metabolic rate, resulting in higher heat production by the chickens. In the event of future chronic heat stress, the heat therapy may cause a metabolic and stress response, suggesting a potential increase in thermotolerance. These results are in line with those found by several authors (Narinç et al., 2016; Al-Rukibat et al., 2017; Al-Zghoul, 2018; Saleh et al., 2020). These authors applied respectively 39.6 °C for 6 hours daily from day 10 to day 18 of incubation, 38.5°C and 40°C for 6 hours at day 16, 9 hours at day 17, and 12 hours at day 18 of incubation; 38.5°C, 39°C, 39.5°C and 40°C for 6 hours from day 12 to day 18 of incubation; 39°C for 18 hours daily from day 10 to day 18 of incubation. They all concluded that thermal manipulation improved the thermotolerance of chicks. Al-Zghoul et al. (2019) added that the dynamics of heat shock proteins (HSPs) and heat shock factors (HSF) mRNA expression were changed by heat treatment, and this was linked to an increased development of thermotolerance.

Table 1. Effects of thermal manipulation on hatching parameters of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters \ Treatments	T0	T1	p-value
IP time (h)	450.7 ± 3.14^{a}	446.5 ± 2.87^{a}	0.33
EP time (h)	461.5 ± 3.14^{a}	457.0 ± 3^{a}	0.33
Total incubation duration (h)	473.2 ± 3.03^{a}	467.5 ± 3.4^{a}	0.20
Duration between IP and EP (h)	10.77 ± 0^{a}	10.5 ± 0^{b}	0.03
Duration between EP and Hatching(h)	11.7 ± 0^{a}	10.5 ± 0^{b}	0.03
Duration between IP and Hatching (h)	22.47 ± 0^{a}	21 ± 0^{a}	0.34
Cloacal temperature of chicks (°C)	37.98 ± 0.22^{b}	39.99 ± 0.06^{a}	< 0.001
Tona score	96.55 ± 0.47^{a}	96.02 ± 0.58^{a}	0.74

IP: Internal pipping, EP: External pipping, h: Hour, P-value: Probability. All results are presented as mean ± SD; ^{a,b} Means with different superscripts are significantly different in a row, T0: Control group, T1: Thermal manipulated group

Weight loss, hatching rate, and mortality rate

Table 2 shows the results of thermal manipulation on weight loss from incubated eggs, hatching rate, and mortality. No significant difference was recorded in terms of weight loss ($p > 0.05$) but raising the temperature to 38.5°C affected the early mortality rate ($p < 0.05$) and the late mortality rate ($p < 0.05$). Lower, early, and late mortality rates were recorded in treatment T1. The hatching rate of batch T1 was higher than that of the T0 group ($p < 0.05$).

Table 2. Effect of thermal manipulation on weight loss, hatching, and mortalities rate of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters (%)	Treatments		p-value
	T0	T1	
Weight loss	13.02[a]	13.98[a]	1
Hatchability	85.43[b]	89.22[a]	0.03
EM	5.6[a]	4.25[b]	0.04
LM	8.87 [a]	6.35[b]	0.01

EM: Early mortality, LM: Late mortality. All results are presented as mean ± SD; [a,b] Means with different superscripts are significantly different in a row, T0: Control group, T1: Thermal manipulated group.

The weight of the pipping muscle and the high level of T3 (triiodothyronine) in T1 group chicks can be used to explain the hatching rates obtained. Chicks' pipping muscles are crucial in the process of hatching. The mechanical strength needed for the chick to break the eggshell and come out is supplied by the pipping muscles (Pulikanti et al., 2010). Heat stress resulted in an increased thyroid hormone T3 and corticosterone concentration in the T1 group. These hormones play an important role in the hatching process, providing the chicks the energy they need to hatch. The higher the T3 and T4 concentrations, the higher the chicks' energy level. For the control of metabolic processes, T3 and T4 are crucial. They affect the turnover of lipids and carbohydrates, protein synthesis, and basal metabolic rate. They promote the mobilization of energy reserves, such as lipids and proteins, needed to sustain energy during the hatching phase. This mobilization is crucial if the embryo is to complete the hatching process with sufficient energy (Al-Zghoul, 2018).

Compared to the chicks in the T0 group, which had a lower concentration of T3, the highly active chicks in the T1 group hatched earlier. Delayed hatching can cause chick mortality within the egg, leading to a lower hatching rate. These findings contradict those reported by Al-Rukibat et al. (2017), who found that thermal manipulation did not affect the hatching rate. The discrepancies between studies could be due to genetic differences. The higher embryonic mortality in the chicks of the control batch (T0) could be explained by the low weight of the pipping muscle, allowing the chicks to spin inside their shells, rip the membrane, and break the shell.

Absolute weight of chicks, heart, liver, and pipping muscle

Table 3 shows the effects of heat treatment on the absolute weight of day-old chicks, heart weight, liver weight, and pipping muscle weight. The weight of chicks in T1 was significantly higher than that of the chicks in the T0 group ($p < 0.05$). The same tendency was observed for the pipping muscles ($p < 0.05$). However, there was no difference in the weight of the heart ($p > 0.05$) and liver ($p > 0.05$). These outcomes (high chicks' weight and pipping muscle in the T1 group) could be explained by the fact that high temperatures are known to speed up not only the metabolic rate but also the growth and development of muscle tissues (Meltzer, 1983). This result confirms the findings reported by Piestun et al. (2015). Piestun et al. (2015) applied a heat treatment of 39.5°C from day 7 to day 16 of incubation for 12 hours. It was concluded that the thermal manipulation had a positive effect on embryo growth with an improved chick's weight at the hatch. This result can also be explained by the effective use (due to accelerated metabolism) of the energy reserves in the egg which resulted in body tissue enlargement (Piestun et al., 2015). In addition, the heat treatment influenced hormone regulation by increasing T3 levels in the T1 batch. These hormones are like growth hormones. Higher levels of T3 can promote the growth of body tissue in chicks, leading to larger size at hatch. The results confirm those reported by Abuoghaba et al. (2018) and Al-Rukibat et al. (2017) but contradict those reported by Yahav et al. (2004) and Tona et al. (2004), who found that a thermal manipulation of 38.5°C applied between ED16 and 18 for 3 hours did not affect the hatching weight of Cobb chicks. This could be explained by the period of application and the type of boiler used.

Table 3. Effect of thermal manipulation on the absolute weight of chick, heart, liver, and pipping muscle of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters	Treatments		p value
	T0	T1	
Chick (g)	36.03 ± 0.59[b]	38.26 ± 0.56[a]	< 0.001
Heart (g)	0.086 ± 0.01[a]	0.092 ± 0.00[a]	0.74
Liver (g)	0.76 ± 0.06[a]	0.86 ± 0.07[a]	0.18
Pipping muscle (g)	0.14 ± 0.02[b]	0.20 ± 0.03[a]	0.02

[a,b] Means with different superscripts are significantly different in a row; All results are presented as mean ± SD, T0: Control group, T1: Thermal manipulated group.

Relative weight of chicks, heart, liver, and pipping muscle

Table 4 shows the result of heat treatment on the relative weight of day-old chicks, heart weight, liver weight, and pipping muscle weight. At the setting, the weight of the eggs was similar across the treatments ($p > 0.05$). The weight of chicks in batch T1 was higher than that of the chicks in the T0 group ($p < 0.05$). The same tendency was observed for the weights of the liver ($p < 0.05$) and pipping muscles ($p < 0.05$). However, there was no difference in the weight of the heart ($p > 0.05$). These results (high chicks' weight and pipping muscle in the T1 group) could be explained by the fact that heat is known to accelerate the growth and development of muscle tissues as well as the metabolic rate (Meltzer, 1983). This result confirms the findings reported by Piestun et al. (2015). In addition, there is a positive correlation between the liver's weight and body weight (Hassan, 2009). These results contradict those reported by Yalcin et al. (2008) who found a lower absolute liver and heart weight under the same heat treatment conditions (38.5°C for 6 hours, from incubation day 10 to day 18). The difference here could probably be due to genetic factors. Cobb500 which is a fast-growing broiler was used in their study while in this study a slow-growing breed was used.

Table 4. Effect of thermal manipulation on the relative weight of chick, heart, liver, and pipping muscle of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters	Treatments		p value
	T0	T1	
Egg's weight (g)	49.34 ± 0,59[a]	48.42 ± 0,48[a]	0.24
Relative chick weight (%)	73.02 ± 0.01[b]	79.01 ± 0.00[a]	< 0.001
Relative heart weight (%)	0.25 ± 0.01[a]	0.24 ± 0.01[a]	0.57
Relative liver weight(%)	1.99 ± 0.01[b]	2.39 ± 0.01[a]	< 0.001
Relative pipping muscle'weight (%)	0.39 ± 0.02[b]	0.52 ± 0.01[a]	< 0.001

[a,b] Means with different superscripts are significantly different in a row, All results are presented as mean ± SD, T0: Control group, T1: Thermal manipulated group.

T3, T4 concentration, corticosterone, and heterophils/lymphocytes ratio

Table 5 shows the effect of high heat treatment on stress hormones T3 and T4 and the heterophils/lymphocytes H/L ratio. Blood serum T3 was higher in group T1 ($p < 0.05$) and corticosterone concentration was also higher in group T1 ($p < 0.05$), compared to the T0 group. The heat treatment did not affect the H/L ratio and T4 concentration. Compared to T0, the higher blood serum T3 concentration in T1 chicks at hatch suggested that less T3 was required for oxidative metabolism, which reduced the amount of T3 absorbed by the cells and increased the blood serum T3 concentration over time. In addition, the increasing metabolic rate is known to increase T3 levels in the blood. When there was an increase in metabolic rate, the T3 rate also increased in the blood. There was no major difference in T4 concentration since the conversion of T4 to T3 occurred more quickly in T1 than in T0 chicks throughout embryonic development (Tona et al., 2004). The decrease in hepatic Deiodinase (D3) expression may be a contributing factor to the rise in blood serum T3 levels. The breakdown of T3 by D3 is a significant cause of determining serum T3 level, even if the hepatic D3 level has not been assessed (Decuypere and Kuhn, 1985; Darras et al., 2000). Under the action of D3, the conversion of T4 to T3 is reduced, which decreases the quantity of T3 in the blood. In addition, the conversion of T3 to T2 by D3 directly reduces the concentration of active T3 (Maia et al., 2005). High levels of hepatic D3 show increased conversion of T3 to T2 and T4 to rT3. This suggests that blood T3 levels may be reduced as the enzyme reduces the amount of active T3. Low hepatic D3 levels show decreased inactivation of T3 and conversion of T4 to rT3. This suggests that blood T3 levels may be relatively higher

Table 5. Effect of thermal manipulation on stress hormones concentration and H/L ratio of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters	Treatments		p value
	T0	T1	
T3 (Pmol/l)	5.54 ± 1.33[b]	9.98 ± 2.12[a]	0.02
T4 (Pmol/l)	7.11 ± 0.87[a]	5.1 ± 0.13[a]	0.2
Corticosterone (ng/ml)	0.40 ± 0.00[b]	0.54 ± 0.01[a]	< 0.001
Ratio H/L	6.63[a]	4.33[a]	0.42

[a,b] Means with different superscripts are significantly different in a row, All results are presented as mean ± SD; T3: Triiodothyronine; T4: Thyroxine, T0: Control group, T1: Thermal manipulated group. Ratio H/L: Heterophils/lymphocytes H/L ratio

Biochemical parameters

Table 6 shows the effect of heat treatment on biochemical parameters. The heat treatment decreased the concentration of uric acid ($p < 0.05$) and increased LDH ($p < 0.05$) in group T1. In addition, there was no difference in the protein content. Heat can increase the metabolism of

embryos, accelerating the processes of purine degradation and the conversion of uric acid into other metabolic compounds (Al-Kharusi et al., 2012; Loyau et al., 2016). This could explain the lower uric acid levels observed. These outcomes confirm those reported by Moraes et al. (2003), who also got a reduction in uric acid in heat-treated batches. Heat has the potential to interfere with metabolic processes. In order to generate energy, cells might shift to a more anaerobic metabolism, which raises the synthesis of lactate, an LDH substrate.

Table 6. Effect of thermal manipulation on biochemical parameters of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters	Treatments		p value
	T0	T1	
Uric acid (mg/l)	75.81±1.87[a]	65.98±1.45[b]	0.02
Lactate Dehydrogenase (UI/L)	982±1.73[b]	1260±1.16[a]	< 0.001
Total protein (g/l)	39.47±7.27[a]	37.58±6.32[a]	0.85

[a,b] Means with different superscripts are significantly different in a row; All results are presented as mean ± SD, T0: Control group, T1: Thermal manipulated group.

CONCLUSION

Applying heat treatment for 6 hours at 38.5°C from ED10 to ED18 of embryogenesis increases the hatching rate, the pipping muscle, and the chick's weight at hatch. Moreover, it did not affect the embryonic development from ED 10 to ED18. Additional investigation is important to clarify the underlying mechanisms and to assess the impact of these thermal manipulations on poultry production on a larger scale.

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Authors' contributions

Rachida Tankouano, Povi Evi Lawson, and Kokou Tona did the design of this study. Meteyake Hezouwe contributed to the conceptualization, and data analysis of the present study and drafted the manuscript. Oyegunle Emmanuel Oke helped to improve the English of the manuscript. All authors approved the final version of the manuscript.

Availability of data and materials

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

Ethical considerations

No sentence in this manuscript has been copied. The manuscript has not been submitted for editorial review, accepted for publishing, or published anywhere else. There is no fabrication or falsification of the data.

Competing of interests

The authors of this work declare no competing interests

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Effects of Aqueous Extracts of Neem Leaf and Ginger Rhizome on Growth Performance and Haematological Parameters of Pure and Crossbred Chickens

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ABSTRACT

Neem leaf and ginger rhizome contain numerous chemical components that are biologically active and are widely utilized in medications to treat various illnesses. The purpose of the current study was to assess the effect of aqueous neem leaf and ginger rhizome extracts on the growth performance and hematological parameters in the three breeds of chicken. A total of 360 one-day-old chicks from 3 genetic groups consisting of 120 Noiler chicks, 120 Heavy Ecotype chicks, and 120 main cross chicks were considered for this study. Each breed of chickens was randomly distributed into four groups, with three replications per group. Each replication consisted of eight females and two males, raised in a deep litter system. A 3×4 factorial arrangement was employed, involving four levels of plant extracts: a control group receiving the basal diet without any extract, a group receiving 200 ml of neem extract (NE200), a group receiving 200 ml of ginger extract (GE200), and a group receiving 100 ml of neem + 100 ml of ginger extract (NE100+GE100). The chickens were evaluated for growth parameters such as initial weight (IW), final weight (FW), average daily gain (ADG), average feed intake (AFI), feed conversion ratio (FCR) as well as some haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC), platelet (P), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Noiler chickens receiving NE100+GE100 and GE200 showed the highest final body weight and daily weight gain. The results of the haematological indices revealed that the interaction effect of genotype and plant extracts on all the treatment groups were significantly different for haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), and platelet (P). Some haematological indices such as Hb, PCV, WBC, and platelet were better for chickens receiving GE200 and NE100+GE100 compared to NE200 and control groups. In conclusion, the interaction of GE200 and NE100+GE100 with Noiler and main cross chickens was beneficial, with no adverse effects on the physiological traits and health status of the chickens 16 weeks of age.

Keywords: Haematology, Heavy ecotype, Heterosis, Noiler, Performance, Plant extracts

INTRODUCTION

The significant increase in chicken production to meet the growing demand for poultry products in developing countries has led to a corresponding rise in the use of antibiotics as growth promoters. These synthetic and semi-synthetic antibiotics positively impact poultry by improving appetite, increasing feed conversion, stimulating the immune system, increasing vigor, and modulating intestinal microflora, all of which contribute to higher survival rates (Ayalew et al., 2022). However, their use comes with several drawbacks, such as high production costs, negative effects on bird health, long withdrawal times, risks of accumulation in tissues and eggs, and ensuing human cancer risks. In line with these findings, the European Union (2006) recommended alternatives categorized as natural growth promoters (NGPs). Nigerian researchers have used a wide variety of

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herbs and plant parts from seeds, fruits, and tree barks to leaf meals and extracts as replacements for conventional feeds, feedstuff, growth boosters, and antibiotics. Plant extracts from therapeutic plants, including neem and ginger, are safe, affordable, and full of various bioactive compounds, or secondary metabolites, and are, therefore, among the possible substitutes (Oluwafemi et al., 2020; Mukherjee et al., 2024).

Dogonyaro, also known as neem (*Azadirachta indica*), is a fast-growing native tropical tree that grows well throughout Nigeria, especially in poor, shallow, stony, or sandy soils where agricultural crops yield little (Ogbuewu et al., 2011). Due to its extensive therapeutic potential, including its antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective, and anticoccidal effects in poultry and other animals (monogastric and ruminant species), neem trees have drawn attention from all over the world. Since the meal from neem leaf contains some bioactive components (limonoids, tannin, and azadirachtin) that negatively impact nutrient consumption, its application is restricted (Islas et al., 2020). Furthermore, the high fiber content of neem leaf meal presents challenges for digestion and consumption in chicken diets (Oloruntola et al., 2019).

To overcome these limitations, the use of neem leaf extracts offers a promising solution, allowing the nutritional and therapeutic benefits of neem leaf meal to be fully utilized without the associated drawbacks (Tibebu et al., 2017). Neem leaf extract contains several bioactive compounds, including nimbin, nimbinene, 6-desacetylnimbiene, nimbadole, nimbolide, and quercetin (Miltra et al., 2000). Studies have shown that neem leaf infusion stimulates growth and enhances haematological parameters, immunological response, and growth performance in chickens (Egbeyale et al., 2021). Furthermore, Egbeyale et al. (2021) reported that administering aqueous neem leaf infusions at concentrations of up to 0.3% in drinking water did not adversely affect the growth performance, carcass traits, or meat quality of broiler chickens, making it a viable alternative to antibiotics.

Zingiber officinale, a perennial blooming plant, is utilized extensively in both culinary and medicinal applications. It facilitates faster digestion and has antibacterial, anti-inflammatory, and therapeutic qualities. In many households, ginger serves as a preservative, spice, condiment, while also being employed for a variety of additional therapeutic uses (Sachan et al., 2018). The primary bioactive constituents responsible for ginger's distinctive flavor and pharmacological effects are gingerols, including 6-, 8-, and 10-gingerol (Alsherbiny et al., 2019). These gingerols, a class of phenolic compounds present as a yellow oil at room temperature, exhibit a wide range of biological activities, such as anti-inflammatory, anti-allergic, antioxidant, anti-cancer, and antimicrobial properties. They are also used in treating different disorders of the central nervous system (Semwal et al., 2015). It has been demonstrated that gingerols can reduce animal oxidative stress brought on by heavy metals, mycotoxins, age, etc (Li et al., 2019). Ginger's immunostimulant properties enhance the body's ability to respond to future challenges from pathogenic organisms by activating cell-mediated immune responses. In vitro studies further suggest that ginger extract may have anti-diabetic effects and regulate the amount of free radicals and lipid peroxidation (Morakinyo et al., 2011). When immuno-suppressed birds are fed neem leaves and ginger extracts, their humoral and cell-mediated immune responses are boosted (Sadekar et al., 1998).

There has been research on the use of several medicinal plant extracts as growth promoters for antibiotics (Lukanov et al., 2018). Still, no published studies have specifically examined the effects of neem leaf and ginger extracts on Noiler chickens, Nigerian Heavy Ecotype chickens, and their crossbreeds. If the biological properties of ginger and neem leaf extracts are demonstrated to improve the growth and hematological parameters in these chickens without adversely affecting their physiological traits and health, these extracts could serve as promising growth-promoting supplements in poultry diets, contributing positively to animal production. Therefore, this study aims to evaluate the effects of neem leaf and ginger extracts on the growth performance and haematology of Noiler chickens, Nigerian Heavy Ecotype chickens, and their crossbreeds.

MATERIAL AND METHODS

Ethical approval

This research was carried out in accordance with the recommendations of research ethics for scientific researchers involving animal subjects. The animals were handled in line with the principles set forth by the Animal Experimentation Ethics Committee of the University of Nigeria, Nsukka (No: UNN/C031ARO12.02.07.2023) following the Research Ethics Committee Recommendations (2013).

Location and duration of the study

The study was conducted from August 5, 2023, to November 25, 2023, at the Poultry Unit of the Department

of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka, Nigeria. Nsukka lies within longitude 6° 45′E and 7° E and latitude 7° 12.5 ′N at an altitude of 447 m above sea level. The climate of the study area is typically tropical, with relative humidity ranging from 65% to 80% and a mean daily temperature of 26.8 °C (Okonkwo and Akubuo, 2007). The present experiment lasted for 16 weeks.

Preparation of extracts

Fresh neem leaves were picked from neem trees inside the premises of the university environment while ginger roots were purchased. They were repeatedly rinsed under running tap water to remove any remaining dirt. In order to lower the moisture level without destroying the chemical content, the ginger roots were peeled, chopped into chips, and oven dried at 50 ^{o}C. The neem leaves were allowed to air dry for five to six days, during which time they crisped up and kept their greenish hue. After being dried and powdered to a 1 mm mesh size, the neem and ginger leaves were kept apart in airtight plastic containers. To prepare the aqueous extracts, 100 g of each fine powder was added to 1 liter of sterile distilled water in a 1:10 ratio. After allowing the mixtures to infuse for eight hours, shaking them, and letting them cool at room temperature, the aqueous extracts were obtained by filtering the infusion, which was subsequently stored at 4^{o}C (Khan et al., 2023).

Experimental birds and management

A total of 360 one-day-old chicks, with an average weight of 35.49±0.82 g, were used in this study. The chicks were from three genetic groups: 120 chickens from a cross between Noiler cocks × Noiler hens (NN), 120 chicks from a cross between Heavy Ecotype cocks × Heavy Ecotype hens (HH), and 120 chickens from a cross between Noiler cocks × Heavy Ecotype hens (MC). The birds were randomly assigned to four treatment groups, with 30 chickens per treatment (6 males and 24 females). Each treatment group was divided into three replicates, with 10 chickens (8 females and 2 males) per replicate. The chicks were raised on deep litter in pens measuring 2.6 m wide by 3 m long. The temperature was kept at 22 ^{o}C until the end of the study. The humidity ranged from 70%–75% in the first week and 55%–65% in the second week. They were provided with unlimited access to feed and water. All groups were managed under the same environmental conditions, including temperature, light, and vaccination programs. All chicks were vaccinated with the Newcastle disease vaccine (Lasota) on day 7 of hatching. Their vaccination program also included the infectious bursal vaccine (Gumboro) on day 14, the infectious bursal (Gumboro booster) vaccine on day 21, and the Newcastle disease (Lasota booster) vaccine on day 28. The third Newcastle disease vaccine (Komarov strain) was administered at week 10, followed by the Fowl pox vaccine at week 12. A 3×4 factorial design was used to administer four dietary treatments based on aqueous plant extracts, which were allocated as follows.

Control = Chickens received the basal diet without any extract.
NE200 = Chickens received 200 ml of neem extract per liter of water.
GE200 = Chickens received 200 ml of ginger extract per liter of water.
NE100+GE100 = Chickens received 100 ml of neem + 100 ml of ginger extract per liter of water.

Feed ingredients and chemical analysis

Chemical analyses of the feeds were done at the Department of Animal Science Biochemistry and Nutrition Teaching Laboratory, University of Nigeria, Nsukka. Samples were randomly selected from each feed ingredient (maize grain, soybean meal, fish meal, and wheat) and their chemical composition was assessed following the Association of Official Analytical Chemists (AOAC) protocol (method 930.15; AOAC, 2016). Based on the results, an experimental ration was formulated.

