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PROOF

RESEARCH PAPER

Assessment of Physicochemical Properties and Microbial Quality of Water on Broiler Farms in Bosnia and Herzegovina**

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Abstract

Water plays a critical role in broiler production. The use of contaminated water can result in infections, contamination of animal products, and is unsuitable for drug administration. This study assessed the physicochemical and microbiological quality of water used on poultry farms producing broilers in the northeastern region of Bosnia and Herzegovina. The majority of broiler farms had private wells (n =58) or public water supplies (n = 34). Mean values for turbidity (NTU), pH, KMnO4 consumption (mg/L), chlorides (mg/L), ammonia (mg/L), nitrates (mg/L), nitrites (mg/L) and, conductivity (µS/cm) were as follows: 0.16±0.04; 7.51±0.25; 1.37±0.64; 11.91±10.49; 0.09±0.31; 12.78±13.64; 0.06±0.09 and 612.61±216.40, respectively. Ammonia and nitrates exceeded the recommended standard in one sample. Total coliform, E. coli and Enterococcus sp. were detected in 41.30 %, 22.83% and 34.78% samples, respectively. We did not find significant differences in microbial load between poultry farms with public water service to private water wells. The water used on poultry farms has satisfactory physicochemical properties but a high microbial load and could represent a potential source of pathogens. This indicates that water from poultry farms needs more treatment to improve its microbiological quality. Regular monitoring of water utilized in poultry farms is essential for preventive measures.

Introduction

Over the past 30 years, meat production has doubled, and it is expected to double again by 2050 (Shahbandeh, 2019). One of the primary global sources of meat production is poultry (Savin *et al.,* 2021). In 2022, more than 69,094 tons of poultry, of which 67,007 tons from broilers and 2,087 tons from other poultry were processed in Bosnia and Herzegovina (Annual report MVTEO, 2023). Prioritizing hygiene and sanitation in poultry farms is essential for sustainable and disease-free production, ensuring the health of both the flock and the workers involved in the process. Water is a necessary component of blood and tissues as well as a vital physiological ingredient for digestion, absorption, enzyme activity, nutrition delivery, thermoregulation, and waste disposal (Abdullah, 2011). Because of its polarity and hydrogen bonding, water has unique chemical characteristics that enable it to dissolve, absorb, or suspend a variety of substances (Singh *et al.,* 2023). Water consumption has a significant influence on the health and productivity of broilers because it is roughly twice as much as feed intake (King, 1996; Maharjan *et al*., 2016). Consequently, it is anticipated that poor-quality water will have a greater impact on hens than tainted or poor-quality food (Talha *et al.,* 2008). Broilers that are not provided with good qualitydrinking water tend to have poor feed conversion

ratios, and overall performance (Boumedous et al., 2017). Poultry farms frequently encounter health issues with their broilers for unknown reasons. The majority of these instances involve issues with hygiene and water quality (Maharjan et al., 2017). Water quality regulations for drinking water for poultry in Bosnia and Herzegovina have been adopted from Bosnia and Herzegovina Regulation (40/10) and European Council Directive 98/83/EC on the quality of water designed for human use (European Commission 1998). The health and productivity of chickens are significantly impacted by the microbiological and physicochemical quality of their drinking water. Water's mineral and microbiological composition has an impact on chicken performance (King, 1996). This study's goals were to evaluate the main physicochemical water parameters and the presence of fecal coliforms (FC), *Escherichia coli* (EC), and fecal *enterococci* (FE) in water samples taken from 92 farms in the northeastern (NE) region of Bosnia and Herzegovina that had access to either a public or private water source.

Materials and Methods

Sample Collection

Water samples were collected between November and December 2023 at 92 poultry farms producing broilers in northeastern (NE) region of Bosnia and Herzegovina. Majority of broiler farms had private wells (n =58) or public water supplies (n = 34). Before they were aseptically collected into sterilized bottles, water was let to run for roughly 30 seconds. Water was brought to the lab at 4°C after being collected, leaving about 3 cm of space at the top for aeration. For microbiological analysis, samples were processed in 24 hours, and for physicochemical analysis, in 48 hours. An overview of the location of poultry farms included in the study is presented in Figure 1.