Benzoic acid was used as a calibration reference in an adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd., UK) to calculate total metabolizable energy. Nitrogen (N) content was determined using the Kjeldahl technique, and crude protein was calculated as N × 6.25. The ether extract was examined using the AOAC protocol (method 920.39; AOAC, 2016). The standard approach (method 2002.04; AOAC, 2016) was followed for the analysis of crude fiber.

For mineral analysis, samples were first ashed and digested with HCl. Then, using Thermos Jarrell equipment (method 968.08D; AOAC, 2005), the concentrations of calcium and phosphorus were measured using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The WinFeed program (Cambridge, UK) was utilized to formulate the experimental diet. The diets were prepared in accordance with NRC guidelines to ensure that the chickens' nutritional needs were met for the starter phase (0 to 8 weeks) and the grower phase (9 to 16 weeks) (NRC, 1994; Table 1). Feed and water were provided *ad libitum* throughout the study.

Table 1. Ingredient composition and chemical composition of experimental diet

Ingredient (kg/1000 kg)	Starter diet (0-8 weeks)	Grower diet(9-16 weeks)
Maize	539.7	523.5
Soybean meal	140.6	62.8
Fish meal	35.1	15.7
Wheat	231.3	348.9
Dicalcium phosphate	18.8	14.6
Calcium carbonate	26.5	26.5
Mineral and vitamin premix	2.5	2.5
NaCl	2.5	2.5
L-lysine	1	1
DL-methionine	1	1
L-threonine	1	1
Total	1000	1000
Calculated nutrient content		
Metabolizable energy, Kcal/kg	3005.87	2864.26
Crude protein, %	18.24	15.31
Ether extract, %	3.55	3.57
Crude fibre, %	3.96	4.43
Lysine, %	1.47	1.62
Methionine, %	0.77	0.55
Calcium, %	1.46	1.36
Phosphorus, %	0.45	0.35

Growth performance

Live weight (g)

Initial weight (IW) and final body (FW) weights were obtained by weighing chickens at the beginning and at the end of the experimental period.

Weight gain (g)

The birds were weighed at the beginning of the experiment and weekly thereafter in order to determine the body weight gain (BWG) that corresponded to each treatment group. During the experiment, BWG was calculated by subtracting the initial weight from the final weight (BWG = FW – IW). Additionally, daily weight gain (DWG) was determined by dividing the BWG by the number of days in the experimental period.

Feed intake (g/chicken)

Each replicate received a known quantity of feed (X) in the morning and evening. The amount consumed was calculated by weighing the leftover feed (Y) in the following morning. The difference between X and Y (X-Y) was recorded as the quantity of feed consumed by each replicate.

Feed conversion ratio

The ratio between the amount of feed consumed and the weight gained during the same period was used to calculate the feed conversion ratio: FCR = feed intake (g) / total weight gain (g).

Haematological analysis

At the end of the study, three chickens from each genotype group within each treatment were randomly selected for blood analysis. Using a syringe and needle, approximately 3 ml of blood was drawn from the chickens' wing veins and immediately poured into ethylene diamine tetra-acetate (EDTA) sample vials for the analysis of haematological indices. Haemoglobin concentration (g/dl) was measured using a hemoglobinometer (Patil et al., 2013). To calculate the total amount of red blood cells ($x10^9$/L) and white blood cells ($x10^9$/L), a Neubauer hemocytometer was utilized (Abuoghaba, 2018). Packed cell volume (PCV) (%) was measured with a Microhematocrit Capillary Tube and subsequently centrifuged at 10,000 RPM for five minutes (Duah et al., 2020). Compound microscopes were used to count platelets ($x10^9$/L) (Mayengbam et al., 2020). Mean cell volume (MCV, μm^3), mean cell hemoglobin (MCH, pg), and mean cell hemoglobin concentration (MCHC, g/dl) were calculated using formulas provided by Odunitan-Wayas et al. (2018).

Statistical analysis

All data were subjected to a 3 × 4 factorial analysis with the following model in a completely randomized design using SAS (2013) software.

$Yijk=\mu+ai+\beta j+(\alpha\beta)ij+\varepsilon ijk$

where Yijk is the response variable, μ is the overall mean, and ai is the effect of the ith genotype (i= NN, HH, and MC). βj represents the effect of the jth level of plant extracts (j= 0, NE200, GE200, and NE100+GE100), (αβ)ij is the effect of the interaction between the level of plant extracts and the genotype, and εijk is the random error due to experimentation. Where necessary, mean separation was performed using Duncan's New Multiple Range Test in the same statistical package with significance accepted at the 5% level. Data are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Growth performance

The results of the effects of aqueous neem leaf and ginger extracts on the growth performance of pure and crossbred chickens are presented in Table 2. The interaction between genotype and plant extracts levels showed no significant differences ($p > 0.05$) in initial weight (IW), average feed intake (AFI), and feed

conversion ratio (FCR). However, final weight (FW) and average daily gain (ADG) were significantly affected ($p < 0.05$) by the treatments. As shown in Table 2, Noiler chickens (NN) fed GE200 and NE100+GE100 had the highest ($p < 0.05$) FW (2253.78 g and 2205.07 g, respectively) and ADG (19.80 g and 19.37 g, respectively), followed by the main cross (MC), while the Heavy Ecotype (HH) recorded the lowest values.

The highest body weight in NN may be attributed to the genetic potential of this breed as a commercial chicken, which tends to outperform others in terms of growth. The average body weight of MC was higher than HH indicating that the genetic potential of NN might be responsible for the higher body weight of the MC observed. Additionally, the therapeutic properties of ginger might also be the reason for the improved FW observed in NN chickens receiving GE200 and NE100+GE100. Ginger contains zingibain, a proteolytic enzyme known to aid digestion, which might have enhanced nutrient utilization and growth in these chickens. These findings corroborate those of Arkan et al. (2012), who found that adding ginger to chicken diets significantly improved body weight. From the result, it is evident that the interaction between genotype and plant extracts levels contributed to the variations in body weight observed in different breeds. This finding indicates that, alongside inherent breed differences, environmental factors such as feeding and management conditions play a crucial role in determining the body weight of chickens (Muller, 2018). The sole administration of ginger extract (GE200) and the combined use of neem and ginger extracts (NE100+GE100) significantly ($p < 0.05$) improved ADG compared to other treatments. The enhanced ADG in NN chickens treated with NE100+GE100 and GE200 may be explained by genetic selection aimed at enhancing the breed's growth rate. Also, it may be attributed to the stimulatory effects of ginger extract on the microbiota, digestive secretions, and nutrient absorption in the digestive tract (Sa'aci et al., 2018). This could be explained by ginger's ability to improve feed palatability and promote faster digestion, leading to earlier emptying of the digestive tract and stimulating additional feed intake. Ginger has been shown to enhance the release of digestive enzymes such as lipase, disaccharidase, and maltase (Zhang et al., 2009). Furthermore, Herawati (2010) reported that the enhanced performance in chickens may be attributed to the two digestive enzyme types found in ginger, lipase and protease, which are part of the plant's natural defense mechanisms.

According to Zhao et al. (2011), ginger enhances gastric secretion, enterokinesia, and digestive enzyme activity, leading to improved nutrient digestion and absorption in animals. Similarly, bioactive compounds such as flavonoids, alkaloids, and saponins found in neem leaves may aid in improved nutrient utilization, thereby improving the growth performance of birds on the NE100+GE100 treatment. On the other hand, Nidaullah et al. (2010) observed that weight gain varied insignificantly across broiler groups fed aqueous infusions of therapeutic herbs such as neem leaves, ginger rhizomes, and garlic bulbs. In the present study, the interaction between neem and ginger extract had no significant effect on FI and FCR. The findings also demonstrated that the plant extracts did not impede the availability, digestion, absorption, or utilization of nutrients. The obtained result was consistent with Landy et al. (2011) study, which indicated that feed intake was not significantly affected by adding neem leaf powder to broiler diets at a rate of 7 or 12 grams/kg at 42 days of age.

Table 2. Effect of aqueous neem leaf and ginger extracts on growth performance of pure and crossbred chickens aged 16 weeks

Parameters	IW (g)	FW (g)	ADG (g)	AFI (g)	FCR
NN × Control	36.66±0.65	1729.15±38.4^{c}	15.11±1.96^{c}	45.41±1.37	2.95±0.63
NN × NE200	35.36±0.08	2035.23±57.6^{b}	17.84±1.03^{b}	51.00±3.36	2.62±0.09
NN × GE200	35.80±0.48	2253.78±42.1^{a}	19.80±0.36^{a}	51.53±5.78	2.61±0.34
NN × NE100+GE100	36.14±0.57	2205.07±62.2^{a}	19.37±0.55^{a}	41.66±2.01	2.33±0.01
HH × Control	34.15±0.60	730.33±46.6^{g}	6.06±0.65^{g}	30.42±0.91	5.05±0.39
HH × NE200	35.02±0.56	913.99±20.1^{f}	7.84±0.17^{f}	33.55±1.34	4.27±0.07
HH × GE200	35.50±0.70	942.14±42.2^{f}	8.24±0.56^{f}	34.28±1.22	4.18±0.42
HH × NE100+GE100	35.14±1.47	1007.92±37.3^{f}	8.69±0.32^{f}	32.96±1.21	3.79±0.14
MC × Control	34.62±1.50	1265.06±4.74^{e}	10.99±0.02^{e}	34.23±7.94	3.11±0.71
MC × NE200	35.83±2.00	1209.03±19.4^{e}	10.47±0.15^{e}	33.08±3.03	3.15±0.34
MC × GE200	35.44±0.47	1417.75±49.5^{d}	12.28±0.88^{d}	30.28±1.18	2.46±0.80
MC × NE100+GE100	36.23±1.03	1411.73±50.9^{d}	12.33±0.43^{d}	30.30±3.22	2.47±0.15
P-value	0.112	0.000	0.000	0.284	0.181

$^{a,b,c,d,e,f \text{ and } g;}$ Means with different letters in the column represent significant differences at $p < 0.05$. Control: Chickens on 0 ml of extract; NE200: Chickens on 200 ml neem extract; GE200: Chickens on 200 ml ginger extract and NE100+GE100: Chickens on 100 ml of neem + 100 ml ginger extract. IW: Initial weight, FW: Final weight, ADG: Average daily gain, AFI: Average feed intake, FCR: Feed conversion ratio

Table 3. Effect of aqueous neem leaf and ginger leaf extract on haematological indices of pure and crossbred chickens aged 16 weeks

Parameters	Hb (g/dl)	PCV (%)	RBC ($x10^9$/L)	WBC ($x10^9$/L)	Platelet ($x10^9$/L)	MCV (μm^3)	MCH (pg)	MCHC (g/dl)
NN×Control	7.40±0.22^{e}	21.85±0.62^{d}	1.75±0.74	9.00±1.38^{bc}	85.50±8.08^{a}	146.19±33.1	49.95±11.1	33.86±0.06
NN×NE200	8.85±0.50^{cd}	29.80±0.72^{bc}	2.45±0.16	7.95±0.62^{c}	70.95±3.06^{b}	148.65±1.54	41.71±1.52	28.05±0.72
NN×GE200	8.00±0.22^{d}	31.93±5.12^{b}	2.35±0.17	8.10±0.68^{c}	58.35±2.82^{c}	154.95±16.3	48.51±0.12	31.56±3.22
NN×NE100+GE100	9.00±0.92^{c}	27.50±5.19^{cd}	1.35±0.28	8.80±1.02^{bc}	45.20±3.34^{d}	198.15±34.6	40.17±1.65	37.71±8.81
HH×Control	7.05±0.50^{e}	28.81±2.34^{c}	3.00±0.46	8.65±0.04^{bc}	88.50±5.88^{a}	96.86±7.08	24.12±5.44	24.69±3.80
HH×NE200	7.15±0.51^{e}	30.94±3.78^{b}	3.50±1.02	9.85±0.28^{b}	67.85±2.94^{b}	97.24±39.6	22.22±8.08	23.21±1.16
HH×GE200	10.20±0.34^{b}	36.20±1.14^{a}	3.35±0.28	13.10±0.34^{a}	55.55±5.82^{c}	96.85±23.6	26.46±0.72	28.45±6.20
HH×NE100+GE100	10.20±0.80^{b}	36.69±1.44^{a}	3.05±0.62	12.25±0.62^{a}	53.30±8.54^{cd}	123.56±20.8	34.75±8.36	27.85±2.04
MC×Control	7.20±0.81^{e}	28.78±4.26^{c}	2.85±0.40	7.55±0.04^{c}	85.65±5.70^{a}	104.14±29.6	25.34±0.76	25.74±6.62
MC×NE200	8.65±0.86^{cd}	29.32±0.72^{bc}	3.45±0.98	10.00±2.18^{b}	63.05±8.70^{bc}	92.01±32.8	27.26±10.2	29.45±0.62
MC×GE200	11.40±0.80^{a}	36.40±2.18^{a}	3.35±0.28	11.65±1.44^{a}	68.30±8.76^{b}	89.59±9.92	24.05±2.76	26.83±0.10
MC×NE100+GE100	10.20±0.34^{b}	35.53±1.38^{ab}	3.10±0.46	12.20±0.68^{a}	55.65±6.06^{c}	116.04±12.7	33.16±2.32	28.67±1.14
P-value	0.000	0.001	0.81	0.000	0.000	0.90	0.50	0.55

$^{a,b,c,d \text{ and } e;}$ Means with different letters in the column represent significant differences at $p < 0.05$. Control: Chickens on 0 ml of extract; NE200: Chickens on 200 ml neem extract; GE200: Chickens on 200 ml ginger extract and NE100+GE100: Chickens on 100 ml of neem + 100 ml ginger extract. Hb: Haemoglobin, PCV: Packed cell volume, RBC: Red blood cell, WBC: White blood cell, MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentration

Haematological indices

The results regarding the effects of aqueous neem leaf and ginger extracts on the haematological indices of pure and crossbred chickens are presented in Table 3. The study revealed no significant interaction effects ($p > 0.05$) on red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentration (MCHC). However, hemoglobin (Hb), packed cell volume (PCV), white blood cells (WBC), and platelets exhibited significant differences ($p < 0.05$) among treatments.

The highest hemoglobin levels were observed in the main cross (MC) on GE200 (11.40 g/dl), followed by the Heavy Ecotype chickens on GE200 (10.20 g/dl), NE100+GE100 (10.20 g/dl), and the main cross on NE100+GE100 (10.20 g/dl). In contrast, Noiler chickens recorded the lowest hemoglobin levels suggesting that the different genotypes had different Hb concentrations for oxygen consumption. Similarly, the highest PCV values were found in the main cross on GE200, the Heavy Ecotype on GE200 and NE100+GE100, which were comparable to the main cross on NE100+GE100, Heavy Ecotype on NE200, and Noiler on GE200. Moreover, both the main cross and the Heavy Ecotype chickens receiving GE200 and NE100+GE100 had significantly higher ($p < 0.05$) WBC counts than the other treatment groups. This might account for the "hardiness" or strength of the local chicken. The current study's findings are in line with those of Vivian et al. (2015), who hypothesized that increases in key haematological components such as PCV, Hb, RBC, and WBC in birds fed ginger-supplemented diets suggest enhanced oxygen-carrying capacity in cells, which, in turn, increases the availability of nutrients for the birds to use, ultimately contributing to overall better health and a stronger immune system in the chickens. The capacity of ginger to enhance immunity could be ascribed to its antioxidant properties as well as the presence of naturally fragrant active ingredients such as shogoals and gingerol in ginger (Khan et al., 2012). Additionally, according to Ali et al. (2008), ginger has specific anti-inflammatory and anti-oxidant properties that indirectly boost the immunity of the birds. Chickens given aqueous neem leaf and ginger extracts (NE200, GE200 and NE100+GE100) exhibited a significant ($p < 0.05$) decrease in blood platelet count compared to the control group. According to Muhammad and Lakshmi (2007), adding ginger to a fatty diet may help prevent the conversion of arachidonic acid to thromboxane and reduce platelet susceptibility to aggregating agents. This finding suggests that due to its inhibitory effects on platelet aggregation, ginger may help enhance blood circulation. The main haematological indices of the birds (RBC, MCV, MCH, and MCHC) showed a non-significant ($p > 0.05$) interaction effect among all the treatments studied, indicating that the plant extracts had no adverse effects on the formation of blood cells, their function, and their constituents. However, the values

obtained were within the reference ranges for clinically normal chickens (1.35-3.50 x10^9/L RBC; 89.59-154.94 µm^3 MCV; 24.05-34.75 pg MCH; 23.21-37.71 g/dl MCHC) (Abdulazeez et al., 2016). The significant decrease in haematological markers observed in all birds treated with higher levels of neem is likely due to the triterpenoid found in neem leaves, as noted by Singh et al. (2015). The results of the current study showed that all the haematological parameters investigated fall within the normal reference range for domestic chickens, as defined by Abdulazeez et al. (2016). The findings also indicate that the treatments administered did not have any adverse effect on the chickens.

CONCLUSION

At 16 weeks of age, the aqueous plant extracts utilized in this study improved the growth performance and haematological parameters of the birds without causing any detrimental effects on their health. It can, therefore, be concluded that NN and MC chickens administered GE200 and NE100+GE100 performed well and that these treatments can serve as suitable alternatives to antibiotic growth promoters without having negative impacts on the physiological traits of the birds. This finding may aid in the selection of superior chickens for genetic improvement, better feed efficiency, promoted growth, and improved health. To achieve the best results, the inclusion of these levels of ginger or neem extracts in chickens' drinking water is recommended.

DECLARATIONS

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Authors' contribution

Anizoba Nnenna Winifred performed the experiments, collected data, and interpreted data, writing and editing. Ikeh Nnanna Ephraim and Machebe Ndubuisi Samuel participated in data collection, and data interpretation and they designed the research methodology. Ezenwosu Celestine and Onuorah Samuel Ifeanyichukwu reviewed the literature. Amaefule Bright Chigozie and Regina Damian-Ozoke drafted the article and participated in data collection. Ugwu Chekwube Maureen and Chukwudi Prosper collected the data and revised the manuscript. Ugwuoke Jervas Ikechukwu and Madu Patricia Onyemaechi participated in the data analysis. Udeh Fredrick Ugochukwu and Nwoga Cornelius Chijioke supervised the data collection and revised the manuscript. Ugwu Simeon Ogochukwu, Ndofor-Foleng Harriet Mbunwen, and Onyimonyi Anselm Ego conceptualized and supervised the study. All authors confirmed the final version of the manuscript.

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Availability of data and materials

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

Competing interests

The authors have declared no conflict of interest.

Ethical consideration

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.

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Identification and Antibiotic Resistance of *Pasteurella multocida* Isolated from Infected Layer Chickens in West Java, Indonesia

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ABSTRACT

Bacterial infections, such as those caused by *Pasteurella multocida* serotype A, pose significant threats to poultry farming. The use of antibiotics to treat these infections can lead to antibiotic resistance. The present study aimed to identify *Pasteurella multocida* from 14 Hisex Brown layer chicken hen farms, with chikens aged 25-55 weeks, in West Java, Indonesia, and to evaluate their resistance to various antibiotics. Three samples from each farm were collected from dead chickens having symptoms of fowl cholera. Initially, the study involved isolating and identifying isolates from liver, heart, and lung organs via polymerase chain reaction. The colony was then tested for antibiotic resistance using the disk diffusion method. The results showed that 13 samples were *Pasteurella multocida* and nine were serotype A. The test results also indicated that all isolates were resistant to colistin (10 µg) and sensitive to tetracycline (30 µg), amoxicillin (25 µg), enrofloxacin (5 µg), sulfamethoxazole (25 µg), lincomycin (109 µg), and ciprofloxacin (5 µg). The study concluded that none of the *Pasteurella multocida* type A isolates were any longer sensitive to colistin, with some isolates still sensitive to tetracycline, amoxicillin, enrofloxacin, sulfamethoxazole, lincomycin, and ciprofloxacin, and two isolates showing multidrug resistance patterns.

Keywords: Antibiotic, Fowl cholera, *Pasteurella multocida*, Layer chicken

INTRODUCTION

Animal protein is an essential nutritional requirement of humans, and poultry is one of the most affordable sources of this protein. As such, the availability of poultry products needs to be increased to meet the growing demand (Choi et al., 2023). However, the poultry farming sector faces several challenges, including the threat of avian cholera. Avian cholera, also known as fowl cholera, is a poultry disease caused by a contagious bacterial infection that is widespread worldwide (Singh et al., 2014). This disease is caused by infection with the bacterium *Pasteurella multocida* (Mohamed and Mageed, 2014).

Pasteurella multocida (*P. multocida)* is a bacterium that can survive with or without oxygen and is classified under Gram-negative bacteria. while *P. multocida* exhibits robust growth on blood and chocolate agar, it fails to cultivate on MacConkey agar, Eosin Methylene Blue (EMB) agar, or other selective differential media. *P. multocida* is classified into five serogroups based on its capsule type, namely A, B, D, E, and F, and sixteen serotypes ranging from serotypes 1 to 16. Serotypes of *P. multocida* with capsules exhibit higher virulence as compared to non-capsulated serotypes. Diseases resulting from this infection can be caused by several serotypes, such as capsule type A (Harper et al., 2006). Among various *P. multocida* serotypes, serotype A is most frequently associated with fowl cholera Serotype (Wilkie et al., 2012). Infections with *P. multocida* may cause pathological lesions in several organs including the heart, intestines, kidneys, and liver, which are often characterized by petechiae and white spots (Zainuddin, 2008).

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Fowl cholera affects not only poultry livestock but also pet birds, turkeys, and ducks. Birds affected by cholera have shown two types of symptoms, including acute and chronic. Acute cholera symptoms, including fever, anorexia, mucus discharge from the beak, diarrhea, cyanosis, and increased respiratory rate, occur shortly before the death of the bird. In contrast, chronic symptoms may occur after the acute phase (Blakey et al., 2019). Fowl cholera is commonly managed by administering broad-spectrum antibiotic preparations mixed into the birds' feed and drinking water (Gray et al., 2021). Most antibiotics are used for infected cases of avian cholera. Inappropriate long-term antibiotic use can lead to antibiotic resistance. Antibiotic resistance to pathogenic bacteria in livestock is a significant health concern that needs attention (Dashe et al., 2013). Some antibiotics that should be considered in antibiotic resistance testing include penicillin, β-lactam/β-lactamase inhibitor, cephalosporin, fluoroquinolone, tetracycline, and macrolide groups (Kapoor et al., 2017). The several antibiotics used for pasteurellosis therapy have exihibited varying degrees of effectiveness and sensitivity. Aminoglycoside antibiotics, *in vitro*, are the least effective against *P. multocida* (Hurtado et al., 2020). Additionally, vancomycin and clindamycin are resistant to *P. multocida*. However, *P. multocida* is highly susceptible to fluoroquinolone and oxazolidinone groups. Isolates of *P. multocida* from animals also show resistance to tetracycline. Currently, penicillin and expanded-spectrum cephalosporin are the preferred antibiotics for treating infections by *P. multocida* (Huang et al., 2009; Hurtado et al., 2020).

The bacterium responsible for avian cholera can bring about substantial financial losses in the poultry industry. Hence, identifying and characterizing *P. multocida* bacteria are essential steps for accurate poultry therapy. It is also essential to assess the resistance profile of each tested isolate in order to establish the efficacy of antibiotics in treating this bacterial infection.

In this line, the present study aimed to identify *P. multocida* bacteria and characterize the antibiotic susceptibility profile from cases of fowl cholera in layer chickens.

MATERIALS AND METHODS

Ethical approval

Ethical approval for this study was obtained from the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Science, IPB University, Indonesia, under the approval number 121/SKE/X/2023.

Samples

The samples were collected between 2016 and 2020 from 14 suspected *P. multocida* archives isolated from Hisex Brown layer chicken farms in Sukabumi, West Java, where the chickens were aged 25-55 weeks. The cases involved chickens exhibiting symptoms of fowl cholera, such as cyanosis, fever, mucous discharge, diarrhea, and sudden deaths. These were the cases with a high mortality rate. Archive isolates were obtained from liver, heart, and lung organs showing abnormalities or lesions combined based on the original sample pens. The organs were washed with sterile phosphate buffer saline (PBS) at pH 7.4, and swab samples were collected from the inner parts using sterile cotton swabs. The swabs were then mixed with 2 mL of sterile PBS and cultured on blood agar (Oxoid, UK). The colonies grown on blood agar were observed macroscopically, and Gram staining was performed to observe bacterial morphology microscopically, confirming the presence of bipolar Gram-negative coccoid-shaped bacteria (Desem et al., 2023). The suspected isolates were subsequently stored in freeze-dried ampoules.

Culture, identification, and confirmation of *Pasteurella multocida*

To culture the bacteria, 300 µL of Brain Heart Infusion (BHI) broth media was mixed with *P. multocida* from each freeze-dried ampoule of archive isolates and homogenized by shaking. The homogenized solution was then inoculated on the edge of blood agar and MacConkey agar media (Oxoid, UK), streaked, and incubated at 37°C for 18-24 hours (Desem et al., 2023). The grown colonies were observed both macroscopically and microscopically using Gram staining to determine purity. Macroscopically, *P. multocida* colonies appear round, shiny, and whitish-grey, with varied sizes. Microscopically, however, The isolates exhibit a characteristic bipolar coccoid morphology and are Gram-negative. Pure colonies obtained from each isolate were further subjected to biochemical tests, such as catalase, oxidase, indole tests, and molecular confirmation through polymerase chain reaction (PCR, Nugroho et al., 2022).

Phenotypic colony identification

The bacterial identification method followed the protocol outlined by Nugroho et al. (2022). Pure colonies from each sample were subjected to the catalase test. The colonies were taken and mixed with 200 µL of 3% H_2O_2 on a glass slide; the formation of gas bubbles indicated a

positive result. Meanwhile, the oxidase test was conducted by adding a single colony loop needle to an oxidase paper; a color change of the paper to purple indicated a positive result. The indole test involved adding Kovacs reagent to the media inoculated with *P. multocida* bacteria; the formation of a red ring on the top of the growth media showed a positive result.

Total bacterial DNA extraction

The DNA extraction process was conducted to get genetic material from *P. multocida* cell samples previously used for testing. The boiling method was employed for DNA extraction, in which several bacterial single colonies from a blood agar culture were combined with 1 mL of PBS in a 1.5 mL microtube. The mixture was then homogenized using a vortex. The suspension was then centrifuged at 10,000 rpm for 10 minutes to pellet the bacterial cells. Subsequently, 100 µL of the pellet was taken and placed into a 1.5 mL microtube. Then, 200 µL of nuclease-free water was added and homogenized using a vortex, and finally incubated at 95 ℃ for 10 minutes. The mixture was centrifuged again at 10,000 rpm, and a 100 microliter portion of the supernatant containing the extracted DNA was collected for potential PCR analysis.

Detection of capA gene specific to *Pasteurella multocida* Serotype A

To detect the capA gene specific to *Pasteurella multocida* Serotype A, a total of 5 µl of extracted DNA was mixed with 25 µl of MyTaq™ HS Red Mix master mix, which included 2 µl of capA forward primer (5′-TGCCAAAATCGCAGTGAG-3′), 2 µl of capA reverse primer (5′-TTGCCATCATTGTCAGTG-3′) with an amplification size of 1044 bp (Townsend et al., 2001; Nugroho et al., 2022), and 16 µl of nuclease-free water. The master mix and the mixture of bacterial DNA extract were then placed into a thermal cycler for DNA amplification. The PCR process was run for 30 cycles. The PCR condition process involved a denaturation step at 95°C for 15 seconds, an annealing step at 55°C for 15 seconds, and an extension step at 72°C for 10 seconds. The PCR product was subsequently analyzed using gel electrophoresis. The amplified samples were observed by electrophoresis, utilizing a 1.5% agarose gel, and stained at a concentration of 0.5 µg/ml ethidium bromide (EtBr). A 100 base pair marker (VC 100 base pair Plus DNA Ladder Vivantis) was employed as a reference for size determination. The electrophoresis procedure was conducted for 35 minutes at a voltage of 80V.

Antibiotic resistance test

The antibiotic resistance test was conducted using the disk diffusion Kirby Bauer method. Mueller Hinton Agar (MHA; Himedia, India) was the media utilized for this assay. Prior to inoculation with bacterial colonies, the agar media was incubated at 37°C for 10-20 minutes. A suspension was prepared from bacterial isolates on Trypticase Soy Agar media (Oxoid, UK) diluted with physiological NaCl and homogenized with a vortex mixer. Turbidity levels were compared with the McFarland 1 standard. A 100 µl suspension was taken and dropped onto MHA media, spread evenly with a sterile cotton bud, and left for 10 minutes (Cappuccino and Welsh, 2018). Antibiotic discs (Oxoid, UK) each containing 25 µg amoxicillin, 30 µg tetracycline, 5 µg ciprofloxacin, 5 µg enrofloxacin, 10 µg colistin, 109 µg lincomycin, and 25 µg sulfamethoxazole were placed on the media inoculated with bacteria, ensuring a minimum distance of 24 mm between the discs. The media was then incubated at 35°C ± 2°C for 18-24 hours (Hudzicki, 2009). After incubation, the diameters of the inhibition zones were measured using a caliper or ruler with a millimeter scale. The results of the antibiotic sensitivity testing were compared with standard inhibition zone diameter values for antibiotics, as outlined in Table 1.

Table 1. The antibiotic resistance parameters in *Pasteurella multocida* from Hisex Brown layer chickens

Group of antibiotics	Antibiotics	Dose	Inhibition zone diameter (mm)			Reference
			Sensitive	Intermediate	Resistance	
Penicillins	Amoxicillin	20/10 µg	≥ 27	–	–	CLSI M45 (2015)
Tetracyclines	Tetracycline	30 µg	≥ 24	–	≤ 24	CLSI M45 (2015)
Fluoroquinolones	Ciprofloxacin	5 µg	≥ 27	–	≤ 27	EUCAST (2024)
	Enrofloxacin	5 µg	≥ 21	17–20	≤ 16	CLSI VET 01S (2015)
Polymyxins	Colistin	5 µg	≥ 17	–	≤ 11	CLSI M45 (2015)
Sulfonamides	Sulfamethoxazole	1,25/ 23,75 µg	≥ 24	–	–	CLSI M45 (2015)

RESULTS

Culture, identification, and confirmation results of *P. multocida*

The results of culture, identification, and confirmation of 14 isolates are detailed in Table 2. Out of 14 archive isolates grown on blood agar media, 13 exhibited colony characteristics with varied sizes, accompanied by round, shiny, and whitish-grey colony formations (Figure 1A). In contrast, no colony growth was observed on MacConkey agar media (Figure 1B). Microscopic examination of all the 13 archive isolates showed conformity with the characteristic features of *P. multocida* bacterial cells, namely a bipolar coccoid shape and a Gram-negative nature (Figure 1C).

The same results were also obtained in the oxidase, catalase, and indole tests for all archive isolates. The 13 isolates exhibited characteristics typical of *P. multocida*. Specifically, pure colonies from each isolate produced gas bubbles upon the addition of H_2O_2 in the catalase test (Figure 1D). They resulted in a color change to purple when tested on an oxidase paper (Figure 1E). In the indole test, all isolates showed the presence of a red ring after being dripped with Kovacs reagent (Figure 1F).

Molecular testing through PCR revealed that 9 out of 13 suspected isolates were confirmed as *P. multocida* serotype A with an amplification size of 1044 bp using specific capA primers (Figure 2).

Antibiotic sensitivity by disk diffusion method

A total of 9 out of 13 isolates, which were confirmed positive for *P. multocida* serotype A through PCR testing, were then subjected to sensitivity testing using the disk diffusion method. The sensitivity test results were evaluated based on the formation of inhibition zones (Figure 3). Isolates tested via the disk diffusion method exhibited different resistance patterns. Each isolate's resistance profile was compared against Clinical and Laboratory Standards Institute (CLSI) standards (2015) for amoxicillin, tetracycline, and sulfamethoxazole, CLSI standards (2015) for enrofloxacin, and European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards 2024) for ciprofloxacin and colistin.

The sensitivity patterns exhibited by each isolate varied, as shown in Table 3. The isolate with the freeze-dried ampoule code B001 demonstrated the highest level of resistance, while the isolates with ampoule codes B0018, B020, B073, and B077 showed the lowest resistance. The antibiotics used in the disk diffusion test exhibited different sensitivities. Table 4 shows that three isolates were resistant to ciprofloxacin, three were resistant to amoxicillin, and one isolate was resistant to tetracycline, enrofloxacin, and lincomycin. The antibiotic resistance patterns indicate the presence of multidrug resistance in several tested isolates. Specifically, two isolates exhibited resistance to more than three types of antibiotics, as illustrated in Table 5.

Table 2. Culture results, identification, and confirmation of *Pasteurella multocida* from Hisex Brown layer chickens using various test methods

No.	Isolate code	Macroscopic	Gram staining	MCA	Catalase Test	Oxidase Test	Indole Test	PCR CapA
1	B001	+	+	–	+	+	–	+
2	B008A	+	+	–	+	+	+	+
3	B009A	+	+	–	+	+	+	–
4	B010A	+	+	–	+	+	+	–
5	B018	+	+	–	+	+	+	+
6	B020	+	+	–	+	+	+	+
7	B036	+	+	–	+	+	+	+
8	B071	+	+	–	+	+	+	+
9	B072	+	+	–	+	+	+	–
10	B073	+	+	–	+	+	+	+
11	B074	–	–	+	–	–	+	–
12	B075	+	+	–	+	+	+	+
13	B076	+	+	–	+	+	+	–
14	B077	+	+	–	+	+	+	+

Isolate code: Bacterial isolate that coded in freeze-dried ampoules; MCA: Mac Conkey Agar

Table 3. Sensitivity pattern of *Pasteurella multocida* from Hisex Brown layer chickens to antibiotics

Sample code	Susceptibility of *Pasteurella multocida* to antibiotics						
	TE	AML	ENR	SXT	LCS	CIP	CT
B001	R	R	R	S	S	R	R
B008A	S	S	S	S	S	R	R
B018	S	S	S	S	S	S	R
B020	S	S	S	S	S	S	R
B036	S	S	S	S	S	R	R
B071	S	R	S	S	S	S	R
B073	S	S	S	S	S	S	R
B075	S	R	S	S	R	S	R
B077	S	S	S	S	S	S	R

TE: Tetracyclin; AML: Amoxicillin; ENR: Enrofloxacin; SXT: Sulfamethoxazole; LCS: lincomycin; CIP: Ciprofloxacin; CT: Colistin; S: sensitive; R: resistance

Table 4. Number of *Pasteurella multocida* isolates from Hisex Brown layer chickens in West Java, Indonesia that are resistant to antibiotics

Antibiotics	Number of isolates based on resistance category		
	Sensitive	*Intermediate*	Resistance
Tetracyclin	8	0	1
Amoxicillin	6	0	3
Enrofloxacin	8	0	1
Sulfamethoxazole	9	0	0
Lincomycin	8	0	1
Ciprofloxacin	6	0	3
Colistin	0	0	9

Table 5. Antibiotic resistance based on the number of *Pasteurella multocida* isolates that cause fowl cholera Hisex Brown layer chickens

Amount of Antibiotic	Amount of isolates[a]	Antibiotics [b]
1	4	CT
2	2	CIP, CT
	1	AML, CT
3	1	AML, LCS, CT
4	0	-
5	1	TE, AML, ENR, CIP, CT
6	0	-
7	0	-

[a] isolates that are resistant to antibiotics. [b] resistance to ≥ 3 types of antibiotics is referred to as multiresistance. TE: Tetracyclin; AML: Amoxicillin; ENR: Enrofloxacin; SXT: Sulfamethoxazole; LCS: lincomycin; CIP: Ciprofloxacin; CT: Colistin

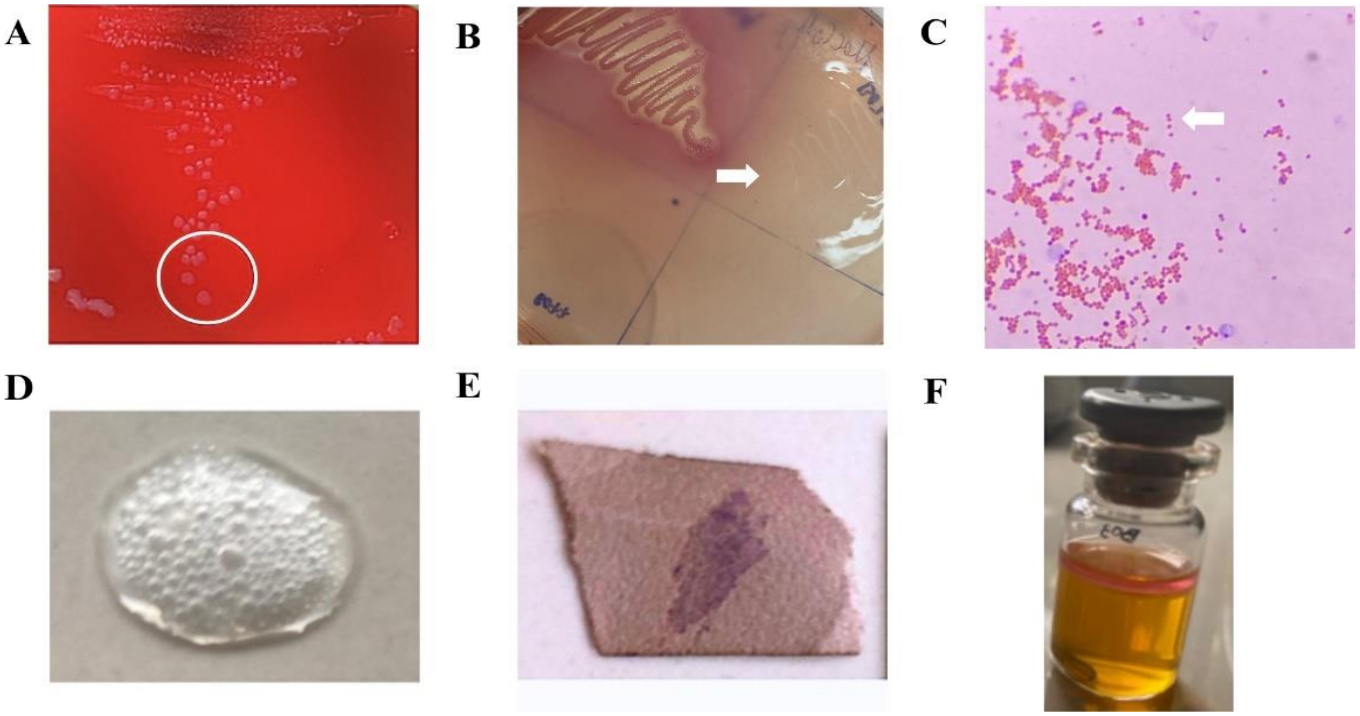

Figure 1. Culture and identification results using various methods. **A:** Morphology of *Pasteurella multocida* colonies on blood agar media (circle); **B:** Absence of bacterial colonies on MacConkey Agar media (arrow); **C:** *Pasteurella multocida* bacteria observed under the microscope (magnification 400x) (arrow); **D:** Catalase test showing bubble formation when *P. multocida* reacts with H_2O_2; **E:** Purple color formation due to *P. multocida* streaks on the oxidase paper; **F:** Red ring in the indole test confirming *P. multocida* isolate

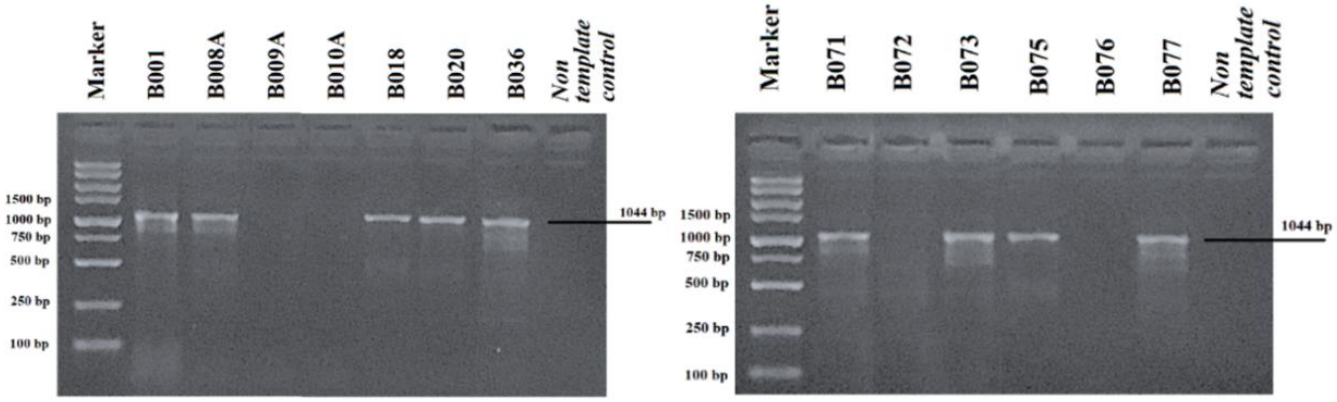

Figure 2. PCR results targeting 1044 bp against *Pasteurella multocida* isolates. Marker: VC 100 bp Plus DNA Ladder Vivantis

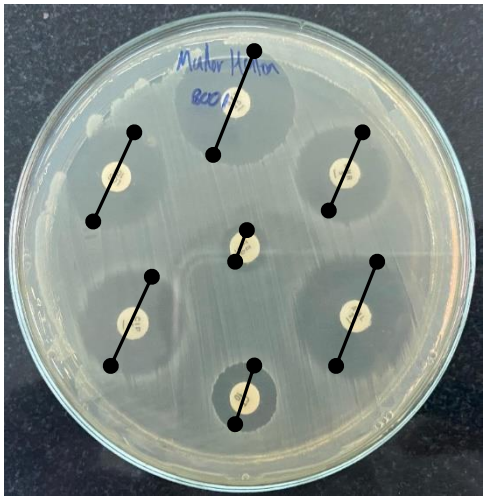

Figure 3. Measurement of the diameter of the inhibition zone in the disk diffusion test. Line marks with rounded edges indicate the apparent diameter of the inhibition zone

DISCUSSION

Fowl cholera has been identified as a significant concern in the commercial poultry business, prompting the use of various techniques to investigate the diversity and transmission patterns of *P. multocida* strains globally (Subaaharan et al., 2010).

Serotypes A, D, and F of *P. multocida* have enzymes capable of producing glucuronic acid and glucosamine, which are modifications of hyaluronic acid, whereas *P. multocida* type B lacks the *hyaC* and *hyaD* genes (Pasomboon et al., 2021). These genes are critical in the synthesis of glucuronic acid and hyaluronic acid. According to Guan et al. (2020), the difference between *P. multocida* serotypes A, D, and F, and type B lies in their capsular components. Serotypes A, D, and F consist of glycosaminoglycan (GAG), while type B consists of non-GAG-like components.

Antibiotic resistance in bacterial infections is a significant global challenge (Frieri et al., 2017). Based on its mechanisms, antibiotic resistance is classified into four categories, including modification of antibiotic molecules, preventing antibiotics from reaching their targets, bypassing antibiotic targets, and cell adaptation to antibiotics (Sabtu et al., 2015; Munita and Arias, 2016).

The antimicrobial resistance patterns in this study align with those of some previous research. Sarangi and Panda (2011) studied the antibiotic sensitivity test of *P. multocida* isolates and found that the organisms were sensitive to enrofloxacin. In the current study, eight out of nine isolates showed sensitivity to enrofloxacin, a fluoroquinolone antibiotics commonly used as a broad-spectrum antibiotic class for various infections (Redgrave et al., 2014). A study by Furian et al. (2014) indicated high antibiotic resistance to enrofloxacin. Resistance to the quinolone group can occur due to type IV topoisomerase

mutations targeting these antibiotics (Redgrave et al., 2014). Another quinolone that was tested in the present study was ciprofloxacin. In this study, six out of nine isolates were sensitive to ciprofloxacin as another quinolone.

In contrast to Sarangi and Panda (2011), the organisms in the current study were sensitive to sulfamethoxazole, an antibiotic widely used in humans and commonly used to treat bacterial infections in pigs and cattle (Vila-Costa et al., 2017). Resistance can occur through several mechanisms, including changes in membrane permeability, less sensitive enzymes, changes in bacterial enzyme targets, mutations in enzyme targets, and inherent resistance (Huovinen, 2001).

Shivachandra et al. (2004) reported significantly elevated levels of resistance (tetracycline 24.39%) in a study that examined 123 strains of *P. multocida*. These strains were collected from outbreaks of fowl cholera in different avian hosts in various regions of India. In this study, eight out of nine isolates were sensitive to tetracycline.

In the present study, six out of nine isolates were sensitive to amoxicillin. A study by Dieb et al. (2020) indicated high resistance of *P. multocida* isolates to amoxicillin. Resistance to beta-lactam antibiotics occurs when PBP undergoes modification or structural changes. Penicillin-binding protein (PBP) is an enzyme that plays a crucial role in the biosynthesis of bacterial cell walls as a peptidoglycan precursor (Halawa et al., 2023).

Lincomycin and colistin were also among the antibiotics examined in the present study. In Table 4, eight out of nine isolates showed sensitivity to lincomycin. Lincomycin is a lincosamide antibiotic derived from several *Streptomyces* (*S.*) species, such as *S. lincolnensis*, *S. roseolu*, *S. caelestis*, and *Micromonospora halphytica*. Lincosamide antibiotics are commonly used as therapeutic agents against anaerobic bacterial infections and some protozoan species. These antibiotics work by inhibiting bacterial protein synthesis, slowing bacterial growth, or killing the bacteria (Spízek et al., 2004). The antibiotic activity against *Pasteurella multocida* indicates fairly good sensitivity. Resistance mechanisms can occur in three ways including modification of antibiotic targets, bacterial efflux pumps, and drug inactivation (Leclercq, 2002).

All isolates examined in the current study displayed resistance to colistin. This finding aligns with a study by El-Demerdash et al. (2023), which reported that 60% of *P. multocida* isolates from birds were resistant to colistin. The primary mechanism of resistance to colistin is typically a chromosomal mutation in genes related to altering the lipid A of lipopolysaccharides (LPS). Such modifications alter the target site of colistin, serving as an adaptive defense mechanism against the antibiotic.

The results of resistance tests indicated that several isolates exhibit multidrug resistance patterns, as shown in Table 5. Multidrug resistance patterns complicate the treatment of bacterial infections using antibiotics (Frieri et al., 2017). Bacterial multidrug resistance to antibiotics can arise from the accumulation of plasmid or transposon genes that confer resistance (R-plasmids) to a particular antibiotic and/or from efflux pumps expelling more than one type of antibiotic (Nikaido, 2009). In addition, the presence of small plasmids has been associated with antimicrobial resistance in *P. multocida* (Rosenau et al., 1991). The simultaneous presence and dissemination of these small plasmids have led to the development of *P. multocida* isolates with multi-resistance (San Millan et al., 2009) and specific resistance to ampicillin (Rosenau et al., 1991), tetracycline (Kehrenberg et al., 2001), and streptomycin (Wu et al., 2003).

CONCLUSION

The isolation and identification of suspected fowl cholera cases in Hisex Brown layer chickens from farms in Sukabumi, West Java, indicated that 13 out of 14 isolates are positive for *Pasteurella multocida*, with 9 out of 13 isolates positive for *P. multocida* serotype A. Antibiotic resistance testing revealed that all nine isolates of *P. multocida* serotype A were resistant to colistin. Still, some isolates remained sensitive to tetracycline, amoxicillin, enrofloxacin, sulfamethoxazole, lincomycin, and ciprofloxacin, with two isolates showing multidrug resistance patterns.