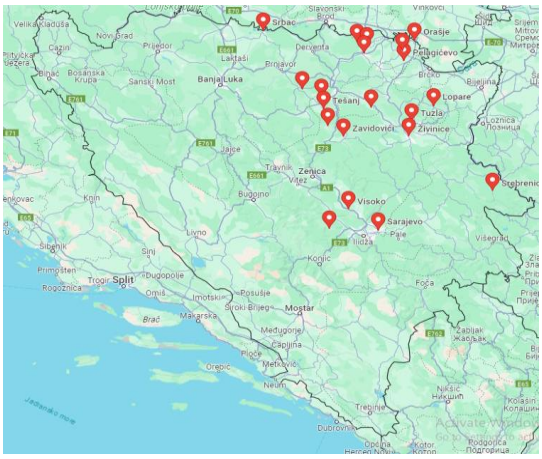

Figure 1. Location of study area and sampling poultry farms

All parameters were analyzed using an appropriate ISO method accredited according to the requirements of BAS EN ISO/IEC 17025:2008. Following physicochemical parameters were analyzed: color, odor, taste, turbidity, pH value, KMNO4 consumption, chlorides, ammonia, nitrates, nitrites and conductivity. Analytical reagent-grade chemicals were employed for the preparation of all solutions.

The Platinum-Cobalt Scale was used for color measurement of water (BAS EN ISO 7887:2013), while determination of the threshold odor (TON) and taste number (TFN) was conducted according to BAS EN 1622:2008. The pH, turbidity and conductivity of the collected samples were determined using pH/turbidity/conductivity meter as described by appropriate ISO methods. Prior to analysis, all instruments were calibrated according to manufacturer's recommendations. The method used calculate $KMNO_4$ consumption involved heating a sample in a boiling water bath containing potassium permanganate and sulfuric acid for a predetermined amount of time. Part of the permanganate was then reduced by oxidizable material in the sample, and the amount of consumed permanganate was then determined by adding an excess of oxalate solution and titrating with permanganate. Using the Mohr's method, silver nitrate titration with chromate indicator was used to determine the chloride content. The manual spectrometric approach was used to determine the ammonium. The yellow chemical produced by the interaction of sulfosalicylic acid (made by adding sulfuric acid and sodium salicylate to a sample) with nitrate and then treating the mixture with alkali was used to quantify the nitrate ion in water using spectrometric measurement at the 655 nm wavelength. The amount of nitrite was determined using molecular absorption spectroscopy.

Following microbiological parameters were analyzed: detection and enumeration of *E. coli,* fecal coliforms and fecal streptococci *Enterococcus* spp. Analyzes were performed using membrane filtration technique according to BAS EN ISO 9308-1/A1:2018 and BAS EN ISO 7899-2:2003 thus 100 mL of the sample was filtered using 0.45 µm pore size membrane filter (Sartorius, Germany). The filter were then aseptically placed on Chromogenic Coliforms Agar (CCA) ISO (Condalab, Spain) and incubated at 37°C for 21±3 h for detection of *E. coli* and fecal coliforms. *E. coli* count is presented as metallic blue to violet colonies while presumptive coliform colonies (pink to red in color) were confirmed through an oxidase-negative reaction. For the determination of the fecal *Enterococci*, the filter was placed on Slanetz and Bartley's agar (Condalab, Spain) and incubated at 37°C for 24 – 48 h. Presumptive colonies of *enterococci* (red, maroon or pink color) were placed on Bile Esculin Agar (Remel, USA) and incubated at 44°C for 4 hours. Plates were examined for blackening of medium around and under the colonies. A descriptive analysis was carried out (min, max, mean, and standard deviation).

The median microbiological counts (FC, EC, and FE) for various water sources (public vs. private) were calculated compared using an Microsoft Excel tool, assessing the microbial load by the presence or absence of bacterial contamination. Statistical significance was established when the p-value was below 0.05, as determined by the t-test.

Results and Discussion

Physicochemical parameters of water samples are summarized in Table 1. The requirements for the physicochemical quality of the analyzed water samples were not met by 1 of the total 92 samples, for ammonia and nitrates quality parameters (Table 1). The results of microbiological analyses are presented in Table 2. Of the total of 92 examined samples. 21 samples (41.30%) did not meet the microbiological quality regarding the presence of *E. coli*. These samples had exceeded the allowed 0 cfu/100 ml, according to the recommendations from the Guidelines on Microbiological Criteria for Water in B&H, which are adapted to EU regulations Directive 98/83/EC. 38 samples had coliforms present (22.83%) and the presence of fecal *enterococci* was recorded in 32 (34.78%) samples.