DECLARATIONS

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Authors' contributions

Agustin Indrawati acquired the funds, conceptualized and supervised the work, and revised the manuscript. Titiek Sunartatie, Safika, Herjuno Rafi Abhirama, Citra, Ryan Septa Kurnia, Muhammad Ade Putra, Christian Marco Hadi Nugroho, and Ni Luh Putu Ika Mayasari conducted the experiments, collected and analyzed the data, and prepared the manuscript. All authors read and approved the last manuscript version.

Competing interests

The authors declared that there are no competing interests.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by the authors.

Availability of data and materials

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

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Effects of *Salvia officinalis* on Production Characteristics of Laying Hens

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ABSTRACT

Due to the extreme importance of the poultry industry in securing animal proteins for humans, it is necessary to expand the research related to increasing egg production without resorting to antibiotics, which pose significant drawbacks. This study explored the impact of sage plant extracts, known for their bioactive compounds, on the production indicators of laying hens. Thirty chickens were randomly assigned to three groups, including a control group and two experimental groups (T1 and T2) receiving sage plant aqueous extract at 0.1% and 0.2% in their diets, respectively. The egg production percentage, egg weight percentage, percentage of daily feed consumption, feed conversion coefficient, and blood calcium concentrations were measured. The results indicated that supplementation of sage extract in the diet of the laying hens under study increased daily egg production percentage and daily egg yield significantly in group T2 (87.63%, 59.7 eggs/day) and improved average egg weight (68.23 grams) in group T1. Moreover, there was no significant difference in daily feed consumption among the tested hens. A notable reduction was also observed in the feed conversion ratio to 2.09 in group T2.

Keywords: Feed additive, Laying hen, Plant extract, Productivity, Sage

INTRODUCTION

The poultry industry plays a vital role in meeting the growing global demand for animal protein owing to the rapid growth rate and efficiency of poultry in food conversion, especially considering the possibility of large numbers of high-density farming in relatively small areas. In line with the global trend, it is recommended to limit the use of antibiotics as growth-stimulating agents in poultry feed due to concerns over residual effects in poultry products and associated side effects that harm human health (Grashorn, 2010). In addition to the emergence of bacterial strains showing resistance to antibiotics, extensive research has been conducted on the use of natural plants and their extracts as safe and effective alternatives to antibiotics. These alternatives aimed to enhance the immunity of poultry (Mustafa and Ihsan, 2022a) and thus improve their productivity represented by growth rates and increased egg production.

Recent studies have indicated that aromatic plants and their extracts, when added to poultry diets, can effectively address current challenges in laying hens' productivity (Galamatis et al., 2021) primarily due to their antioxidant and antimicrobial properties (Khater, 2022).

The use of medicinal plants dates back many centuries, with certain species actively integrated into human life (Datta and Patil, 2020). Nowadays, however, medicinal plants are widely recognized as phytogenic feed additives in poultry nutrition (Karaskova et al., 2015). It worth noting that Sage is considered a medicinal plant that has been known since ancient times for its healing properties in poultry due to its rich profile of active compounds, particularly its polyphenols.

The European Union has banned the use of antibiotics as growth stimulators due to the emergence of bacterial resistance to them. Moreover, using antibiotics has led to the destruction of beneficial intestinal microbes, which has spurred research efforts to find alternative approaches

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(Palamidi et al., 2017) in poultry nutrition to improve the productive qualities of growth and increase egg production without having negative consequences for human or avian health. As a result, medicinal plants have become essential additives in poultry diets. The investigation of the effect of medicinal plants (Aroche et al., 2018), including powder, essential oils, and oil extract as growth-stimulating substances, antioxidants and immune system enhancers, is an active area of poultry research (Mustafa and Ihsan, 2022b).

Therefore, the present study aimed to evaluate the effect of adding an aqueous extract of sage on the egg production parameters, as well as its influence on feed consumption, feed conversion coefficient, and blood calcium concentrations in laying hens.

MATERIALS AND METHODS

Ethical approval

The present study was conducted and approved by the Animal Care and Use Committee of Saydnaya Poultry Facility, General Poultry Corporation, Ministry of Agriculture, Damascus countryside, Syria.

Experimental design

The research was carried out from December 2021 to January 2022 in the poultry field in Saydnaya, which belongs to the public poultry organization, Saydnaya Poultry Facility, General Poultry Corporation, Damascus countryside, Syria. Thirty white laying hens, approximately 32 weeks old and weighing around 1.65 kg each (commencing production at six weeks), with an average production rate of 66% across all groups, were selected for the study.

The hens were randomly assigned to three groups, each consisting of 10 laying hens included a control group and two treatment groups (T1 and T2). Group T1 received an aqueous extract of sage in their diet at a concentration of 0.1% of diet while Group T2 received the extract at 0.2% (Alduri et al., 2016).

The experimental units of laying hens were kept in sheds with each unit having a floor area of 4 m^2. Throughout the experiment, the hens were subjected to a daily lighting program (14 hours of light and 10 hours of darkness). Daily temperatures were recorded and maintained between 20-22 degrees Celsius. The hens had access to feed and water throughout the experimental period.

The feed, provided in the form of crushed pellets, was formulated according to the specifications given in Table 1 by the Department of Medical Biotechnology, National Commission for Biotechnology, Damascus, Syria. Each hens received 115 g of feed per day. Each shed was equipped with an automatic hanging plastic waterer and a hanging plastic feeder.

Table 1. The components of laying hens' diet used in the present study

Diet component	Weight (kg)	Chemical composition	%
Soya	590	Protein	18.70
Corn	230	Fat	4.10
Bran	50	Fiber	22.9
Egg white concentrate	30	Ash	8.50
Sand	100	Carbohydrate	35.10
Antifungal	1	Moisture	8.30
Salt	0.5	Dry matter	97.10
Organic acids	0.5	Energy (kcal/kg)	2273.33
Sage Exract	0.1 - 0.2%		

Preparation of sage aqueous extract

The sage plant (*Salvia officinalis*), belonging to the Labiatae family, was a perennial herbaceous growing plant that can grow up to 60 cm in height. Leaves were collected in the spring from the Damascus countryside, dried at room temperature, and ground into a fine powder. To prepare the extract, 50 grams of dried, ground *Salvia officinalis* leaves were mixed with 250 ml of distilled water and stirred for 24 hours. The mixture was then filtered and subjected to rotary evaporation. Jakovljevi´c et al. (2019) have characterized the chemical composition of *Salvia officinalis* extract as having 49 components including camphor (25.14%), α-thujone (18.83%), 1,8-cineole (14.14 %), viridiflorol (7.98%), β-thujone (4.46%), and β-caryophyllene (3.30%) as the main components, determined by gas chromatography-mass spectrometry.

Measurement of parameters

The 120-day experiment was divided into four equal productive periods of 30 days each (Ceylan et al., 2003). The mortality percentage in each group was calculated using the following equation.

$$\text{Mortality (\%)} = \frac{\text{Total number of hens}}{\text{Number of dead laying hens}}$$

Percentage of egg production

The productivity indicators were studied after collecting the produced eggs and weighing them daily via the following equation.

$$\text{Daily production ratio} = \frac{\text{Number of eggs produced per week}}{7}$$

The eggs were collected once a day at 1:30 in the afternoon for the duration of the experiment and then according to the egg production rate. Hen Day Production

HDP (%) was The number of eggs produced during the experimental period/the number of live chickens at the end of the term × the length of the term in days ×100 (Ceylan et al., 2003).

Percentage of egg weight

The eggs produced at the end of each of the four trial periods were weighed for three consecutive days for each of the treatment groups, where the average weight of the eggs, and mass of eggs produced per hen per day, was calculated using the following equations respectively:

$$\text{Average weight of the eggs} = \frac{\text{Total weight of eggs produced per week}}{\text{Number of eggs produced in the same week}}$$

$$\text{Mass of eggs produced (hen/day)} = \frac{\text{Average daily weight} \times \text{Daily production ratio}}{100}$$

Percentage of daily feed consumption

The average daily feed consumption per chick was consumed weekly (taking into account the subtraction of the value of the weight of the wasted feed that was collected and weighed on a daily basis (Ceylan et al., 2003).

Feed conversion coefficient

The feed conversion coefficient was calculated using the following equation.

$$\text{Feed conversion coefficient (kg)} = \frac{\text{Amount of feed consumed per day}}{\text{Number of eggs produced per day}}$$

Blood calcium concentrations

Blood was collected using the anterior heart-puncture method (Blalock, 1956), and all calcium determinations were made according to the method described by Fales (1953).

Statistical analysis

The results were reported as the mean ± SD for ten replicate measurements. Statistical analysis was conducted using one-way ANOVA, followed by Tukey's test to compare treatment means, employing MINITAB software 2016 (version 14). Statistical significance was set at $p < 0.05$.

RESULTS

The indicators of productive efficiency in laying hens were studied separately.

Percentage of egg production

Table 2 shows the average percentage of daily egg production during the four weeks of the experiment. Both groups of laying hens that consumed the aqueous extract of sage plant at 0.1% and 0.2% concentrations showed higher daily egg production percentages compared to the control group.

Percentage of egg weight

Table 3 illustrates the daily egg mass per hen, which was higher in both groups of laying hens that consumed the aqueous extract of sage plant at 0.1% and 0.2% concentrations compared to the control group. Additionally, the average egg weight in these groups was also higher, as shown in Tables 4 and 5, respectively.

Percentage of daily feed consumption

As is shown in Table 6, the average of daily feed consumption in both groups of laying hens that consumed the aqueous extract of the sage plant at 0.1% and 0.2% concentrations was higher as compared to the control group.

Feed conversion coefficient

Table 7 indicated that the feed conversion coefficient was lower in both groups of laying hens that consumed the aqueous extract of sage plant at 0.1% and 0.2% concentrations compared to the control group.

Blood calcium concentrations

Table 8 showed that blood calcium concentrations were higher in both groups of laying hens that consumed the aqueous extract of sage plant at 0.1% and 0.2% concentrations compared to the control group.

Table 2. The effect of *Salvia officinalis* extract on the percentage of daily egg production in laying hens

Group	Breeding period (weeks)				Average	p value
Control	66.81 ± 0.89^{b}	65.42 ± 0.33^{c}	64.15 ± 0.16^{d}	67.79 ± 0.18^{a}	66.04 ± 1.47	0.001
T1	86.3 ± 0.06^{c}	86.96 ± 0.18^{b}	87.02 ± 0.33^{b}	89.48 ± 0.04^{a}	87.44 ± 1.39	0.001
T2	86.41 ± 0.16^{d}	87.02 ± 0.13^{c}	87.91 ± 0.2^{b}	89.21 ± 0.11^{a}	87.63 ± 1.21	0.001

The values with the same letters on the same row are not statistically different ($p > 0.05$).

Table 3. The effect of *Salvia officinalis* extract on the egg mass per day in laying hens

Group	Breeding period (weeks)				Average	p value
Control	42.36 ± 5.31^{ab}	41.89 ± 4.87^{ab}	40.33 ± 5.12^{b}	42.97 ± 4.38^{a}	41.88 ± 1.12	0.05
T1	58.86 ± 4.21^{a}	60,31 ± 3.99^{a}	58.46 ± 4.76^{a}	61.00 ± 5.23^{a}	59.65 ± 1.19	0.03
T2	58.31 ± 5.11	60.16 ± 6.09	59.87 ± 4.63	60.49 ± 4.44	59.70 ± 0.96	0.12

The values with the same letters on the same row are not statistically different (p > 0.05).

Table 4. The effect of *Salvia officinalis* extract on the average of egg weight in laying hens

Group	Breeding period (weeks)				Average	p value
Control	63.42 ± 0.28^{a}	61.04 ± 0.85^{b}	62.87 ± 1.16^{a}	63.39 ± 0.72^{a}	62.68 ± 1.12	0.001
T1	68.21 ± 0.77^{c}	69.36 ± 0.38^{a}	67.19 ± 0.49^{b}	68.18 ± 0.26^{bc}	68.23 ± 0.88	0.001
T2	67.49 ± 0.38^{c}	69.14 ± 0.33^{a}	68.11 ± 0.23^{b}	67.81 ± 0.36^{bc}	68.13 ± 0.71	0.001

The values with the same letters on the same row are not statistically different (p > 0.05).

Table 5. The effect of *Salvia officinalis* extract on the average egg weight in laying hens

Group \ Age	End of month 1	End of month 2	End of month 3	End of month 4	Average	p value
Control	61.6 ± 0.12	62.1 ± 0.33	61.4 ± 0.22	60.8 ± 0.42	61.47 ± 0.53	0.61
T1	67.6 ± 0.16	68.1 ± 0.19	68.4 ± 0.12	69.2 ± 0.36	68.32 ± 0.67	0.43
T2	67.3 ± 0.14	67.9 ± 0.28	68.1 ± 0.65	68.8 ± 0.51	68.02 ± 0.61	0.51

Table 6. The effect of *Salvia officinalis* extract on the daily feed consumption of laying hens (Gram/ Hen / Day)

Group	Breeding period (weeks)				Average	p value
Control	121.31 ± 5.3^{a}	124.87 ± 6.27^{a}	120.83 ± 5.12^{a}	122.09 ± 5.19^{a}	122.28 ± 1.81	0.001
T1	122.21 ± 4.21^{b}	127.24 ± 5.09^{a}	123.49 ± 6.46^{b}	123.52 ± 6.03^{b}	124.12 ± 2.17	0.001
T2	122.61 ± 5.11^{d}	126.96 ± 6.19^{c}	125.78 ± 4.83^{b}	124.12 ± 4.94^{a}	124.87 ± 1.9	0.001

The values with the same letters on the same row are not statistically different (p > 0.05).

Table 7. The effect of *Salvia officinalis* extract on the feed conversion coefficient of laying hens

Group	Breeding period (weeks)				Average	p value
Control	2.86 ± 0.34	2.98 ± 0.28	2.99 ± 0.32	2.84 ± 0.41	2.91 ± 0.07	0.41
T1	2.08 ± 0.21	2.1 ± 0.21	2.11 ± 0.16	2.02 ± 0.23	2.07 ± 0.04	0.93
T2	2.1 ± 0.11	2.11 ± 0.09	2.1 ± 0.13	2.05 ± 0.14	2.09 ± 0.02	0.97

Table 8. The effect of *Salvia officinalis* extract on blood calcium concentrations in laying hens

Group \ Age	End of the first month	End of the second month	End of the third month	End of the fourth month	Average	p value
Control	9.78 ± 0.72^{b}	9.41 ± 0.35^{a}	9.26 ± 0.29^{a}	10.81 ± 0.12^{a}	9.81±0.69	0.004
T1	12.6 ± 0.13	12.10 ± 0.19	11.98 ± 0.54	12.2 ± 0.33	12.22 ± 0.26	0.33
T2	11.9 ± 0.11	12.72 ± 0.28	12.1 ± 0.65	12.6 ± 0.51	12.33 ± 0.39	0.34

The values with the same letters on the same row are not statistically different (p > 0.05).

DISCUSSION

The present study demonstrated that herbal supplementations with *Salvia officinalis* extract may reduce the stress caused by increasing the number of chickens in the same area in different conditions. Specifically, the use of 0.2% sage extract significantly ($p \leq 0.05$) increased egg production to 87.63%, compared to 66.04% in the control group. This finding aligns with previous research indicating a significant ($p < 0.01$) decrease of approximately 3% in egg production when chicks density increased to 10 birds/m^2 without supplementations (Mustafa and Ihsan, 2022a). It has been demonstrated that Plants from the *Labiatae* family promote stability in animal production, also shown in poultry meat and eggs. Table 4 shows data regarding the effect of sage extract on egg weight measurements. Treatments with 0.2% herbals led to a significant ($p \leq 0.05$) increase in egg weight (68.13 g) compared to the control group (62.68 g).

The results in the present study corroborate the findings of Mustafa and Ihsan (2022a) and were consistent with those of Cabuk et al. (2014) who reported increased egg mass in quails fed with sage powder, while parameters such as egg shape index and shell thickness showed non-significant effects (Alduri et al., 2016). Additionally, Alduri et al. (2016) suggested that using sage extracts in layer feed significantly increased egg weight compared with the control group.

The results presented in Table 7 demonstrated that the feed conversion coefficient, which indicated the efficiency of converting feed consumption (Table 6) into egg production during the specified period, was improved with herbal supplementation, consistent with findings by Alduri et al. (2016). This improvement was further supported by Mustafa and Ihsan (2022a), who showed that medicinal herbs from the *Lamiaceae* family, such as sage, enhance feed conversion coefficients in laying hens.

The enhancement in feed utilization and growth performance due to sage supplementation may be attributed to improvements in the metabolic system, balancing beneficial and pathogenic bacteria, increasing enzymatic activity in digestion, and enhancing liver function Farhadi et al. (2020). Additionally, the appealing aroma of sage could potentially increase palatability, encouraging higher feed intake. Furthermore, sage contains antioxidants that scavenge radicals and mitigate lipid oxidation, thereby reducing the feed conversion coefficient in laying hens.

As for the effect of sage aqueous extract on calcium concentrations in blood plasma, its use has been observed to enhance calcium digestion and absorption processes, similar to the findings of Rasouli et al. (2019). Overall, this study emphasized the efficiency of aqueous sage extract in improving egg production and overall health in laying hens (Cabuk et al., 2014), which finally revealed that the intake of aqueous extract of the sage plant may achieve the economic goal sought by poultry breeders.

CONCLUSION

Based on the results of the study, it can be concluded that adding 0.1% (T1) and 0.2% (T2) sage extract to the feed of the laying hens under study was beneficial for several tested parameters involving daily egg production percentage, number of eggs produced per day, and blood calcium concentrations. Notably, group T2 showed superior results compared to group T1. Additionally, the study highlights the positive impact of adding 0.1% (T1) and 0.2% (T2) sage extract to feed diet on increasing average egg weight in laying hens, with group T1 demonstrating higher weights compared to group T2. However, no significant difference was observed in the daily feed consumption of the tested hens. Further investigation into the effects of other medicinal plants known for their high phenolic and flavonoid content, and consequently high bioactivity, on the production characteristics of laying hens was recommended.

DECLARATIONS

Competing interests

The authors declare that there is no conflict of interest.

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Authors' contributions

Frdoos Al Fadel acquired the funds and conceptualized and supervised the work. Rafan Abd Al Hadi collected and analyzed the data, and prepared the manuscript. All authors read and approved the last version of the manuscript.

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Ethical considerations

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

Availability of data and materials

The data and materials are available upon demand from authors.

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Heritability and Genetic Correlations of Carcass and Meat Quality Traits in White and Brown Strains of Japanese Quail

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ABSTRACT

Successful breeding programs for Japanese quails rely on accurately estimating genetic parameters linked to economically important traits such as body weight, carcass characteristics, and meat quality. The objective of the present study was to evaluate body weight (BW) characteristics, carcass attributes, and their genetic correlations with select meat quality traits in two strains of Japanese quail (white and brown). A total of 530 quail chicks, with 265 from each strain, were included in the analysis. At six weeks of age, the quails were slaughtered, and carcass traits as well as amino acid profiles were measured. For BW traits, the heritability (h^2) estimates ranged from 0.27 at d 1 to 0.36 at d 42. The h^2 estimated for carcass traits ranged from 0.19 for liver weight, to 0.42 for carcass yield (CY). The h^2 estimated for drip loss (DL) of meat quality was 0.21, and the h^2 estimate was 0.35 for the meat's ultimate Ph (Phu). White quail quails recorded the heaviest weight of all carcass traits. Also, white quails had the highest water-holding capacity (WHC), yellowness (b*), and lightness (L*) with the lowest level of DL, cooking losses (CL), and redness (a*) in muscles compared with brown quails. A high genetic correlation of 0.32 was noted between CW carcass weight (CW) and b*. For the pHU, a negative correlation of -0.11 was exhibited with BW. In contrast, L* appeared to have a positive but smaller relationship with CW and CY. High negative correlations were noted for b* with CY -0.27. The CW showed a moderate relationship (0.19) with CL. In conclusion, the current study revealed that the white quail strain had high BW, as well as the finest carcass traits and meat quality. Therefore, white plumage Japanese quail might be preferred as a meat-producing strain.

Keywords: Amino acid, Carcass, Genetic correlation, Meat quality, Heritability, Quail

INTRODUCTION

The primary goal for poultry producers is to maximize the genetic improvement of the productive traits of chicks (Saghi et al., 2022). Among poultry species, Japanese quails are recognized for their exceptional productivity, particularly in terms of meat and egg production (Minvielle, 2004; El-Attrouny et al., 2020).

Additionally, Japanese quail can be used as animal models in breeding programmed for some attributes, such as lower feed intake, small body size, early maturation, quick life cycle, elevated reproductive efficiency, strong disease resistance, and low production costs (Minvielle, 2004; Narinc et al., 2009; 2013; Molino et al., 2015; Saghi et al., 2022).

Meat consumers have shown a global interest in Japanese quail meat, an ideal protein source for humans due to the quality and quantity of the essential amino acid contents, which are critical in evaluating meat quality (Sabow, 2020). Compared with other poultry species, Japanese quail meat has low lipid content with a high proportion of unsaturated fatty acids, with beneficial effects on human health as atherosclerotic preventatives (Nasr et al., 2017) compared with those of white meat including broiler chicken (Ioniță et al., 2011) and red meat (Boni et al., 2010). Genchev et al. (2008) demonstrated that consuming two quails per day can supply around 40% of the daily protein requirements for humans, equating to approximately 11 grams of essential amino acids. This amount is comparable to consuming 125-130 grams of red

meat. Therefore, Japanese quail meat offers a cost-effective and valuable source of animal protein (Vali, 2008), hence it can be considered as a cheaper alternative for chicken meat, especially in developing countries.

To develop a breeding program aimed at enhancing carcass traits in Japanese quail, it is essential to estimate the genetic parameters of body weight, carcass characteristics, and meat quality traits. This forms the foundation for determining the potential for direct selection of these traits (Lotfi et al., 2011; El-Attrouny et al., 2021). Selection has primarily led to improvements in traits with high heritability such as body weight and carcass traits (Khaldari et al., 2010; Zerehdaran et al., 2012). However, a previous study by Narinc et al. (2013) showed that, despite the successful selection for increased carcass yield, the impact on meat quality remains unclarified. Selection for growth rate, as an important economic trait, could lead to various changes in the meat quality of broiler chickens (Chomchuen et al., 2022). Meat quality is a crucial factor for the poultry industry, as alterations in meat quality could result in a significant economic loss.

Determining the genetic correlations between meat quality traits and other traits is crucial for identifying the direction and magnitude of changes in meat quality before selecting for growth and carcass traits. However, this approach is not well recognized for Japanese quail, as measuring these traits follows a complex process and involves sacrificing a large number of chicks (Le Bihan-Duval et al., 2003; 2008). Thus, the main objectives of the current study were to estimate the heritability and the genetic correlation coefficients between body weight (BW), carcass traits, and meat quality traits, which can be used as a selection criterion in breeding programs of Japanese quail and explore the differences in growth performance, carcass traits and meat quality of two different strains of Japanese quail.

MATERIALS AND METHODS

Animal welfare and ethical approval

The study was carried out at the Poultry Research Facility of the Faculty of Agriculture, Benha University, Egypt, and received approval from the Scientific Ethics Committee of the Animal Production Department, Faculty of Agriculture, Benha University, Egypt (BUAPD-20212).

Housing

In the current study, data were collected from 530 Japanese quail chicks (*Coturnix coturnix japonica*) of two distinct plumage colors, including 265 white and 265 brown. These chicks were obtained from 140 sires and 280 dams. The experiment began in May 2023 and lasted for two months. Each strain of quail, consisting of 265 quails, was sourced from 140 sires and 280 dams. Breeding pairs were housed in individual breeding cages (25 × 35 × 40 cm^2), with one selected male and two females per cage. The practice of housing one male with two females in a breeding cage was common in poultry breeding, including quail, to ensure efficient reproduction and maximize egg production (Shanaway, 1994). The cages had sloped floors to facilitate the collection of pedigreed eggs. Once the eggs were collected, each egg was labeled with the sire and dam's identification. Dams were distinguished by a specific eggshell color pattern within each cage.

After hatching, the chicks were housed in brooding cages at a density of 10 in 10 cm around 100 cm^2 per quail, and they were wing-banded after hatching. The temperature in brooding cages was not fixed because chicks require different thermal environments as they grow. Therefore temperature was initially set at 35°C using electric heaters for the first five days to maintain body warmth due to their inability to regulate temperature. Their ability to control body temperature improved as they developed, so the temperature was gradually reduced to 32°C, 29°C, and 26°C during the first, second, and third weeks, respectively, to prevent overheating and encourage proper growth. From the fourth week onward, the temperature was maintained between 20°C and 22°C for the remainder of the experiment as the chicks were capable of thermoregulation.

Following the brooding period, the quails were transferred to grower cages, with a density of 150 cm^2 per quail (Shanaway, 1994). Throughout the experiment, the quail had unlimited access to feed and water, and the lighting remained on for 24 hours a day. All quails were fed the same basal diet following recommendations from the Nutrient Requirements of Poultry by the National Research Council (NRC, 1994; as outlined in Table 1).

Table 1. Ingredient, composition, and calculated chemical analysis of the basal diets for growing quails

Ingredients	g/kg DM of Feed
Yellow corn	556.0
Soybean meal (44%CP)	288.0
Corn gluten meal (60% CP)	105.0
Vita. and Min. mix.†	3.0
DL-Methionine	1.0
L-lysine	4.0
Wheat bran	20.0
Limestone	19.0
Salt (NaCl)	4.0
Calculated chemical composition (%)	
ME (kcal/kg)	2902.4
CF	3.87
CP	24.01
Na	0.17
Ca	0.82
Available phosphorus	0.41
Methionine	0.56
Lysine	1.39

† Vitamin and trace mineral mixture: Composition per 3 kg, Vit. A 12,000,000 I.U.; Vit. D3 2,000,000 I.U.; Vit. E 10,000 mg; Vit. K3 1000 mg; Vit. B1 1000 mg; Vit. B2 5000 mg; Vit. B6 1500 mg; Vit. B12 10 mg; Niacin 30,000 mg; Biotin 50 mg; Folic acid 1000 mg; Pantothenic acid 10,000 mg; Choline chloride 500,000 mg; Zinc 50,000 mg; Manganese 60,000 mg; Iron 30,000 mg; Copper 10,000 mg; Iodine 1000 mg; Selenium 100 mg; Cobalt 100 mg; Calcium carbonate to 3 kg.

Body weight, carcass traits, and meat quality

Body weight (BW) was recorded individually at hatch, 3, and 6 weeks of age, namely BW0, BW3, and BW6. Also, BW gain (BWG) was calculated during the period from 3 to 6 weeks of age (WG 3-6). At 6 weeks of age, the feed was withdrawn for 7 h, quails (n = 120) were slaughtered and then weighed after bleeding (slaughter weight (SLW), empty carcass (including the skeletal structure and muscle tissue). The heart, liver, and gizzard were carefully removed, cleaned of any excess fat and moisture, and weighed individually using a digital scale with a precision of ± 0.01 g, then according to Inci et al. (2015), the carcass was kept at 2-4 ^{o}C for 24 h for further analyses. Carcass yield (CY) was determined as a correlation between carcass weight and live body weight.

The pectoral and thigh muscles were extracted from the chilled carcass to assess the physical meat quality, which included ultimate pH (pHu), redness (a*), yellowness (b*), lightness (L*), drip loss (DL), water holding capacity (WHC), and cooking loss (CL) as per Nasr et al. (2017). The ultimate pH (pHu) was measured following the method described by Korkeala et al. (1986). In brief, 24 hours after chilling, 1 gram of both breast muscle (PM) and thigh muscle (TM) was homogenized with 10 ml of 5 mM iodoacetate for 30 seconds using a Knick digital pH meter (Broadly Corp., Santa Ana, CA, USA). Muscle color was evaluated using a colorimeter (Lovibond CAM-system 500) with the CIE a* b* L* system, where a* denotes redness, b* indicates yellowness, and L* represents lightness. Cooking loss was measured by placing 25 g of muscle in aluminum pans and cooking them in a preheated electric oven at 200°C for 15 minutes until an internal temperature of 70°C was reached, as described by Cyril et al. (1996).

Water-holding capacity was assessed following the method outlined by Bouton et al. (1971). A muscle sample weighing 3-4 grams was centrifuged at 10,000 g for 30 minutes in a stainless-steel tube. The released juice was quickly decanted to prevent reabsorption by the meat. The muscle sample was then removed, blotted dry with tissue paper, and reweighed to calculate the amount of liquid loss. To measure thawing and cooking losses, the breast muscle was thawed overnight at 4°C, cooked in a water bath at 85°C for 15 minutes until the internal temperature reached 70°C, and then cooled in crushed ice for 20 minutes. Thawing and cooking loss was calculated as a percentage of the initial fresh muscle weight

Chilled pectoral muscle PM without fat was used to estimate the amino acid profile after acid hydrolysis under vacuum in 6 molars HCl at 110 ^{o}C for 24 h. Chemical analysis of muscle amino acid profiles was assessed using High-performance liquid chromatography (HPLC; Agilent HP 1200 series; USA). The utilized analytical column was Supelcosil C18 (5 µm particle and 80 Ao pore size). Samples and amino acid standards (Purchased from Thermo Fisher Scientific) were injected into the Supelcosil C18 column with 5 µm particle size and 80 Å pore size for separation by HPLC. Amino acid contents in the breast muscle were determined as described by Salah et al. (2019).

Statistical analysis

Descriptive statistics of the productive traits (growth traits, carcass characteristics, meat quality, and amino acid profile) were calculated using the univariate procedure of the SAS software (version 9.4, 2004, SAS Institute). Differences were considered significant at $P \leq 0.05$ and significant differences between means were tested by Duncan's multiple range test (Duncan, 1955). The following model was used:

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

Where Y_{ij} = is the observation of the j^{th} trait on the i^{th} quail strain, µ = is the overall mean, P = is the fixed effect of the i^{th} quail strain (with different plumage color, 1 and 2) and e_{ij} = is the residual random effect.

Data on growth traits, carcass characteristics, and meat quality were analyzed using the following multi-trait animal model:

$$y = Xb + Z_a u_a + e$$

where, y= the vector of observing all traits, b = the vector of fixed effects of strain (two levels), Ua = a vector of random additive genetic effects for each bird in the pedigree, X and Z_a are incidence matrices corresponding to fixed and additive random effects of the chicks, respectively, e is a vector of random residual effects. The VCE6 software was used to estimate the variance components of random effects, heritabilities, and genetic correlations among all combinations of traits (Groeneveld, 2010).

RESULTS AND DISCUSSION

The descriptive statistics, including the mean, standard deviation, and coefficient of variation, along with the minimum and maximum values for the analyzed traits, were summarized in Table 2. All traits were normally distributed. The average of BW was 7.38 g, 108.6 g, and 207.3 g at 0, 3, and 6 weeks of age, respectively. The average BWG in Japanese quail was 98.3 g during the intervals from 3 to 6 weeks of age (Table 2). The values of BW and BWG were similar to those of Zerehdaran et al.

(2012), and Nasr et al. (2017), and higher than those of Oguz et al. (2004). Minvielle (2004) reported that BW for Japanese quail may differ among flocks.

The carcass yield, which was an important economic trait, was determined to be 81.4% of BW (Table 2). The average values for carcass traits (Table 2) were consistent with those reported in the literature, with slaughter weight (SLW) ranging from 163 to 195 g, carcass weight (CW) from 140 to 170 g, and carcass yield from 69 to 81% (Kaye, 2014; Nasr et al., 2017). In contrast, the current results were higher than those of Caron et al. (1990) and Zerehdaran et al. (2012), who revealed that the carcass yield CY was 60-70% of BW. The liver (5.62g) and gizzard (5.29g) weights of quail chicks in the present study were within the range reported in the literature of liver (2.19-5.95 g) and gizzard (2.2-4.7 g; Kaye 2014; Shafik et al., 2022). Kaye (2014) found that the weight of a quail's heart ranged from 1.1 to 4.3 grams, which was consistent with the findings of this study. In this study, the average ultimate pHU of breast meat was 6.14, which was comparable to the values reported by Karakaya et al. (2005) and Genchev et al. (2008), who found pHU levels of 6.17 and 6.38, respectively.). However, Remignon et al., (1998) and Gevrekci et al. (2009) reported lower values of pH_U in quail meat 5.59 and 5.94, respectively, than those reported in the present study. Generally, for broiler chicken meats, the normal pH_U that does not exhibit any quality problems ranges between 5.7 and 6.1 (Barbut, 1997; Zhang and Barbut, 2005).

The current study reported an average value of 47.53, 7.49, and 9.25 for L*, a*, and b*, respectively, for Japanese quail meat are presented in Table 2. Oguz et al. (2004) showed that the means of L*, a*, and b* were 54.92, 9.70, and 5.59, respectively. Similarly, Gevrekci et al. (2009) revealed that the average L*, a*, and b* values of breast meat were 54.87, 9.68, and 3.23, respectively. In a study on Japanese quail by Narinc et al. (2013), the authors determined the breast meat parameters of L*, a*, and b* to be 43.09, 19.24, and 7.74, respectively. Zerehdaran et al. (2012) presented values of 53.88 (L*), 5.52 (a*), and -1.69 (b*) for Japanese quail's breast meat at 42 d of age. Based on the literature review for the meat quality of broiler chicken, the optimum L* ranges between 46 and 53 (Zhang and Barbut, 2005). Meats with an L* value below 46 tend to have a darker color, are firmer, and drier, exhibit high water-holding capacity (WHC), and have a shorter shelf life. The a* and b* values for broiler chicken breast meat typically range from -0.96 to 4.50 for a* and from 6.7 to 13.5 for b*, according to studies by Fletcher et al. (2000), and Le Bihan-Duval et al. (2001; 2008). Higher a* values, ranging between 7.5 and 11, were observed in the breast meat of native chicken breeds (Yue et al., 2010; Jiang et al., 2011).

Table 2. Descriptive statistics and heritability estimate for body weight, carcass traits, and meat quality of two quail strains

Trait	Mean	SD	CV%	Minimum	Maximum	$h^2 \pm SE$
Body weight and gain						
BW at hatch	7.38	1.11	15.04	5.14	11.21	0.23 ± 0.03
BW at 3 weeks	108.6	8.65	7.96	51	132	0.27 ± 0.04
BW at 6 weeks	207.3	35.8	17.26	143	254	0.36 ± 0 .04
Weight gain from 3 to 6 weeks	98.3	6.2	6.30	64	145	0.31 ± 0.05
Carcass traits						
Slaughter weight (g)	198.5	22.3	11.23	170.2	250.6	0.34 ± 0.06
Carcass weight (g)	167.6	16.4	9.78	134.2	1985.1	0.38 ± 0.06
Carcass yield (%)	81.4	7.3	8.96	72.4	86.5	0.42 ± 0.05
Liver weight (g)	5.62	0.52	9.25	4.6	6.8	0.19 ± 0.02
Gizzard weight (g)	5.29	0.25	4.72	4.11	7.3	0.27 ± 0.03
Heart weight (g)	1.97	0.15	7.61	0.98	2.25	0.24 ± 0.03
Meat quality						
Ultimate Ph (Phu)	6.14	0.92	14.8	5.01	7.12	0.35 ± 0.04
Water Holding Capacity (%)	25.55	3.21	12.56	25.3	48.7	0.29 ± 0.04
Cooking loss%	24.20	2.44	10.08	14.3	35.4	0.27 ± 0.03
Drip loss (%)	3.21	0.34	10.59	1.74	5.11	0.21 ± 0.02
Lightness (L*)	47.53	3.51	7.38	34.4	56.6	0.32 ± 0.04
Redness (a*)	7.49	0.52	6.94	4.21	15.6	0.28 ± 0.05
Yellowness (b*)	9.25	0.86	9.29	8.60	12.5	0.33 ± 0.04

SD: Standard deviation; CV: Coefficient of variation

Genetic parameters

Heritability (h^2) estimates for all studied traits are presented in Table 2. The h^2 estimates for BW ranged from 0.23 to 0.36 at BW0 and BW6, respectively, while for meat quality traits h^2 estimates were 0.21 for DL and 0.35 for Phu.

The current h^2 estimates for body weight (BW) align with findings from previous studies on Japanese quail (Saatci et al., 2006; Khaldari et al., 2010; Narinc et al., 2010). Additionally, several researchers have reported high h^2 estimates for BW in Japanese quail (Oguz et al., 2004; Narinc et al., 2010; 2013). The h^2 estimates of carcass traits reported in Table 2 were moderate to high ranging from 0.19 (LW) to 0.42 (CY). A high h^2 estimated of 0.38, and 0.42 for CW and CY, respectively was reported in the current study. However, many researchers reported low heritability estimates ranging from 0.12 to 0.19 for CY in quail (Vali et al., 2005; Narinc et al. 2010; Lotfi et al. 2011). The current results agreed with those reported by Daikwo et al. (2013) who revealed that the heritability of CW was 0.42. Estimated h^2 for liver, gizzard, and heart weight were presented in Table 2. The h^2 estimates for liver, gizzard, and heart weight were 0.19, 0.27, and 0.24, respectively, which were similar to those (0.11 and 0.27) reported by Daikwo et al. (2013), but diverged from those found by de Gaya et al. (2006).

Based on the findings of the current study, pH_U was considered the highest heritable trait (0.35). Oguz et al. (2004) presented a high h^2 estimate (0.48) for pH_U. Gevrekci et al. (2009) reported a moderate h^2 estimate of 0.24 for pH_U. In broiler chicken, the pH_U was considered highly heritable as the estimates range between 0.34 and 0.49 Le Bihan-Duval et al. (2001; 2008). However, for commercial turkey lines, low heritability estimates for pH_U in the breast muscle ranging from 0.12 to 0.21 were reported by Le Bihan-Duval et al. (2003).

Meat pH plays a key role in determining the color of poultry meat. According to Fletcher (1999), muscle pH was primarily influenced by the biochemical condition of the muscle at the time of slaughter. As a result, pHu and L* values showed stronger direct additive genetic effects compared to other traits studied. This suggests that these traits may respond well to selection, as their expression was largely driven by additive genetic factors.

The h^2 estimated for water-holding capacity (WHC) was 0.29, closely matching the findings of Rance et al. (2002). However, the h^2 estimates for cooking loss, at 0.31 and 0.35, differed from those reported by Zerehdaran et al. (2012) and Le Bihan-Duval et al. (2008). In terms of cooking loss and drip loss, the h^2 estimates of 0.27 and 0.21, respectively, were higher than those observed in broilers for these traits, as noted by de Gaya et al. (2011).

Table 2 showed that the h^2 estimates for breast meat color traits, including L*, a*, and b*, were 0.32, 0.28, and 0.33, respectively. Oguz et al. (2004) and Gevrekci et al. (2009) reported h2 estimates for L*, a*, and b* at 0.23 and 0.24, 0.45 and 0.35, and 0.22 and 0.15, respectively. Additionally, Le Bihan-Duval et al. (2001; 2008) demonstrated that breast meat color traits were notably heritable, with h^2 values ranging from 0.25 to 0.81 in broiler chickens. These estimates indicated that heritability for meat quality traits ranges from moderate to high, emphasizing the significance of genetic selection in improving meat quality traits in Japanese quail, particularly L*, which was the primary determinant of meat color in this species

Least square means

Table 3 shows the BW of the two Japanese quail strains. Noticeable significant variations were noticed ($p < 0.05$) between the means of BW and BWG of the two Japanese quail strains. The white quail had the highest BW (226.7 g) compared to that of the brown quail (195.2 g) at 6 weeks of age.

On the contrary, Inci et al. (2015) reported that the BW did not vary between different quail strains on the first day of post-hatch. White plumage Japanese quail showed the highest BW compared with the brown strain. Ojo et al. (2014) revealed that the BW of white quails was higher than of brown plumage quails at weeks 2 and 4 of age. Islam et al. (2014) also reported that the white plumage strain of quail had a greater body weight (BW) at 5 weeks of age compared to the brown strain. These differences may be attributed to two factors involved firstly, the effect of recessive gene action, which tends to have a depressive impact on BW, particularly in black and brown quails (Minvielle et al., 2007); and secondly, the enhanced feed conversion efficiency and reduced mortality rate observed in the white strain (Islam et al., 2014). However, inconsistent findings have been reported in the literature on the variations of BW among Japanese quails with different plumage colors. Several studies reported significant differences (Genchev et al., 2008), while some studies showed no differences (Mahmoud et al., 2014). The present results were consistent with studies that demonstrated significant differences in the body weight of quails with varying plumage colors, except on the first day of age.

Table 3. The Least squares mean (± Standard error) of body weight, carcass traits, and meat quality in two quail strains

Trait \ Quails with different plumage color	White	Brown	p-value
Body weight (g)			
BW at 0 week	7.40 ± 0.78	7.30 ± 0.78	0.320
BW at 3 weeks	119.4 ± 3.56[a]	101.3 ± 3.21[b]	0.001
BW at 6 weeks	226.7 ± 5.31[a]	195.2 ± 4.62[b]	0.001
Weight gain from 3 to 6 weeks	105.4 ± 3.1[a]	92.6 ± 2.32[b]	0.001
Carcass traits (g)			
Slaughter weight	218 ± 6.52[a]	187 ± 6.52[b]	0.001
Carcass weight	180 ± 4.69[a]	151 ± 4.69[b]	0.001
Liver weight	6.10 ± 0.75[a]	5.62 ± 0.75[b]	0.001
Gizzard weight	5.72 ± 0.64[a]	4.96 ± 0.64[b]	0.001
Heart weight	2.32 ± 0.21[a]	1.86 ± 0.21[b]	0.001
Carcass yield (%)	82.3 ± 3.84[a]	81.4 ± 3.84[b]	0.015
Meat quality			
Ultimate pH	6.22 ± 0.59	6.10 ± 0.59	0.081
WHC (%)	26.21 ± 2.23[a]	25.14 ± 2.23[b]	0.041
Drip loss (%)	2.12 ± 0.21[b]	2.30 ± 0.21[a]	0.031
Cooking loss (%)	23.74 ± 2.59[b]	24.62 ± 2.59[a]	0.013
Lightness (L*)	48.20 ± 5.63[a]	46.61 ± 5.63[b]	0.001
Redness (a*)	7.38 ± 0.63[b]	7.74 ± 0.63[a]	0.001
Yellowness (b*)	9.40 ± 0.91[a]	9.17 ± 0.91[b]	0.021

[a,b] Means in the same row with different superscript letters are significantly different at $p < 0.05$

Table 3 presents the carcass traits of the two different quail strains. High Significant differences ($p < 0.05$) were shown between the means of the two strains of quails for traits of slaughter weight, carcass weight, carcass yield, liver, gizzard, and heart weight. The white plumage quails recorded the highest slaughter and carcass weights, liver, gizzard, heart, and carcass yield, compared with those of the brown plumage quails (Table 3). This variation between the two strains could be related to the variance in BW at slaughter, which was influenced by intrinsic factors such as genotype. Nasr et al. (2017) described that carcass traits varied between Japanese quail strains. Inci et al. (2015) revealed that carcass characteristics were significantly affected by the feather colors of Japanese quails. In the current study, white quail recorded the heaviest slaughter and carcass weights (218 and 180 g, respectively), which was out of the range of those reported by Kaye (2014) and Sabow (2020).

The CY of Japanese quail was influenced by several factors such as strain, line, gender, and slaughter age of chicks (Genchev et al., 2008). A higher CY of Japanese quail was indicative of their exceptional efficiency capacity for meat production. Kaye (2014) reported that the percentages of CY ranged between 72-88.1%, which agrees with those reported in the current study for white (82.3%) and brown (81.4%) quails. However, Caron et al. (1990) presented lower values of CY percentage (67–70%) for Japanese quail, compared to those reported in the current study. In general, means of liver, gizzard, and heart weights in white (6.10, 5.72, and 2.32 g) and brown (5.62, 4.96, and 1.86 g) quails were higher than the range of 2.19-6.63, 2.2-5.53 g and 1.1 and 4.3 g, respectively, reported by Kaye (2014) and Nasr et al. (2017). These findings could be related to the variation of BW, which affects the internal organs weight (Kanlisi et al., 2024). The current study revealed a significant difference in all meat quality estimates between Japanese quail with different plumage colors. The white quail strain had the highest Ph_U, WHC, L*, and b* with the lowest level of DL, CL, and a* compared to the brown quail strain (Table 3). The Ph_U of meat for both white and brown quail strains fell within the reported range of 5.30-6.58 for Japanese quail (Genchev et al., 2008; Narinc et al., 2013; Sabow, 2020). Barbut (1997) noted that a decrease in meat pH levels leads to reduced water-holding capacity (WHC) and tenderness, causing the meat to become pale, soft, and exudative, and it increases the percentage of cooking loss. In the present study, the meat from white plumage quails demonstrated a higher WHC compared to that of brown plumage quails. However, the detected levels of both

strains were approximately similar to those levels for breast muscles (21.68-22.39) and thigh muscles (25.08-26.91) reported by Genchev et al. (2008), Ribarski and Genchev (2013) and higher than the levels (17.7-20.3) reported by Kaye (2014). The present study showed that the CL percentage was within the range (19.9-21.5%) reported in the literature (Zerehdaran et al., 2012), with the lowest CL percentage observed for white plumage quail strain. In contrast, other studies reported higher CL percentages ranging from 13.7 to 34.2% (Narinc et al., 2013) and 27.3 to 31.1% (Kaye, 2014) compared to the findings reported in this study. The present study showed Japanese quail strain has a significant influence on drip loss, where the white plumage quail strain recorded the lowest drip loss compared with the brown plumage strain, hence better meat quality for the white quail strain.

Amino acid profile

The content of protein and amino acids profile of breast muscle meat from both Japanese quail strains was illustrated in Table 4. The total protein content of Japanese quail breast meat revealed highly significant differences ($p < 0.05$) based on the strain. In this study, the total protein content observed was slightly higher than the values reported by Genchev et al. (2008), who found protein levels of 22.23 g in quail breast. Additionally, the amino acid profiles of both Japanese quail strains closely resembled those reported by Genchev et al. (2008), with the white plumage quail displaying the highest amino acid levels.

The current study showed that lysine and glutamic acid levels were the highest, while threonine and methionine levels were the lowest. These findings agree with those reported by Nasr et al. (2017) and Sabow (2020). The current study showed that white plumage quail exhibited the heaviest BW and superior carcass traits and meat quality. These findings contrast with the findings of Zerehdaran et al. (2012) who revealed that selecting Japanese quail for heavier BW and better carcass composition could decrease the meat quality.

Table 4. Total protein and amino acid profile of breast and thigh muscle in two quail strains

Trait \ Quails with different plumage color	White	Brown	MSE	p-value
Indispensable amino acids (g/100 g protein)				
Lysine	2.41[a]	2.16[b]	0.18	0.001
Leucine	2.12[a]	2.04[b]	0.19	0.021
Isoleucine	1.77[a]	1.39[b]	0.05	0.011
Valine	1.27[a]	1.18[b]	0.05	0.001
Threonine	1.11[a]	0.93[b]	0.04	0.024
Methionine	0.84[a]	0.63[b]	0.03	0.025
Phenylalanine	1.13[a]	0.88[b]	0.07	0.001
Total	10.65[a]	9.21[b]	1.25	0.012
Dispensable amino acids (g/100 g protein)				
Glycine	1.15[a]	0.95[b]	0.04	0.001
Tyrosine	2.42[a]	2.13[b]	0.08	0.032
Serine	1.32[a]	1.19[b]	0.05	0.001
Aspartic	2.18[a]	1.89[b]	0.07	0.021
Glutamic	3.37[a]	3.04[b]	0.31	0.001
Alanine	1.17	1.08	0.08	0.031
Arginine	1.61[a]	1.47[b]	0.06	0.001
Total	13.22[a]	11.75[b]	2.10	0.021

[a, b] Means in the same row with different superscript letters are significantly different at $p < 0.05$. MSE: Mean standard error.