We did not find significant differences ($p < 0.05$) in microbial load between poultry farms with public water service to private water wells**.** The physicochemical and microbiological results of this study were compared with those from the European Council Directive 98/83/EC on the quality of water meant for human consumption (European Commission 1998), as there is no special legislation for water used in animal production. Quality of drinking water is a crucial health determinant for animal health and production. Water which plays an important role in poultry farms can easily be contaminated with microorganisms and transmitted to the animals through water consumption. It is thus important to provide a microbial contamination free water source. These waterborne pathogens may cause infections and could also be responsible for development of antimicrobial resistance, which is a major public health issue (Jacobs *et al.*, 2008). The majority of tested poultry farms were dependent on private wells source of water. Physicochemical parameters assessed included color, odor, taste, turbidity, pH value, $KMNO_4$ consumption, chlorides, ammonia, nitrates, nitrites and conductivity of the water samples. The color of water indicates the presence of suspended particles or dissolved substances while the odor can result from various sources, including organic matter, minerals, or chemical contaminants. Factors such as high mineral content, chemical residues or microbial contamination can alter the taste of water.

The appearance, taste and odor of tested samples were satisfactory. Turbidity of water measures the presence of suspended particles that could potentially serve as reservoirs for bacteria and viruses (Mann *et al.,* 2007). All water samples used on poultry farms had turbidity values below 1 NTU with a mean of 0.16 NTU (Table 1). This implies that water used on broiler farms has low levels of suspended particles (Mann *et al.,* 2007). The degree of acidity or alkalinity caused by dissolved ions in water is measured by its pH. The pH of water is an important consideration; the optimal pH range for drinking water is between 5 and 7 (Saleh *et al.,* 2023). pH values below 5 can impact water consumption, potentially leading to parasitic infections due to altered conditions favoring certain organisms. Conversely, elevated pH values may suggest the presence of salt pollution, which could impact the assimilation of crucial nutrients such as calcium, phosphorus, potassium, and magnesium (Vermeulen *et al*., 2002).

Table 1. Physicochemical properties of water used on broiler farms

Physicochemical parameters	Ref.value	min	max	mean ± SD	median
Color	No change	ND	ND	ND	ND
Odor	No change	ND	ND	ND	ND
Taste	No change	ND	ND	ND	ND
Turbidity (NTU)	1.0	0.1	0.32	0.16±0.04	0.16
pH	≥ 6.5 ≤ 9.5	7.05	8.02	7.51±0.25	7.47
$KMNO_4$ consumption (mg/ L O_2)	5,0	0.30	2.89	1.37±0.64	1.20
Chlorides (mg/ L)	250	0.71	77.81	11.91±10.49	11.28
Ammonia (mg/ L)	0,50	<0.01	1.70	0.09±0.31	0.02
Nitrates (mg/ L)	50	0.97	80.56	12.78±13.64	7.73
Nitrites (mg/ L)	0.5	<0.002	0.34	0.06±0.09	0.01
Conductivity (µS/cm)	2500	208.00	1665.00	612.61±216.40	595.00

Table 2. Microbiological properties of water used on broiler farms

Microbiological properties	Ref. value	No of isolates	Public water	Private well
E. coli	0 cfu/100 ml	21	6	15
Total coliforms	0 cfu/100 ml	38	18	20
Fecal enterococci	0 cfu/100 ml	32	14	18

It's essential to maintain a balanced pH level for both water quality and health considerations. Most of the tested water samples were neutral to slightly alkaline with average values 7.51±0.25. These findings are accordance to similar research (Abd-El-Kader *et al*. 2009, Mohebi *et al*., 2023, Haddad and Masoud, 2024). An essential water quality parameter, the permanganate index is used to calculate the amount of oxidizable materials (both organic and inorganic) in water. The values of this index were in range 0.30 to 2.89 mg/L and despite this variation remained lower than the permissible limit. In water and wastewater, one of the main inorganic anions is chloride, specifically the chloride (Cl^-) ion. Research has demonstrated that elevated amounts of chloride may cause disruptions in metabolism (Coetzee *et al.,* 2000). Elevated amounts of chloride have been shown to affect digestion, decrease feed consumption, and increase water intake (Ayoub *et al*., 2017). Concentrations of chloride in water in this study remained under the permissible limit and ranged between 0.71 and 77.81 %.

These results are similar to findings in well water in Nigeria (Taiwo *et al*. 2011). The presence of ammonia, nitrites and nitrates in groundwater is a criterion for water pollution with nitrogenous organic substances. It is likely that contamination of groundwater is from the farm as manure removed from the buildings is stored on the ground near the production buildings. In spring months, when the environment temperature is higher, more intensive are the processes of ammonification of organic matter in manure and the levels of ammonia, nitrites and nitrates in groundwater increase. High levels of agricultural activity are typically associated with elevated ammonia concentrations in groundwater (Liu *et al.,* 2020). Generally, nitrites and nitrates are natural components of water, originating from sources like fertilizers, organic matter decomposition but excessive levels can be harmful to poultry. For nitrites, concentrations above 10 ppm can be toxic to poultry, leading to conditions like methemoglobinemia which affects the bird's ability to transport oxygen (Casey *et al.,* 1998). Nitrate levels below 100 ppm are generally considered safe for poultry, although levels above this threshold may cause health issues such as reduced growth rates and reproductive problems (Saleh *et al,* 2023). Our results do not align with the study on water samples from broiler farms in Bulgaria, where the nitrate concentration was 71.6 mg/L (Stefanova *et al.,* 2012), which is above the reference standard. This suggests that sanitation measures in broiler farms in Bosnia and Herzegovina may be quite successful in controlling certain water quality issues. All water samples in our study had electrical conductivities within the acceptable range, with a mean value of 612.61 µS/cm. Our results are slightly higher compared to a study conducted in Ghana, where the conductivity of 100 samples ranged from 23.6 to 1114.0 µS/cm, with an average of 146.7 µS/cm (Osei *et al,* 2019). The lower conductivity values were likely due to low mineralization (Gray, 2004). Water is essential to chicken farms and is readily polluted with bacteria, which the animals can contract by drinking it. However, despite the high bacterial activity, nitrite and nitrate levels might be low if the microbial community is primarily composed of species that do not produce or consume significant amounts of these compounds. Waterborne gastroenteritis is most likely to occur when microorganisms are present in drinking water (Amaral *et al.,* 2004). (In the northeastern part of Bosnia and Herzegovina, we found that fecal *streptococci* (34.78%), *E. coli* (22.83%), and fecal coliforms (41.30%) polluted the water in broiler farms. These waterborne pathogens cause low body weight and high mortality in poultry (Amaral *et al.,* 2004). One bacterium connected to epidemics of colibacillosis disease is *E. coli.* It is important because 95% of the bacteria that comprise the most well-known and researched group of bacteria, fecal coliforms, are formed by it (Gama 2005; Cardozo *et al.,* 2015). Our findings are similar to those from Jordan in the context of the coliform pathogen patterns isolated (Haddad and Masoud, 2024). Both studies observed similar trends in coliform contamination, highlighting the importance of monitoring water quality to prevent potential health risks to poultry. Sewage treatment facilities nearby and insufficient waste management can also contribute to fecal bacteria contamination in well water samples. Fecal bacteria contamination in well water samples can arise from inadequate waste management and sewage treatment plants that are closely located to. To mitigate the risk of microbial contamination in wells located near surface drainage water, it's essential to implement proper well construction practices, including adequate casing depth, sealing, and placement away from potential contamination sources (Cronin *et al.,* 2006). From the above findings, there is an urgent need for the strict monitoring of the microbial quality and physicochemical properties of the various sources of water used in animal husbandry in Bosnia and Herzegovina.

Conclusion

Most poultry farms in the Northeastern region of Bosnia and Herzegovina rely on own well water as their main source of water, followed by public water sources. Most of the analyzed poultry farms has satisfactory physicochemical properties but a high microbial load that could represent a potential source of pathogenic organisms. Most often water samples were contaminated with coliform and *enterococci* bacteria with *Streptococcus* sp. being the predominant isolate. Ensuring access to clean, high-quality water is livestock and poultry production is of major importance to support optimal health, growth, and productivity. Water

quality can impact animal health and performance, so it's crucial to monitor and maintain water sources to meet the specific needs of the animals. Regular monitoring of water used in poultry must be conducted.

Conclusion

Most poultry farms in the Northeastern region of Bosnia and Herzegovina rely on own well water as their main source of water, followed by public water sources. Most of the analyzed poultry farms has satisfactory physicochemical properties but a high microbial load that could represent a potential source of pathogenic organisms. Most often water samples were contaminated with coliform and *enterococci* bacteria with *Streptococcus* sp. being the predominant isolate. Ensuring access to clean, high-quality water is livestock and poultry production is of major importance to support optimal health, growth, and productivity. Water quality can impact animal health and performance, so it's crucial to monitor and maintain water sources to meet the specific needs of the animals. Regular monitoring of water used in poultry must be conducted.