Genetic correlations

Table 5 displayed the genetic correlation (rg) estimates between body weight (BW) and various carcass traits concerning meat quality traits. Generally, these correlations were low. Specifically, rg estimates between BW and carcass traits with pHu and water-holding capacity (WHC) ranged from -0.05 (for HW) to 0.15 (for CW), showing both positive and negative correlations. Notably, low genetic correlation was observed between WHC and BW or carcass traits, suggesting that WHC might be lower in quails with higher carcass and breast

yields, as noted by Van Laack et al. (2000) and Le Bihan-Duval et al. (2001). Low positive genetic correlation estimates were discovered between drip loss with BW, CW, and CY (0.04, 0.07 and 0.17). Color parameters exhibited both negative and positive genetic correlations with body weight (BW) and carcass traits, with rg values ranging from -0.02 to 0.32. Berri et al. (2001) reported that selecting broilers for increased breast meat yield was linked to lower ultimate pH and reduced drip loss, The same researchers, along with Zerehdaran et al. (2012), observed that there was generally a low or negative genetic correlation between BW and color parameters, although a strong association was found between BW and the L* value (Le Bihan-Duval et al., 2001; 2003).

Table 5. Estimates of genetic correlations among body weight and carcass traits with meat quality traits in two quail strains

Trait	PHu	WHC	DL	CL	L*	a*	b*
BW6	-0.11(0.02)	-0.24(0.10)	0.04(0.02)	-0.08(0.01)	-0.06(0.02)	-0.04(0.02)	-0.24(0.04)
CW	0.08(0.01)	0.15(0.09)	0.07(0.01)	0.19(0.07)	0.10(0.03)	0.09 (0.02)	0.32(0.09)
CY	0.04(0.03)	0.11(0.06)	0.17(0.06)	-0.17(0.02)	-0.08(0.02)	0.14 (0.06)	-0.27 (0.05)
LW	-0.09(0.03)	-0.15(0.08)	-0.10(0.04)	-0.31(0.11)	0.17(0.06)	-0.02(0.01)	0.06(0.02)
GIZ	-0.05(0.06)	-0.32(0.11)	-0.09(0.03)	-0.20(0.07)	-0.17(0.08)	-0.18(0.03)	-0.12(0.08)
HW	-0.19(0.02)	0.06(0.01)	-0.21(0.02)	-0.19(0.08)	-0.11(0.05)	0.09 (0.03)	-0.30(0.03)

BW6: Body weight at 6 wks, CW: Carcass weight, CY: Carcass yield, LW: Liver weight, GIZ: Gizzard weight, HW: Heart weight, pHu: Ph Ultimate, WHC: Water Holding Capacity (%), DL: Drip loss (%), CL: Cooking loss, L*: Lightness, a*: Redness, b*: Yellowness.

CONCLUSION

White quails exhibited the heaviest body weight and the best carcass traits. Carcass and meat quality traits of Japanese quail were highly heritable, indicating that these traits could have been enhanced through genetic selection. Moreover, selecting for higher body weight and carcass traits in Japanese quail may have negatively impacted meat quality by reducing redness and ultimate pH, while increasing lightness, cooking loss, and yellowness of the meat. Therefore, it was essential to consider meat quality traits alongside performance traits in the selection index to preserve high-quality meat products in Japanese quail.

DECLARATION

Competing interests

The authors declare that they have no conflicts of interest.

Availability of data and materials

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

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Ethical considerations

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

Authors` contributions

Mahmoud. M. El-Attrouny and Mahmoud. M. Iraqi designed the research project. Mahmoud. M. El-Attrouny and Mahmoud. M. Iraqi experimented and collected data. Mahmoud. M. El-Attrouny and Farid. S. Nassar analyzed the data and interpreted the results. Mahmoud. M. El-Attrouny and Farid. S. Nassar wrote the initial manuscript. The authors revised the manuscript together and prepared the last edition for submission and publication.

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Carcass Characteristics and Blood Biochemical Parameters of Cobb-500 and Hubbard Chicken Strains Fed on Commercial and Farm-Formulated Diets

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ABSTRACT

The limits of commercial diets, their quality, and their rising costs are some of the major challenges to broiler production in Ethiopia. The purpose of this investigation was to evaluate carcass yield characteristics and blood biochemical parameters of Cobb-500 and Hubbard chicken strains fed on farm-formulated diets (T1) and three different commercial diets (T2, T3, and T4). A total of 384 mixed-sex day-old chicks (192 per strain) were randomly assigned to four dietary treatments with four replicates, each consisting of 12 broilers. The experiment was set up as a 2 × 4 factorial design, providing each strain with four diets in a completely randomized design. After 42 days of the experiment, one male and one female of each strain from each pen (eight birds per treatment) were slaughtered for carcass yield and hematological analysis. Although diets had a significant impact on live body weight, feed conversion ratio, and feed consumption among the study treatments, they had no significant effect on the mortality rate of the broilers as a whole. There was a significant effect of strains on the weight of eviscerate, dress, thigh, drumstick, breast, neck, back, and eviscerate yield percentage, with Cobb 500 showing higher values than Hubbard broilers. The farm-formulated diet (T1) significantly increased the weight of non-edible offal compared to the commercial diets, except for the weight of crops and lungs, which were similar to those in commercial diet group T4. The Hubbard strain showed a higher least square mean for packed cell volume than the Cobb-500 strain. Sex was found to have no significant impact on the hematological parameters. The farm-formulated diet (T1) also resulted in a higher marginal return rate than that of the commercial diet (T3) in the Cobb-500 strain. These findings suggest that locally sourced farm-formulated diets could be a viable alternative to commercial diets for broiler chickens in the study area.

Keywords: Broiler chicken, biochemical parameter, Carcass trait, Farm-made diet, Haematology, Profitable

INTRODUCTION

The demand for protein bases to feed the world's growing population has significantly boosted the poultry production industry within the meat-producing agriculture sector (Bogale and Engida, 2022; El-Sabrout et al., 2022). Worldwide, commercial systems are used to produce huge quantities of chickens; however, these systems are not widely employed in developing countries like Ethiopia, where they are primarily limited to urban areas (Habte et al., 2017). In the industrialized world, broiler chickens are typically raised for rapid growth and slaughtered between 6 and 8 weeks of age, or when they reach a body weight of 1.8 to 2.2 kg (Musa et al., 2006).

The carcass yield characteristics, including dressed weight, edible giblet weights, and the weights of the breast, drumstick, thigh, back, and shank are all significantly impacted by strain (Marcu et al., 2013). Correspondingly, Pripwai et al. (2014) reported similar results, showing that sex affected the weight of the thighs, the dressed weight, the meat-to-bone ratio, and the wings. The combined weight of edible and inedible offal in chicken carcasses was a

significant factor for both producers and consumers (Zawacka et al., 2018). According to Uhlíová et al. (2018), age, sex, strain, and diet are the main factors that affect the carcass and the meat quality of broiler chickens. High packed cell volume (PCV) and high haemoglobin (Hb) are indicators of great feed conversion efficiency. Moreover, recognizing the typical physiological standards in a normal state is crucial for the effective management of broiler chickens (Nyaulingo, 2013). According to Ayo-Enwerem et al. (2017), the response of broilers to their internal and external surroundings, including their feeding, is always reflected in their haematological features.

To increase carcass yield, chicken feed in Ethiopia commonly includes oil seed cakes, milling by-products, and cereal grains (FAO, 2019). However, the rising prices of protein and energy sources have led to increasing feed costs, posing a significant challenge for commercial broiler production in developing countries (Abbas, 2013). Since commercial feeds are expensive and are provided in limited supply, small-scale chicken producers often cannot afford them (Wilson et al., 2021). Consequently, one of the main challenges in broiler chicken production in Ethiopia is feed scarcity and the cost of purchasing and transporting broiler feeds. This issue is further exacerbated by the fact that most cereals used as broiler feed are also staple diets for humans and animals. In Ethiopia, maize, soybean meal, noug seed cake, and wheat short are the primary ingredients used in formulating commercial feed (Mengesha, 2012). As a result, smallholder chicken farmers and others have to purchase expensive commercial rations from manufacturing industries due to the lack of affordable alternative feed formulations for broilers. These chicken feed ingredients are mainly produced in the rural areas of Ethiopia, particularly in the western part of the country. However, these raw materials are transported to Addis Ababa and surrounding towns for processing and ration formulation.

The costs associated with transportation, processing, and service charges contribute to the high purchase price of commercial feed. To achieve sustainable diet production and ensure global feed security, alternative substances are increasingly being incorporated into broiler diets (Morgan and Choct, 2016; Tufarelli et al., 2018). There is growing interest in using alternative feed ingredients, such as near-available resources and local diets, to reduce the economic costs of producing carcass-yield meat (El-Deek et al., 2020). In this study, farm-formulated poultry diets were proposed as a cost-effective alternative to expensive commercial diets for comparison.

However, there is limited information on the effects of different commercial and farm-formulated diets, using locally available ingredients, on the carcass yield and blood profile of broilers. Moreover, insufficient research has been conducted on the carcass yield and blood biochemical of strains in Ethiopia using locally available resources and ingredients. Therefore, this study aimed to assess carcass yield characteristics and blood biochemical parameters of Cobb-500 and Hubbard's chicken strains fed on commercial and farm-formulated diets.

MATERIALS AND METHODS

Ethical approval

All procedures involving animal handling, blood collection, and routine manipulations followed the animal care guidelines and protocols approved by the Institutional Review Board of the College of Veterinary Medicine and Agriculture (CVMA), Ethiopia, Animals Ethics Committee (Approval Number: VM/ERC/01/13/12/2020).

Description of the study site

The broiler feeding experiment was conducted at a poultry farm located on the Nekemte campus of Wollega University, Ethiopia, situated at 10° 0' North latitude and 37° 30' East longitude. The study area has an average annual rainfall of 1998 mm, a relative humidity range of 11% to 31%, and average minimum and maximum temperatures of 8 °C and 30 °C, respectively (NMS, 2019).

Experimental diet and treatment

Broilers were fed three commercial diets and one farm-formulated diet in two feeding phases, both of which were isoprotein and isocaloric, 21 days for the starter phase and 21 days for the finisher phase. The commercial diets, labeled A, B, and C, were randomly selected from different manufacturers in Ethiopia. Commercial diets are formulated to be complete, containing balanced levels of protein and calories. The farm-formulated diet (T1) was prepared using locally available feed ingredients such as maize grain, noug seed cake, wheat shorts, soybean meal, and common salt. Limestone, dicalcium phosphate, vitamin premix, L-lysine, and DL-methionine were also added to the diets (Table 1). All diet plans were formulated using Win Feed 2.84 software based on the nutritional recommendations for broilers and the chemical composition of the ingredients (Table 2). The formulated diets were to meet the isocaloric (3100–3200 kcal/ME per kg DM) and isoproteins (18–22% CP) nutrient requirements of broiler chickens (NRC, 1994).

Table 1. Percentage composition of feed ingredients in starter and finisher diets

Phase	Ingredients (%)	Treatments			
		T1	T2	T3	T4
Starter	Maize grain	52.5	51.5	52.5	50
	Soybean meal	22	15	17.5	25
	Noug seed cake	12	10	12	-
	Wheat short	10	-	15.5	-
	Mineral	0.5	0.25	0.1	0.75
	Vitamin premix	0.5	0.25	0.1	0.75
	Limestone powder	1	1	0.5	0.5
	Di-calcium phosphate	0.5	0.25	0.2	-
	L-lysine	0.25	0.25	0.2	-
	DL-methionine	0.25	0.25	0.2	-
	Common salt	0.5	0.25	0.5	-
	Meat and bone	-	5	0.7	-
	Groundnut	-	-	-	9
	Wheat bran	-	16	-	14
	Total	100	100	100	100
Finisher	Maize grain	54.5	52	53.5	50
	Soybean meal	21	16	18	25
	Noug seed cake	10	11	12	-
	Wheat short	11	-	14	-
	Mineral	0.5	0.25	0.1	0.75
	Vitamin premix	0.5	0.25	0.1	0.75
	Limestone powder	1	1	0.5	0.5
	Di-calcium phosphate	0.5	0.25	0.2	-
	L-lysine	0.25	0.25	0.2	-
	DL-methionine	0.25	0.25	0.2	-
	Common salt	0.5	0.25	0.5	-
	Meat and bone	-	5	0.7	-
	Groundnut	-	-	-	8
	Wheat bran	-	13.5	-	15
	Total	100	100	100	100

T1: Farm-formulated diet, T2, T3, and T4: Commercial diets from different sources (A, B, and C), %: Percentage, Vitamin premix: Poultry booster soluble powder, Amoxicillin soluble powder, and Amprolium soluble powder

Experimental broilers and management

This experiment was conducted over 42 days, comprising 21 days for the starter phase and 21 days for the finisher phase. Three hundred and eighty-four mixed-sex day-old chicks (192 per strain), procured from Alema (Cobb-500 strain) and Elere Farms (Hubbard strain) located at Bishoftu, were used for the experiment. Upon arrival, the chicks were kept in 32 separate deep-litter pens, each with five cm of wood shavings (sawdust) litter underneath. Before the chicks arrived, the 2.5 x 1.5 m^2 deep litter floor housings (pens) containing the broilers were thoroughly cleaned, disinfected, and covered with sawdust litter material. All the pens were provided with drinkers, feeders, and a brooding unit (with a 230-watt bulb) placed at the centre of the house. At the hatchery, the chicken received vaccinations against Newcastle (UK, Indonesia, and Korea) and Gumboro (USA strain), as well as against Marek's disease (Turkey, USA, and Europe strains) at 7 and 21 days of age. Throughout the trial, diets were given *ad libitum* up to the end of the experiments. Clean, cold, and fresh drinking water was also available at all times.

Experimental design and treatments

The experiments involved two broiler strains (Cobb 500 and Hubbard) and four treatment diets (one farm-formulated and three commercial diets), assigned to pens in four replicates of 12 chicks each. The study followed a 2 × 4 factorial design, which provided each strain with four diet distributions in a completely randomized design (CRD). Treatment for each of the two strain groups consisted of 48 chick-feeding experiments.

Live body weight and feed consumption

Feed consumption for the broiler chickens was determined by subtracting the amount of feed refused from the amount offered. Refusals were collected and weighed daily, before fresh feed was provided, after removing any contaminants. The quantity of feed provided every three days was adjusted to ensure that all groups of broilers had *ad libitum* access to feed. For every pen, the feed that was provided and refused was recorded. The feed conversion ratio (FCR) was calculated by dividing the mean daily intake of feed by the average daily body weight (Lawrence and Fowler, 1998). The mortality rate was determined by dividing the number of deceased broilers by the total number of broilers at the start of the experiment and multiplying by 100 to express it as a percentage.

Carcass yield characteristics of broilers

At the end of the 42-day finisher period, one male and one female from each pen were slaughtered for carcass characterization, totaling 32 males and 32 females per treatment. Before slaughter, the chickens were randomly selected, weighed, and fasted for 12 hours while having unrestricted access to water to relieve their digestive tracts. To determine the slaughter weight, the chickens' body weights were measured before slaughter. Cervical dislocation, a sharp knife incision to the throat, and five minutes of bleeding were the methods used for slaughtering (Ncobela et al., 2016). After bleeding, the carcass was

scalded in hot water (60 °C) for 45 seconds before de-feathering and eviscerating; the feathers were removed starting from the tail, wing sides, legs, back, and neck regions of the scalded chicken. The carcass was then eviscerated, hung over the evisceration line, and given fifteen minutes to drain before being weighed. The weight of the slaughtered carcass was measured following the removal of the inedible viscera. The eviscerated bodies were separated into six sections including the breast, thigh, drumstick, wings, neck, and back, and their weights were measured. Information on the weight of the back, neck, breast, drumstick, thigh, liver, wing, gizzard, and all other non-edible offal, including the digestive tract (crop, proventriculus, gizzard, small and large intestines) as well as the pre-slaughter live weight was recorded. Additionally, noted were the visceral organs, which included the weight of the lungs, heart, kidneys, and shank. The individual parts of the total non-edible (TNE) offal, such as the heads, shanks, crops, kidneys, heart, lungs, intestines, and abdominal fat were also noted. The total weight of the back, neck, drumsticks, thighs, wings, breast, and edible offal (liver, heart, and gizzard) was used to calculate the weight of all the carcass parts. A cut of each carcass was used to determine the weights of the breast, thigh, drumstick, and wings. The dressing percentage was determined following FAO (2001).

$$\text{Dressing Percentage (\%)} = \frac{\text{Dressed Weight (g)}}{\text{Slaughter Weight (g)}} \times 100$$

According to FAO (2001) guidelines, the dressing percentage was calculated as follows: The dressed weight was computed by summing the weights of the drumsticks, thighs, wings, breast, back, neck, heart, liver, gizzard, feet, head, and viscera (including lungs, pancreas, and intestines). The eviscerated weight was obtained by subtracting the weights of the head, viscera, and feet from the dressed weight. The eviscerated percentage was then calculated by dividing the eviscerated weight by the slaughter weight and multiplying by 100.

$$\text{Eviscerated yield (\%)} = \frac{\text{Eviscerated weight}}{\text{Slaughter Weight}} \times 100$$

Evaluation of the haematological and serum biochemical tests of broilers

The blood and serum biochemical profiles were evaluated at the end of the experimental period (Day 42 of the study). Blood samples were collected from two randomly selected chickens per replication (one female and one male). Five millilitres of blood were drawn from immobilized chickens via the wing veins. Following conventional protocols outlined by Davice and Lewis (1991), half of the blood sample was transferred to vacutainer glass tubes containing ethylenediaminetetraacetic acid (EDTA) for haematological analysis. The remaining blood was placed in the second set of vacutainer glass tubes without EDTA for serum biochemical analysis. The haematological indices assessed included packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Likewise, the concentrations of creatinine, glucose, cholesterol, and total protein in the serum were determined. For serum analysis, the samples were stored at -20 °C. RBC and WBC were counted using a hemocyte meter (Irizaary-Rovira 2004). The values obtained for RBC, Hb, and PCV were used to calculate MCV, MCHC, and MCH, which were computed as described by Irizaary-Rovira (2004).

Partial budget analysis

The partial budget analysis was conducted following the method outlined by Upton (1979) to determine the economic benefit of feed and chicken production. The total variable cost (TVC) for each treatment was calculated by summing the expenses related to feed, veterinary care, labor, and other services incurred during the experimental period for each treatment. Marginal revenue (MR) was calculated by subtracting the total feed cost from the total revenue (MR = TR + TFC). Total return (TR) was computed as the difference between the buying price and the sale price. In other words, the selling price minus their buying price equals TR. The following is how net return (NR) was computed by deducting TVC from TR: TR – TVC = NR. The changes in net return were calculated as follows: ΔTR – ΔTVC = ΔNR. The increase in net return (NR) corresponding to each extra unit of expenditure (ΔTVC) was measured by the marginal rate of return (MRR), which is represented as a percentage.

$$\text{MRR\%} = \frac{\Delta NR}{\Delta TVC} X100$$

Chemical analysis of diets

The dry matter, crude protein, ether extract, crude fiber, and ash of the feed samples used in the study were evaluated in compliance with AOAC (1990). Atomic absorption spectroscopy and the spectrophotometer method were used at Haramaya University Laboratory to assess the levels of calcium and phosphorus, respectively (AOAC, 1998). Using the Wiseman (1987) equation, the metabolized energy values were indirectly determined from the ether extracts (EE), crude fiber (CF), and ash to determine the metabolizable energy of the diets.

Table 2. Chemical feed composition of commercial and farm-formulated diets (percentage on dry matter base)

Phase	DM	CP	CF	EE	Ash	Ca	P	ME (Kcal/kg DM)
Starter (1–21 days)								
T1	89.38	14.64	4.32	5.89	6.96	0.97	0.60	3604.22
T2	91.07	15.37	3.42	5.69	7.17	0.86	0.65	3665.03
T3	90.42	16.18	5.68	5.48	10.52	1.04	0.60	3316.29
T4	89.76	14.22	5.45	5.72	6.93	0.42	0.25	3495.99
Finisher (22–42 days)								
T1	89.43	14.37	4.90	6.16	6.89	0.92	0.58	3570.21
T2	91.11	14.89	4.32	5.91	6.80	0.74	0.62	3612.45
T3	90.62	15.77	5.81	5.79	9.31	1.01	0.61	3370.37
T4	89.80	13.89	5.92	5. 88	5.88	0.40	0.24	3505.61

T1: Farm-formulated diet, T2, T3, T4: Commercial diets from different sources, %: percentage, DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, Ca: Calcium, P: Phosphorous, ME: Metabolisable energy, Kcal: kilocalorie, kg: Kilogram

Statistical data analysis

The Statistical Analysis System (SAS) version 9.4 and the General Linear Model (GLM) techniques were used to analyze the data (SAS, 2016). Duncan's multiple range tests were utilized to separate treatment means (Duncan, 1955). The statistical models for feed consumption and body weight were expressed as following formula.

$$Y_{ijk} = \mu + B_i + F_j + (B*F)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is the response variable, μ is an overall mean, B_i is the fixed effect of the strains (i: Cobb 500 and Hubbard), F_j is the fixed effect of the j^{th} feed-type (j: farm-formulated, commercial diets 1, 2, and 3), $(B*F)_{ij}$ is the interaction effect between chicken strains and feed treatment diets, and ε_{ijk} is the random error term. For carcass yield and blood profile analyses, the statistical model used was as following formula.

$$Y_{ijk} = \mu + B_i + F_j + S_k + \varepsilon_{ijk}$$

Where Y_{ijk} is the response variable i, j, k; μ is the overall mean, Bi is the effect of the strains (i: Hubbard and Cobb 500); F_j is the effect of feed type (j: farm-formulated diet, commercial diets 1, 2, and 3); S_k is the effect of sex (k: male and female); and ε_{ijk} is the random error component.

RESULTS AND DISCUSSION

Live body weight and feed consumption

The effects of diet and strain on feed consumption and living body weight are presented in Table 3. The findings show that strain significantly affected live body weight (LBW) and feed conversion ratio (FCR), but no significant influence on broiler mortality rate (MR) or feed consumption was observed (FC, $p < 0.05$). Cobb 500 broilers outperformed Hubbard broilers in terms of feed conversion ratio and live body weight. Diet had a significant impact on live body weight, feed conversion ratio, and feed consumption, but did not affect the mortality rate ($p < 0.05$). In terms of live body weight, the farm-formulated diet (T1) was comparable to the commercial diet (T4). Similarly, broilers fed with commercial diets T2 and T3 exhibited comparable live body weight and feed conversion ratios, with T2 and T3 showing the best feed conversion ratios (FCR) among the treatments. For the total number of broilers, there was no significant interaction between strain and diet affecting live body weight, feed consumption, feed conversion ratio, or mortality rate.

In comparison, the Hubbard strain had an average live body weight of 1583.43g, overall, while the Cobb-500 strain achieved the highest live body weight at 1975.77g. Cobb 500 broilers also demonstrated a superior feed conversion ratio of 2.43 compared to Hubbard's 3.05. This indicates that Cobb 500 broilers are more efficient in converting feed to meat, as reflected by their lower FCR. The observed variations can be attributed to sex, strains, nutrition, genetics, and environmental factors. At six weeks of age, Cobb-500 broilers consistently maintained a higher live body weight compared to Hubbard broilers. These findings are consistent with those of Udeh et al. (2011), who reported similar results for final body weights at eight weeks of age: Anak (1855 g), Arbor Acre (1880 g), Ross (1812.50 g), and Marshal (1645 g).

Consequently, after six weeks, the live body weight of 2455.58g achieved with diet treatment T2 was lower than the final body weights in previous studies. Mezgebu et al. (2020) reported that the male Sasso T44 broilers' final body weights at 20 weeks of age in Nekemte ranged from 2755.98 g to 3907.42 g. This difference was attributed to the length of feeding and the variation in dietary ingredients. The higher live body weight of the broilers led to an increase in their intake, which in turn produced the highest overall superiority in feed consumption with diet T3 (4515.55g). Among all treatments, T2 and T3 exhibited the highest FCR. Similarly, Alagawany et al. (2021) revealed that FCR was enhanced when lemongrass essential oil was added to quail diets over a maximum of five weeks.

Table 3. Least squares mean for performance and percentage mortality of broilers in overall 42 days of age

Effect and level	LBW (g/bird)	FC/Chick(g/bird)	FCR	MR%
RMSE	860.99	207.14	0.38	0.35
R^{2}	0.32	0.78	0.85	0.00
Strain				
Cobb500	1975.77^{a}	4071.65	2.43^{b}	14.06
Hubbard	1583.43^{b}	4007.90	3.05^{a}	13.02
P-Value	<.0001	0.3926	0.0001	0.7677
Diet				
T1	1102.76^{b}	3596.23^{c}	3.70^{a}	11.46
T2	2455.58^{a}	4122.38^{b}	1.86^{c}	15.63
T3	2175.57^{a}	4515.55^{a}	2.25^{c}	14.58
T4	1384.48^{b}	3924.93^{b}	3.06^{b}	12.50
P-Value	<. 0001	<. 0001	<. 0001	0.8318
Strain* Diet				
Cob*T1	1224.99	3609.07	3.29	13
Cob*T2	2659.31	4080.25	1.65	17
Cob*T3	2404.49	4529.89	2.06	15
Cob*T4	1614.28	4067.40	2.74	13
Hub*T1	980.53	3583.40	4.10	10
Hub*T2	2251.84	4164.52	2.08	15
Hub*T3	1946.65	4501.22	2.44	15
Hub*T4	1154.69	3782.46	3.58	13
P-Value	0.8011	0.3521	0.5069	0.9933

^{a,b,c} Different superscripts within the same column are significantly different at $p < 0.05$, T1: Farm-formulated diet, T2, T3, T4: Commercial diets from different sources, %: Percentage, LBW: Live body weight, FC: Feed consumption, FCR: Feed conversion ratio, MR: Mortality rate, g: Gram: RMSE: Root mean square error, R^{2}: Coefficient of determination, Cob: Cobb-500, Hub: Hubbard

Carcass yield characteristics of broiler chickens

The effects of strain, sex, and diet treatments on the carcass yield of the chickens are detailed in Table 4. The results of the current study indicate that the chickens' strain significantly affected several measurements including thigh weight (TW), drumstick weight (DrW), breast weight (BrW), neck weight (NW), back weight (BaW), dressed weight (DW), eviscerate yield percentage (EY %), and eviscerate weight (EW). For Cobb 500 and Hubbard, there was no significant effect on slaughter weight (SW), carcass weight (CW), dressing percentage (DP), or wing weight (WW), respectively ($p < 0.05$).

When compared to Hubbard strains, the Cobb-500 strain demonstrated the maximum weight for the drumstick, thigh, back, and breast. This is because of the genetic makeup of the strains and their greater capacity for feed intake, feed conversion efficiency, and adaptation to environmental factors. These findings aligned with those of Biazen et al. (2021) who noted that chickens with a higher slaughter weight had heavier breast, wing, neck,

and back weights. Similarly, Mirosław et al. (2021) provided additional evidence on the impact of breed, origin, and diet on slaughter yield and meat quality. Therefore, consumers often prefer chickens with high yields of desirable parts such as breast muscle, drumsticks, and thighs, as these are considered the most valuable carcass sections in broilers raised for meat production (Faria et al., 2010).

Subsequently, comparing the eviscerated weight (1570.25 g) and dressed weight (1815.28 g) of the strains, the Cobb 500 chickens outperformed those of the Hubbard strain. The Cobb-500's larger body size contributes to its higher live and dressing weights, indicating superior carcass yield and visceral weights. The strain variations in the carcass yield and growth performance of broiler chickens make this significant. The study's findings are consistent with those of Fernandes et al. (2013). As the results indicated, there was a variation in the proportion of breast, thigh, drumstick, neck, and back among the strains. This result was similar to previous reports (Ibrahim, 2019; Biazen et al., 2021). The Cobb-500 strain showed higher breast weight compared to the Hubbard strain, attributed to genotype, feeding capacity, and environmental adaption. Compared to meat from other regions of the chicken carcass, breast meat frequently has a higher economic value (Eltazi et al., 2014). This is because there are no bones in the chicken's body and the breast meat has content collection meat. These findings concurred with those of Biazen et al. (2021) and Marapana (2016). In terms of eviscerated percentage, the Hubbard strain (61%) was lower than the Cobb-500 (67.85%), consistent with findings reported by Tesfaye et al. (2013).

For males and females, sex significantly affected slaughter weight, carcass weight, eviscerate yield percentage, dressing percentage, and back weight. However, the effect of sex was not significant on eviscerate weight, dressed weight, wing weight, thigh weight, drumstick weight, breast weight, or neck weight. In this study, male broilers had a greater carcass weight (1868.14g) compared to female broilers (1589.92g). As expected, a larger carcass yield was found in broiler chickens with higher growth potentials or higher live weight, which is comparable to the results of Cruz et al. (2018). The males weighed more in the slaughter, carcass, and back, and the females weighed more in the dressing than their male counterparts. This is due to the hormonal differences between the sexes and feed intake capacity. The dressing percentage for males (70.91%) was lower than for females (77.05%). These variations are influenced by genetics, strain, sex, and dietary factors. The dressing percentage observed in the present study was higher than the 53.7–56.7% reported by Melkamu (2017) for Sasso chickens slaughtered at 56 days of age, reflecting differences due to age and diet.

Regarding eviscerates yield percentage and dressing percentage, diet treatments did not show significant effects. However, other carcass yields were significantly influenced by diet treatment. The weight of the carcass was different depending on the diet treatment, showing that there were significant variations in the yield of the carcass part. This is because different dietary treatments contain different ingredients, which affect carcass yield. These results align with those of Ikusika et al. (2020) and Sanka et al. (2021), who reported a significant influence of the rearing system on carcass yields. Similarly, compared to other dietary treatments in the study, the broiler strains fed on the commercial diet (T2) exhibited greater slaughter, carcass, eviscerates, and dressed weights. This is because the profiles of amino acids and crude proteins of meat and bone meal are higher than those of other diet treatments. In contrast to other dietary treatments, the broiler strain in the farm-formulated diet (T1) showed reduced weights of slaughter, carcass, eviscerate, and dressing. This reduction is likely due to the lower content of meat and bone meal in the farm formulations derived from locally available resources. Therefore, the chickens fed T2 and T3 had the largest yields of carcass components (breast, thigh, and drumstick), while the broilers fed the farm-formulated diet had the lowest carcass yields.

In terms of back weight and wing weight, broilers consuming the farm-formulated diet (T1) had weights comparable to those fed the commercial diet (T4). However, dietary treatments in the current investigation resulted in significantly different weights for the slaughter, dressed, eviscerated, and breast broilers, consistent with findings reported by Seid et al. (2020). These results, on the other hand, contrast with those reported by Shawle et al. (2016). Significant differences in drumstick and thigh weights were observed across the dietary treatments. Variations in age, strains, and dietary composition typically account for these differences. The findings contradicted those reported by Chala et al. (2022). In addition, Marapana (2016) states that some factors, including strain, sex, length of feed withdrawal before processing, distance of hunger before slaughter, the birds' travel distance from the farm to the slaughter plant, their life span, and their rearing system, can all impact dressing percentage and relative meat yield in different carcass parts.

Table 4. The live weight and carcass traits of slaughtered broiler chickens at 42 days of age

Effect and level	SW(g)	CW(g)	EW(g)	DW(g)	EY%	DP	WW(g)	TW(g)	DrW(g)	BrW(g)	NW(g)	BaW(g)
RMSE	384.77	326.61	152.39	170.23	11.66	13.56	8.19	41.44	40.89	75.37	9.83	39.11
R^2	0.64	0.54	0.71	0.69	0.24	0.26	0.31	0.48	0.49	0.60	0.65	0.69
Strains												
Cobb500	2418.14	1786.13	1570.25^{a}	1815.28^{a}	67.85^{a}	75.57	78.12	276.81^{a}	255.53^{a}	431.45^{a}	87.70^{a}	324.86^{a}
Hubbard	2232.96	1671.94	1330.27^{b}	1570.34^{b}	61.00^{b}	72.27	75.81	232.38^{b}	204.7^{b}	388.55^{b}	72.17^{b}	252.95^{b}
P-Value	0.0591	0.1673	<. 0001	<. 0001	0.0223	0.2875	0.2639	<. 0001	<. 0001	0.0265	<. 0001	<. 0001
Sex												
M	2478.23^{a}	1868.14^{a}	1473.95	1721.10	61.25^{b}	70.91^{b}	77.77	258.55	233.11	411.62	80.16	299.34^{a}
F	2172.86^{b}	1589.92^{b}	1426.57	1664.52	67.61^{a}	77.05^{a}	76.16	250.64	227.20	408.38	79.70	278.47^{b}
P-Value	0.0024	0.0012	0.2186	0.1889	0.0332	0.0465	0.4353	0.4483	0.5649	0.8639	0.8521	0.0370
Diets												
T1	1633.99^{c}	1249.08^{c}	1132.79^{c}	1359.20 ^{c}	69.04	76.60	72.19^{b}	204.38^{b}	182.62^{b}	291.72^{c}	63.38^{b}	233.21^{b}
T2	2844.15^{a}	2042.40^{a}	1634.62 ^{a}	1874.20 ^{a}	59.27	68.03	76.61^{b}	270.87^{a}	249.57^{a}	510.35^{a}	90.15^{a}	335.74^{a}
T3	2600.56^{a}	1907.09^{ab}	1573.84^{ab}	1840.20^{b}	61.41	71.92	85.14^{a}	260.78^{a}	237.63^{a}	467.05^{a}	84.97^{a}	318.29^{a}
T4	2223.49^{b}	1717.56^{b}	1459.79 ^{b}	1697.63^{b}	67.97	79.13	73.92^{b}	282.35^{a}	250.81^{a}	370.88^{b}	81.24^{a}	268.38^{b}
P-Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0521	0.0628	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

^{a,b,c} Different superscripts within the same column are significantly different at $p < 0.05$, T1: Farm-formulated diet, T2, T3, T4: Commercial diets from different source, SW: Slaughter weight, CW: Carcass weight, EW: Eviscerate weight, DW: Dressed weight, EY %: Eviscerate yield percentage, DP: Dressing percentage, WW: Wing weight, TW: Thigh weight, DrW: Drumstick weight, BrW: Breast weight, NW: Neck weight, BaW: Back weight, RMSE: Root-mean-square error, and R^2: Coefficient of determination.

Table 5. The non-edible offal weights of slaughtered broiler chickens at 42 days of age

Effect and level	HEW (g)	CRW (g)	LUW(g)	SHW (g)	SIW (g)	LIW(g)	KW(g)	AFW (g)	PRW (g)
RMSE	10.43	1.91	1.62	11.68	9.13	2.30	1.95	4.37	0.99
R^2	0.52	0.15	0.04	0.46	0.42	0.38	0.16	0.25	0.53
Strain									
Cobb500	65.35^{a}	10.13	9.98	70.93^{b}	71.10	14.52	9.61	24.42	6.99
Hubbard	52.41^{b}	9.31	10.37	80.36^{a}	72.07	13.68	9.26	26.47	6.86
P-Value	<.0001	0.0896	0.3450	0.0021	0.6722	0.1471	0.4897	0.0664	0.5926
Sex									
M	60.21	9.78	10.36	75.24	72.13	14.48	9.67	25.42	7.14
F	57.55	9.66	9.99	76.05	71.04	13.71	9.19	25.47	6.71
P-Value	0.3114	0.8039	0.3680	0.7819	0.6340	0.1830	0.3271	0.9625	0.0902
Diet									
T1	45.67^{b}	8.96	10.29	66.76^{b}	63.54^{b}	12.51^{b}	8.66^{b}	23.08^{b}	5.62^{c}
T2	67.05^{a}	10.56	10.42	88.90^{a}	80.39^{a}	16.10^{a}	8.70^{b}	28.83^{a}	8.25^{a}
T3	61.56^{a}	10.06	10.06	79.01^{a}	77.36^{a}	15.27^{a}	10.38^{a}	25.71^{ab}	7.30^{b}
T4	61.25^{a}	9.31	9.9	67.91^{b}	65.06^{b}	12.51^{b}	9.98^{a}	24.16^{b}	6.53^{bc}
P-Value	< 0.0001	0.0885	0.8232	< 0.0001	< 0.0001	< 0.0001	0.0272	0.0029	< 0.0001

^{a,b} Different superscripts within the same column are significantly different at $p < 0.05$, T1: Farm-formulated diet, T2, T3, and T4: Commercial diets from different sources, HEW: Head weight, CRW: Crop weight, LUW: Lung weight, SHW: Shank weight, SIW: Small Intestine weight, LIW: Large Intestine weight, KW: Kidney weight, AFW: Abdominal Fat weight, PRW: Proventicuas weight, RMSE: Root-mean-square error, and $R^{2:}$ Coefficient of determination

Table 6. The edible offal weights of slaughtered broiler chickens at 42 days of age

Effect and level	GW (g)	HW (g)	LW (g)	SkW (g)
RMSE	6.97	1.42	4.08	23.51
R^2	0.74	0.42	0.84	0.78
Strain				
Cobb500	56.44[a]	12.41	53.13[a]	160.19[a]
Hubbard	40.68[b]	12.67	37.33[b]	142.92[b]
P-Value	<.0001	0.4599	<.0001	0.0047
Sex				
M	48.57	12.68	45.85	154.45
F	48.55	12.40	44.61	148.66
P-Value	0.9916	0.4348	0.2289	0.3289
Diet				
T1	35.41[c]	10.67[b]	39.21[c]	97.03[c]
T2	56.62 [a]	13.49[a]	49.28[a]	198.81[a]
T3	53.48[ab]	13.41[a]	48.02[ab]	184.43[a]
T4	48.74[b]	12.60[a]	44.43[b]	125.94[b]
P-Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a,b,c] Different superscripts within the same column are significantly different at $p < 0.05$, T1: Farm-formulated diet, T2, T3, and T4: Commercial diets from different sources, GW: Gizzard weight, HW: Heart weight, LW: Liver weight, SkW: Skin weight, RMSE: Root-mean-square error, and R^2: Coefficient of determination.

Edible offal of the slaughter

The effects of chicken strain, sex, and diet treatment on edible offal are summarized in Table 6. The Cobb 500 strain exhibited significantly higher weights of gizzard, liver, and skin compared to the Hubbard strain, while the heart weight showed no significant difference between the two strains ($p < 0.05$). Therefore, the Hubbard strain's greater susceptibility to these effects could indicate a limited capacity for feeding-related adaptation. This finding is in agreement with Biazen et al. (2021), who observed similar differences in these parameters across chicken breeds. The weight of the edible offal was not significantly affected by the sex of the chickens. This result indicated that there was no difference between the sexes between treatments. These findings were similar to those of Biazen et al. (2021).

The present study revealed that there was a significant effect of diet treatment on the gizzard, heart, liver, and skin weight of the broiler chickens ($p < 0.05$). This difference was due to feed intakes, sex, strains, feed conversion ratio, and environmental conditions. Except for the gizzard weight, the finding on edible offal weight was similar to that reported by Mosebework et al. (2018). These similarities are likely due to dietary treatment ingredients, genotype, and climatic factors.

Non-edible offal of the slaughter

The effects of chicken strain, sex, and diet treatment on non-edible offal are depicted in Table 5. The results reveal that there was no significant strain effect on crop weight (CRW), lung weight (LUW), small intestine weight (SIW), large intestine weight (LIW), kidney weight (KW), abdominal fat weight (AFW), and proventriculus weight (PRW), while a significant effect was observed for head weight (HEW) and shank weight (SHW, $p < 0.05$). The least-square means obtained for HEW were higher for Cobb 500 when compared with those of Hubbard, while SHW values were significantly higher for Hubbard than for Cobb 500. The sex of the broiler chickens did not significantly affect non-edible offal ($p > 0.05$).

The study demonstrated a significant effect of diet treatment on the weights of the head, shank, small and large intestines, kidney, abdominal fat, and proventriculus, except for crop and lung weight ($p < 0.05$). Likewise, broiler strains consuming the farm-formulated diet (T1) exhibited weights for the shank, small and large intestines, and abdominal fat similar to those consuming the commercial diet (T4). Additionally, the farm formulation was similar to the commercial diet (T2) about kidney weight. The broiler strain chickens receiving the commercial diet (T2) had a higher abdominal fat weight among dietary treatments. Therefore, the abdominal fat weight in the farm-formulated diet (T1) was similar to that of the commercial diet (T4) consumed among the treatments for the broilers. The accumulation of unnecessary fat on carcasses, particularly in the abdomen, was the main concern of broiler farmers in the previous studies. This finding highlights the issue of excessive abdominal fat, which is often rejected by consumers and considered waste. Although the statistical results indicated a significant difference in abdominal fat weight among treatments, T2 had the highest abdominal fat weights compared to other dietary groups. This result suggests that the farm-formulated diet (T1) was more effective in reducing abdominal fat compared to any commercial diet. These results are consistent with the findings of Tamasgen et al. (2021). Conversely, the effect of dietary treatments on the small intestine and proventriculus weights was not

supported by Mirosław et al. (2021). The weight of the large intestine varies significantly among treatments based on the diets, which aligns with Abera et al. (2016).

Haematological and serum biochemical study

The impact of chicken strain, sex, and diet treatment on serum biochemical and haematological parameters is shown in Tables 7 and 8. The results of the study revealed that the chicken strain had a significant effect on packed cell volume (PCV, $p < 0.05$). No significant differences were observed for red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), and mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Compared to the Cobb-500 strain, the Hubbard strain had a higher least square mean for the packed cell volume. Sex had no significant impact on the haematological parameters.

Table 7. The haematological parameters of broiler chickens at 42 days of age

Effect and level	PCV (%)	RBC (*10^6/dl)	WBC (* 10^3/dl)	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
RMSE	0.90	0.23	12.87	1.19	5.98	1.76	1.34
R^2	0.42	0.15	0.66	0.27	0.07	0.19	0.24
Strain							
Cobb500	8.80^{b}	3.13	329.19	14.71	135.92	44.99	33.79
Hubbard	9.39^{a}	3.21	324.20	14.57	134.79	44.55	33.32
P-Value	0.0111	0.1488	0.1259	0.6281	0.4526	0.3271	0.1641
Sex							
M	9.07	3.13	328.51	14.74	135.95	45.08	33.65
F	9.13	3.20	324.88	14.54	134.76	44.47	33.47
P-Value	0.7962	0.2322	0.2646	0.4886	0.4269	0.1709	0.5968
Diet							
T1	8.57^{bc}	3.05	315.78^{b}	13.91^{b}	133.28	45.14^{ab}	34.06^{a}
T2	8.41^{c}	3.17	337.24^{a}	14.74^{ab}	136.58	45.46^{a}	33.95^{a}
T3	9.32^{ab}	3.24	348.02^{a}	15.69^{a}	136.40	44.89^{ab}	33.81^{a}
T4	10.10^{a}	3.21	305.74^{b}	14.21^{b}	133.28	43.60^{b}	32.41^{b}
P-Value	<. 0001	0.0910	<. 0001	0.0005	0.3851	0.0228	0.0027

^{a,b,c} Different superscripts within the same column are significantly different at $p < 0.05$, T1: Farm-formulated diet, T2, T3, T4: Commercial diets from different sources, %: Percentage, PCV: Packed cell volume, RBC: Red blood cells, WBC: White blood cells, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, one deciliter (dL): 10^{-10} liters, one femtoliter (fL): 10^{-15} liters, one pictogram (Pg): 10^{-12}, g: Gram, RMSE: Root-mean-square error and R^2: Coefficient of determination.

Table 8. The serum biochemical parameters of broiler chickens at 42 days of age

Effect and level	TP (g/dl)	GLU (mg/dl)	CHO (mg/dl)	CRT (mg/dl)
RMSE	0.61	25.77	15.38	0.13
R^2	0.14	0.06	0.16	0.04
Strain				
Cobb500	3.17	208.13	137.41	0.07
Hubbard	3.26	216.94	138.06	0.11
P-Value	0.5830	0.1770	0.8657	0.1500
Sex				
M	3.31	215.16	142.05^{a}	0.09
F	3.12	209.91	133.42^{b}	0.09
P-Value	0.2325	0.4185	0.0287	0.9050
Diet				
T1	3.03	212.11	139.72	0.08
T2	3.54	207.44	141.34	0.09
T3	3.00	214.96	129.59	0.09
T4	3.28	215.63	140.28	0.10
P-Value	0.0557	0.8011	0.1209	0.9776

^{a,b} Different superscripts within the same column are significantly different at $p < 0.05$, T1: Farm-formulated diet, T2, T3, and T4: Commercial diets from different sources, mg: Milligrams, TP: Total protein GLU: Glucose, CHO: Cholesterol, CRT: Creatinine, one deciliter (dL): 10^{-10} liters, g: Gram, RMSE: Root-mean-square error and R^2: Coefficient of determination.

There was a significant response to diet treatment in the packed cell volume, white blood cells, and haemoglobin, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration ($p < 0.05$). However, there was no significant response observed in the mean corpuscular volume and red blood cells of the broiler chicken strains. These results are similar to those of Gana et al. (2019) and Oluwafemi et al. (2021), and highlight that factors such as species, age, sex, environment, nutrition, infection, and physiological conditions (Hrabčáková et al., 2014) can influence hematological variables.

Similarly, broilers consuming the farm-formulated diet (T1) exhibited higher packed cell volume compared to those on a commercial diet (T2) and were similar to those on commercial diets (T4) concerning white blood cells, mean corpuscular volume, and mean corpuscular hemoglobin concentration.

The white blood cell counts for broilers fed commercial diets (T2 and T3) were significantly higher and comparable to those observed in other treatments. These results might have played a role in the broilers' enhanced performance in both diets, as white blood cells play a crucial role in resisting diseases and fighting infections (Soetan et al., 2013). Furthermore, the study revealed that the mean corpuscular haemoglobin concentration for the farm formulation diet (T1) was comparable to that of the commercial diets (T2 and T3), aligning with the findings of Aikpitanyi and Egweh (2020).

The farm formulation (T1) also showed similar levels of hemoglobin to the commercial diets (T4) although the hemoglobin (Hb), packed cell volume (PCV), and white blood cell (WBC) values for the farm formulation were within the normal range; the commercial diets resulted in higher values for these parameters. This suggests that commercial diets might offer more effective nutrient utilization, enhancing blood formation due to their nutrient composition. This observation is consistent with the findings of Mulatu et al. (2019).

The effects of diet and strain on creatinine, glucose, cholesterol, or total protein were not statistically significant. However, sex had a significant impact on cholesterol levels but no significant effect on total protein, glucose, or creatinine ($p < 0.05$). Cholesterol levels and total protein were lower than those reported in previous studies, consistent with the findings of Alagbe et al. (2019) and Oluwafemi et al. (2021). In the present study, blood glucose levels were within normal ranges in broiler treatments, with values of 212.11, 207.44, 214.96, and 215.63 for T1, T2, T3, and T4, respectively. Thus, the current results, which ranged from 200 to 500 mg/dL, were comparable to the blood glucose levels in healthy birds (Campbell, 2012). The creatinine levels observed in this study are consistent with the findings reported by Aikpitanyi and Egweh (2020).

Partial budget analysis

The effects of diet treatment and strain on the partial budget analysis are presented in Table 9. The partial budget analysis of the total feed consumed per bird (kg) led to the following rankings: T3 > T2 > T4 > T1 for both the Cobb 500 and Hubbard strains. For the Cobb 500 broiler strain, T2 had the best net return, followed by T3, T4, and T1. The highest marginal rate of return was also found in T2, followed by T4, T1, and T3. However, T3 also showed a high marginal rate of return, which was followed by T4, T2, and T1. Additionally, T3, T2, T4, and T1 all showed high values for net returns in the Hubbard broiler strain.

The highest net returns were observed in broiler chickens fed the T2 diet in the Cobb500 strain, followed by T3, T4, and T1. For the Hubbard strain, T3 resulted in the highest net returns, with T2, T4, and T1 following in that order. Variations in net return were due to the differences in feed cost, feed consumption efficiency, strain type, and the selling price of individual broiler chickens in each treatment. Among the experimental diets, the most profitable diets were T2 for Cobb 500 broilers and T3 for Hubbard broilers, respectively, based on net return and marginal rate of return. These findings are in alignment with those reported by Alemayehu et al. (2019) and Tamasgen et al. (2021). The higher net returns observed for Cobb 500 (T2) and Hubbard (T3) compared to the farm-formulated diet (T1) highlight the profitability of these commercial diets. This profitability is linked to the higher carcass weight achieved with these diets. The results of the study corroborate those of Abd El-Hack et al. (2018), who suggested that pigeon peas could boost growth and meat yield in addition to lowering feeding costs without compromising performance. However, Solomon et al. (2017) claimed that the cost of manufacturing each experimental meal with toasted Cajan was comparable to the cost of the diet prepared on a farm. This is not supported by the results of the current investigation. The results of the present study showed that the high income generated by the commercial diets of Cobb 500 (T2) and Hubbard (T3) increased as a result of

increased weight gain and carcass weight, with no adverse effects on the chickens' performance. The greatest economic benefit was obtained when broilers were fed higher levels of a commercial diet than the farm-formulated diet. However, the farm-formulated diet (T1) had a higher marginal rate of return than that of a commercial diet (T3) in the Cobb 500 strain.

Table 9. Effects of commercial and farm-formulated diets on economic analysis of two broiler chickens at 42 days of age

Parameter \ Treatments	T1	T2	T3	T4
Partial Budget Cost (Birr)				
Cobb 500 strain				
Day old chick cost (Et. Birr)	52	52	52	52
Total feed consumed/bird (kg)	3.61	4.08	4.53	4.07
Per unit feed cost (Et. Birr)	30.75	37.08	33.38	34.35
Total feed cost (birr/bird)	111.01	151.29	151.21	139.81
Revenue (Et. Birr)				
Average carcass weight (kg)	1.36	2.16	1.85	1.77
Carcass price (supermarket)	260	260	260	260
Total return (Et. Birr)	353.6	561.6	481	460.2
Net return/bird (Et. Birr)	242.59	410.31	329.79	320.39
Marginal rate of return %	218.53	271.21	218.10	229.85
Hubbard strain				
Day old chick cost (Et. Birr)	57.50	57.50	57.50	57.50
Total feed consumed/bird (kg)	3.58	4.16	4.50	3.78
Per unit feed cost (Et. Birr)	30.75	37.08	33.38	34.35
Total feed cost (birr/bird)	110.09	154.25	150.21	129.84
Revenue (Et. Birr)				
Average carcass weight (kg)	1.14	1.92	1.96	1.66
Carcass price (supermarket)	260	260	260	260
Total return (Et. Birr)	296.4	499.2	509.6	431.6
Net return/bird (Et. Birr)	186.31	344.95	359.39	301.76
Marginal rate of return %	169.23	223.63	239.26	232.41

T1: Farm-formulated diet, T2, T3, and T4: Commercial diets from different sources, %: Percentage, kg: Kilogram, ET. Birr: Ethiopian Birr

CONCLUSION

The result revealed that the farm-formulated diet had effects on the live body weight, feed consumption, and feed conversion ratio comparable to those of the commercial diet in the T4 group. Notably, the farm-formulated diet demonstrated a higher marginal return rate than the commercial diets in T3 group for the Cobb-500 strain. Additionally, the farm-formulated diet showed advantages in several haematological parameters in broiler chickens. Farm-formulated diets were comparable with commercial diets in the T4 group for carcass yields, wing weight, and back weight. Consequently, the Cobb-500 strain had a greater result in carcass yield compared to the Hubbard strain during the experimental study. Overall, farm-formulated diets, which utilize locally available resources, offer a viable and cost-effective alternative to more expensive commercial diets. Therefore, it is feasible to generate a commercial diet for broiler chickens, as an alternative diet, using the feed ingredients that are accessible in the farming locations.

DECLARATIONS

Availability of data and materials

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

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Author's contributions

Bikila Negari created and planned the experiments, collected data, analyzed and interpreted the results, wrote the

manuscript, and confined the report. Yesihak Yusuf, Demissu Hundie, Negassi Ameha, Kefelegn Kebede, Biazen Abrar, and Diriba Diba created and planned the experiments, performed the experiments, and provided materials, reagents, analysis tools, or data. All the authors read and approved the final version of the manuscript.

Competing interests

The authors declare that there are no competing interests.

Ethical considerations

The ethical concerns of plagiarism, permission to publish, misconduct, data fabrication, double publication, and redundancy have all been reviewed by each author.

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Biosecurity Compliance and Its Applications in Poultry Production Sectors

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ABSTRACT

Poultry farming has been recognized as one of the most vital sectors for the economy and revenue generation in many countries. For the production of high-quality freshly hatched chicks, effective cleaning and sanitation of the hatchery environment and hatching eggs were crucial components of proper management and hygiene in chicken hatcheries. The current review aimed to assess the efficient ways of mitigating the risk of disease introduction (external biosecurity) and its subsequent dissemination (internal biosecurity) within and between poultry farms and hatcheries. In addition to identifying the variety of risk categories that are applied to various biosecurity industries, this article clarified the equivalent tools, including checklists and/or questionnaires, that can be used to assess biosecurity compliance. The checklist was aimed to evaluate numerous biosecurity protocol categories, including the farm's infrastructure, employees, their education and training, access control mechanisms, cleaning and disinfection procedures, handling of litter and waste, chick control, registrations, and pest management. In conclusion, external biosecurity was critical to preventing infections from entering hatcheries and poultry farms. Questionnaires or checklists were effective instruments for gathering information on biosecurity and evaluating compliance in poultry farms.

Keywords: Biosecurity compliance, Checklist, Hazard, Poultry sector

INTRODUCTION

Poultry sectors are under threats from numerous kinds of viruses, bacteria, and other microorganisms. To lower mortality and morbidity, several immunization programs have been developed for parent stock, broiler, and layer chickens. However, in an effort to lessen the possibility of bacterial and viral shedding further becoming an issue on the farm and/or on the farm's neighboring, new biosecurity measures have been implemented. Effective use of disinfectants and sanitizers is essential to any biosecurity strategy (Abdelaty et al., 2019).

In poultry farms, biosecurity refers to the health protocols and measures designed to protect a population from transmissible infectious agents. It is the initial line of defense against diseases that could affect food safety, the well-being of animals, and the farm's economic viability. The most commonly practiced biosecurity measures include farm sanitation, infrastructure maintenance, proper cleansing, and efficient disinfection equipment and procedures (Tilli et al., 2022).

The danger of infectious disease transmission in traditional poultry farming poses a significant risk to the health and welfare of the chicks due to factors, such as excessive stocking density, low genetic variation, inadequate ventilation, and immunosuppression (Espinosa et al., 2020). Biosecurity is one of the most effective strategies to remove the risk of disease introduction between farms and subsequent internal and external dispersion (Van Limbergen et al., 2018). Thus, the proper implementation of interior (e.g., cleaning and disinfection, segregation of poultry facilities, and home hygiene lock) and exterior (e.g., feed supply, admission of visitors and vehicles, and farm location) biosecurity should be given top priority (Damiaans et al., 2020).

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The presence of biofilms in the environment of chickens is considered a significant challenge, which has the potential to make any biosecurity program fail. Thus, before disinfecting the poultry house, a step for biofilm removal needs to be introduced (Abdelaty et al., 2019). The disinfection and cleaning program should be carried out as economically and safely as possible, which entails minimizing the frequency of doing so in the shortest possible time, with the least amount of capital spent on labor, chemicals, and energy, creating the least amount of waste, and causing no damage to the machinery. Routines for cleaning and disinfection must be carried out with expertise and experience. The polysaccharide matrix of biofilms acts as a barrier to shield connected cells from disinfectants, making it harder to remove the attached bacteria and biofilm. Additionally, attached cells exhibit greater resistance to biocides compared to planktonic cells. Therefore, it is crucial to take into account the special characteristics of biofilms when developing cleaning techniques (Costerton et al., 1995).

Maintaining high sanitation efficiency through proper cleaning and sanitary maintenance is crucial for both avian production and the reduction of infectious disease spread (Lazarov et al., 2018) using disinfectants such as quaternary ammonium compounds, glutaraldehyde, chlorine, peroxides, phenolic, and formaldehyde at bactericidal concentrations (Narayan et al., 2023). The current study aimed to evaluate efficient tools for mitigating the threat of disease introduction and subsequent dissemination between hatcheries and poultry farms. Additionally, it sought to identify the various categories of hazards applicable to various biosecurity sectors and clarify comparable tools such as checklists and/or questionnaires to assess biosecurity compliance.

The concept of biosecurity

When referring to safeguarding, the term "biosecurity" was primarily utilized for controlling biological weapons. The key goal of biosecurity was to safeguard against the hazards that pathogens and living things pose. Elimination, extermination, and control were the main tools of biosecurity, supported by effective system management, useful policies, and the efficient sharing and safeguarding of critical data, as illustrated in Figure 1. Bakanidze et al. (2010) noted that "when working with potentially contagious microbes and other biological dangers, implementation of laboratory techniques and practices, particular elements of laboratory construction, protective clothing, and appropriate health and safety program" is what is meant by biosafety, which is a supplement to biosecurity.

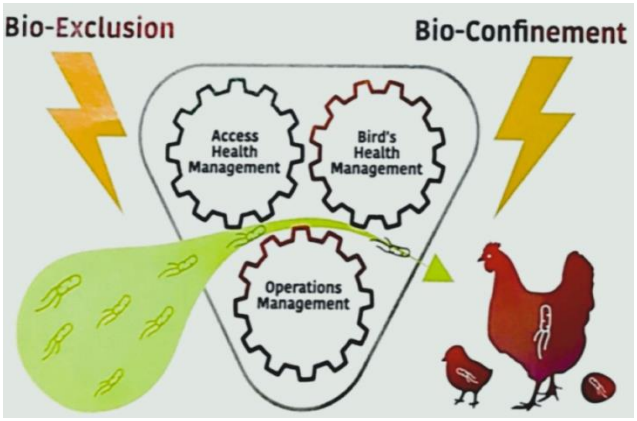

Figure 1. The main goals of biosecurity measures applied in poultry farms

Many countries have embraced the idea of biosecurity, incorporating it into several sector-specific strategic documents. In the context of animal health and production, biosecurity is defined as "a collection of physical and managerial precautions intended to minimize the possibility of animal infections and diseases entering, and spreading within an animal population" (OIE-FAO, 2009). Consequently, there are conflicting interpretations of biosecurity despite efforts to develop a single definition that encompasses "the approaches for evaluating and controlling the risk of diseases that are transmissible, quarantine pests, living altered organisms, and biologic weapons" (Meyerson and Reaser, 2002).

According to OIE-FAO (2009), biosecurity refers to all efforts taken to stop pathogen introduction (bio-exclusion) and to limit their spread (bio-containment). Biosecurity is integral to the concept of health, as it involves inhibiting transmission to humans, pets, plants, and surrounding environments. The definition provided by the WHO and FAO, which takes these factors into account, is appropriate and should be used as a reference point by other stakeholders to highlight the significance of biosecurity for public health, environmental protection, and animal health.

Biosecurity addresses various hazards across several industries, including food safety and human health , pets, and plants. Particular emphasis is placed on biological weapons, invasive alien species, and zoonoses. Thus, the classification of risks and hazards that need to be addressed varies depending on the sector, as shown in Table 1.

Table 1. Different categories of hazards applicable to different biosecurity sectors

Biosecurity Sector	Kinds of hazards
Animal health	Any pathogen that could have a negative impact on an animal's health
Zoonoses	A biological agent that may spread from domestic or wild animals to human beings.
Food safety	A physical, biological, or chemical substance included in food or its present form could have a negative impact on health.
Plant health	Any plant, animal, or pathogenic agent species, strain, or biotype that harms plants or botanical products.
"Biosafety" in relation to plants and animals	A living modified organism (LMO) with a unique genetic profile created by recent biotechnology that could have negative consequences for the preservation and sustainable use of biological diversity while also posing health risks to humans.
"Biosafety" in relation to food	A recurrent DNA organism that exists in food and has the potential impact on human health.

FAO Biosecurity Toolkit. Source: FAO (2007).

Biosecurity compliance of poultry farms

Questionnaires and similar tools, such as checklists, are frequently used to evaluate biosecurity compliance. These tools involve the assessor responding to several inquiries on the biosecurity measures that have been implemented (Renault et al., 2018; Damiaans et al., 2020). Depending on the surveys and the national legislation in existence, the ultimate assessment of both internal and external biosecurity can be either quantitative (Tanquilut et al., 2020) or qualitative (Sahlström et al., 2014). In case of unfavorable results, several alternatives can be used, including the provision of recommendations, imposing fines, or offering training for staff and farmers (Caekebeke et al., 2021). Questionnaires or checklists are effective instruments for collecting information on biosecurity, all-in/all-out production systems in poultry farms and evaluating the compliance of poultry farms both within and outside of the European Union (Van Limbergen et al. 2018; Correia-Gomes and Sparks, 2020; Ornelas-Eusebio et al., 2020). The implementation of biosecurity protocols in chicken farms is governed by national regulations that mandate frequent inspections by official veterinary services. These inspections evaluate compliance with biosecurity standards through the use of nationally standardized checklists (European Commission, 2022). Given that infectious diseases have the potential to seriously disrupt the entire supply chain, biosecurity precautions must be put in place during recurrent avian influenza (AI) epidemics (Mulatti et al., 2017; EFSA, 2021).

It is crucial to have external biosecurity to prevent infections from getting into poultry farms. Studies have shown that compared to internal biosecurity, external biosecurity is marginally more compliant. The most commonly adhered-to measures include the cleaning of "filter zones", which are similar to farm hygienic locks comprising sanitary and cleaning zones, having clean basins and equipment for cleaning (i.e., liquid or bars of soap, disposable or sanitary towels or dryers for hands and clothes storage areas), and footwear cleaning facilities (Chowdhury et al., 2012). Other biosecurity-related variables include access control (e.g., gate/bar closed upon arrival), vehicle disinfection (e.g., spray bay), and animal control. Still other biosecurity-related variables include internal biosecurity variables, such as walls, roofs, washable and disinfectable floors, and intact walls in house premises. All these variables have demonstrated high biosecurity compliance, marking a significant advancement in the application of biosecurity measures. Maintaining intact walls limits the existence of invertebrates, which may otherwise hide in crevices and act as transporters for poultry infections. Suitable cleansing and disinfection techniques are also essential for limiting the transmission of pathogens (Souillard et al., 2014).

Biosecurity Checklists

Some questions are broken up into further sections on the checklists for poultry farms. The objective of every section is to assess various categories of biosecurity protocol, involving the farm's infrastructural features, the number of employees, their education and training, the access control systems, the cleansing and disinfection protocols, the management of litter and manure, the bird control, the registrations, and the pest control. Additionally, the layer checklist includes sections on egg

care. A handful of the questions are open-ended, but the majority need a "yes" or "no" response. During in-person interviews, the official veterinarian has asked the farmer certain questions specific to him or her and, therefore, has depended on his or her credibility. When there are no biosecurity-related non-compliances displayed by the inspected farm, the outcome is deemed positive. In case the result is unfavorable, recommendations or fines are imposed, and corrective actions are documented in the checklist, as shown in Table 2, based on Tilli et al. (2022).

Hierarchy of biosecurity levels

To restrict the entry or limit the transmission of pathogenic agents that cause infectious diseases, a biosecurity program combines physical barriers like fences and mesh wire with targeted actions like footbath use, carwash deep cleansing, and equipment disinfection in the farm (Aiyedun et al., 2018). According to Kouam et al., (2018), traffic control, segregation, and sanitation are the three components of biosecurity measures. Van Limbergen et al. (2017) and Sasaki et al. (2019) further categorize biosecurity into two types: Internal and external. Biosecurity can be structured into three levels: Conceptual, structural, and operational (Maduka et al., 2016).

Farm locations fall under the conceptual category. Structural considerations include building layouts and amenities that ward off intruding wildlife and raptors. Operational considerations include the regular cleaning, sanitation, and work practices that farm workers and guests adhere to Shane (1997). The farms' biosecurity protocols have an impact on the birds' performance (Wijesinghe et al., 2017). The conceptual biosecurity includes elements like the separation between homes and farms, the distance from the main road, the existence of standing water, the type of house, the location of the house, and the construction materials used in the house. The presence of a farm gate and fence, footbaths, tire baths or sprays, restrictions on vehicle entry, visitor sign-on logbooks, and bans on purchasing day-old chickens, and feed. Among the issues raised by the structural framework are a truck sharing space with other farms, continuous rodent control, and a limitation on accessing newly stored litter intended for wild bird control (Ismael et al., 2021). In conclusion, the questions focus on using certain clothing, shoes, masks, and hats, routine washing and disinfection, using high-pressure sprayers, appropriate handling of deceased chickens, absence of other animals on the property, veterinarian advice, intervals between disinfection cycles, preventive care, and immunization, as shown in Table 3.

Table 2. Biosecurity checklist for poultry farms distributed to various sections.

Checklist section	Category
1. The farm's infrastructural features	
1.1 Year of building.	Year
1.2 Surface area of the farm.	m^2
1. Number of brick sheds.	Numbers
1.4 Gender of reared broilers (Male, Female, mixed sexes)	Select Gender
1.5 Farm entrance boundary (presence of gate)	Yes/No
1.6 If the gate/bar is closed on arrival	Yes/No
1.7 Presence of ≥ 1 area for storage materials (e.g. farm equipment, materials, fresh litter, etc.).	Yes/No
2. Boundary of the farm area	
2.1 Presence of other buildings not belonging to the farm	Yes/No
2.2 Presence of vehicles not dedicated to farm activities inside the farm area.	Yes/No
3. Equipment for vehicle cleaning and disinfection	
3.1 Presence of a spray bay with a waterproof floor.	Yes/No
3.2 The disinfection system is adequate.	Yes/No
3.3 Presence of a permanent automated installation for vehicle disinfection	Yes/No
3.4 Equipment for vehicle cleaning is working.	Present/absent
4. Dead-bird disposal	
4.1 Disposal notes are stored in the farm.	Yes/No
4.2 Carcasses loading is always during the production cycle.	Yes/No
4.3 Presence of a refrigerated storage container	Yes/No
5. Litter and manure management	
5.1 Fresh litter is used in the house without being stored.	Yes/No
5.2 No addition of litter during the production cycle.	Yes/No
5.3 A platform for manure storage is present.	Yes/No
5.4 Built-up litter (manure) is stored.	Yes/No
6. Rodent and pest control	
6.1 Managed by the farmer	Yes/No
6.2 The control procedure is dated and signed	Yes/No
6.3 Pesticides used during the cycle or at the end.	Yes/No

Table 3. The indicator points of conceptual, structural, and operation biosecurity levels in poultry farms.

Indicators of biosecurity level	Category
Conceptual level	
Distance of the farm from the main road (m)	%
Distance from the nearest farm (m)	%
Distance from the residential place (m)	%
No standing water near the farm	Yes/No
Premise with modified open side and curtains	Yes/No
Housing position	East-west/ others
Biosecurity training for employee	Yes/No
Structural level	
The presence of a fence and gate	Yes/No
Presence of footbath dip	Yes/No
Farm vehicle parked off the farm	Yes/No
Visitors sign on logbook	Yes/No
No equipment exchange with other farms	Yes/No
Operational level	
Use of special clothes, footwear, masks, hat, and coveralls	Yes/No
Visitors' special clothes, and footwear,	Yes/No
Regular cleaning and disinfection	Yes/No
Proper disposal of dead chickens	Yes/No
Removed litter stored at a cover shade	Yes/No
No access to stored food for rodents	Yes/No
The presence of an isolation room for diseased chicken	Yes/No
Sick birds are regularly examined	Yes/No
Vaccinating chickens for diseases	Yes/No

CONCLUSION

Biosecurity regulations require ongoing implementation and education of employees, as biosecurity compliance in intensive poultry operations was a crucial step in preventing the entry and dissemination of infectious diseases. While the questionnaires had shown to be an effective method for collecting data, they may only capture the state of biosecurity at the time they were completed, potentially missing ongoing efforts.

Safeguarding poultry flocks against microbial contamination was a critical aspect of the modern chicken production. Poultry growers may face severe economic consequences if a highly virulent and contagious disease organism was introduced into their flocks. The efficacy of a programin biosecurity can be maximized by regional involvement. The program will work better as a whole if all poultry growers use the optimal managerial programs, even though any level or degree of biosecurity is beneficial. As a component of any effectively managed program, putting good biosecurity practices into daily practice can help minimize the likelihood of becoming affected by pathogenic agents and, in cases of an outbreak, help prevent the progression of the disease.

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Authors' contributions

Asmaa N. Mohammed was responsible for data collection, study design, writing the article, and approving the final version of the manuscript for publication in this journal.

Competing interests

The author declares no competing interests.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, were addressed by the author.

Availability of data and materials

All data and materials are included in this review article.

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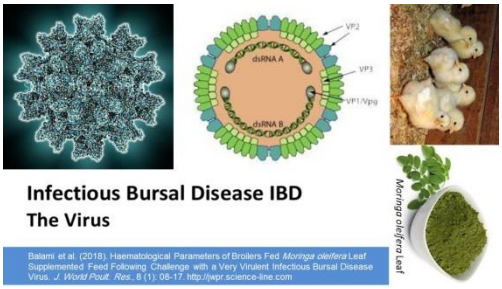

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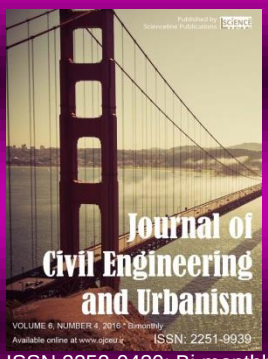

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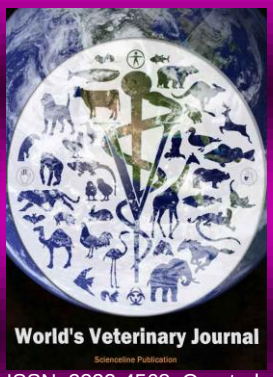

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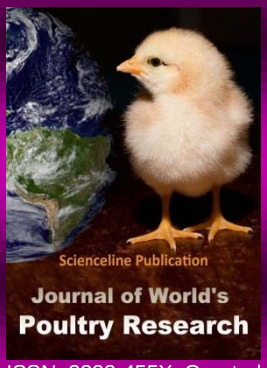

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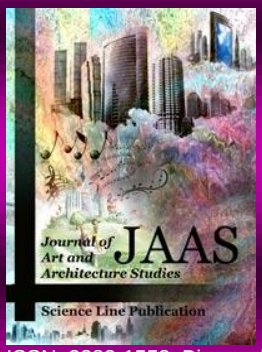

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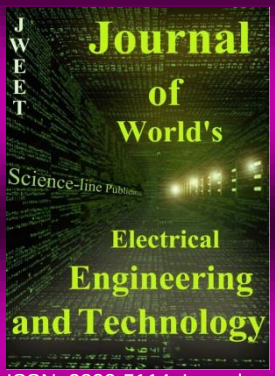

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