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RESEARCH PAPER

Determination of Genotype, Housing System and Age Effect on Egg Production and Quality Traits of Layers

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Abstract

This study was carried out to determine the effects of housing systems (free system-FR, conventional cage-CC and enriched cage-EC) on the egg production and quality traits of layer genotypes (Lohmann Sandy-LS, Lohmann White-LW and Lohmann Brown-LB) at 35, 45, 55 and 65 weeks of age. A total of 180 layers were used for egg production traits. A total of 45 eggs were analyzed for egg quality traits at each age period. The highest feed intake was in FR-reared LS layers at 35 wk of age (P=0.000). The lowest yolk index was in CC, and FR-reared LW layers at 65 wk of age (P=0.042). The lowest haugh unit was in EC-reared LB layers at 65 wk of age (P=0.041). The highest yolk color was in CC-reared LB layers and EC-reared LS and LB layers at 45 wk of age (P=0.009). It can be concluded that feed intake of layers, yolk index, haugh unit and yolk color of eggs are affected by the age and genotype of layers reared in different housing systems. The production and egg quality traits are affected by the age of layers. The genotype of layers influences the production and egg quality traits, excluding egg weight and yolk index. The housing system affected feed intake of layers and some egg quality parameters, except for yolk and shell weight, shell ratio, shell thickness, shape index and yolk color. The study could help breeders look for commercial genotypes for rearing in different housing systems.

Introduction

Concerns of consumers regarding animal welfare are prompting significant global changes to the implementation of cage-free housing systems for laying hens (Rodenburg *et al*., 2022). According to the Council Directive 1999/74/EC, all European countries have the capability to produce table eggs using various housing systems (cage and cage-free; litter, free-range, organic systems) (EU Commission, 2021). While enriched cage and litter systems are used for egg production in European countries, there is a growing interest in exploring alternative systems (Majewski *et al*., 2024). Although new trends emerge for animal-friendly systems in the rearing of hens, approximately 90% of hens used in global commercial egg production continue to be housed in cages (Ledvinka *et al*., 2012). But the traditional cage systems are known to significantly restrict the freedom of chickens and their capacity to exhibit normal behaviors (Lay *et al*., 2011).

Because of their high protein content, ease of preparation, widespread availability, and affordability when compared to other animal-based protein sources, eggs are an essential component of the human diet. The quality of an egg has a significant impact on consumer preferences, product value, and food safety (Hisasaga *et al*., 2020). The main goal in layer breeding is to obtain eggs of sufficient yield and quality at low cost.

Commercial egg layer hybrids are used for rearing to obtain high production performance and egg quality (Tůmová *et al*., 2017; Sokołowicz *et al*., 2018). However, both internal and external variables; including genetics, age, laying cycle, diet, microclimate, management, and housing system, might affect the quality of eggs (Abebe *et al*., 2023; Alig *et al*., 2023). With the spread of alternative housing systems, there are many studies about the effects of rearing systems on egg yield and egg quality. Thus, some studies shown that the differences between layer performance and egg quality characteristics in cage and cage-free systems (Yılmaz Dikmen *et al*., 2016, 2017; Tutkun *et al*., 2018; Philippe *et al*., 2020). Egg quality and eggshell color are essential factors affecting consumer preferences (Scott and Silversides, 2000; Abebe *et al*., 2023). But, using suitable layer genotypes for different housing systems provides significant benefits for productivity (Castellini *et al*., 2016). However, there are limited studies effect of these housing systems on performance and egg quality of different layer genotypes (Sokołowicz *et al*., 2018; Rakonjac *et al*., 2021; Tainika *et al*., 2024; Aygün *et al*., 2025). And there have been few studies comparing differences in housing systems for egg quality attributes over the production cycle (Yılmaz Dikmen *et al*., 2017; Sokołowicz *et al*., 2018). Therefore, this study aimed to determine the effects of housing systems (FR, CC and EC) on egg production and quality traits of laying hen genotypes (LB, LW and LS) at different age periods.

Materials and methods

Practices regarding the care and use of animals for research purposes were in accordance with the laws and regulations of Türkiye and approved by the Animal Use and Ethical Committee of Bursa Uludağ University (Approval Number 2023-05/01). In this study, Lohmann Sandy (LS), Lohmann White (LW) and Lohmann Brown (LB) layer hen were used in free system (FR), conventional cage (CC) and enriched cage (EC) in Bursa Uludağ University, Agriculture Faculty, Research and Application Unit. The CC and EC cage systems were in same hen house. The cage house unit was 120 m from the FR house. The CC system consisted of 3 tiers, cage unit (50×45×45 cm), trough-type feeder, nipple drinker, egg cradle and manure belt. The CC cage provided 450 cm^2/hen. The EC system cages fulfilled the standards of EU Directive 1999/74/EC. The EC cage dimensions were 240×125 cm. The EC system consisted of 2 tiers, trough-type galvanized feeder, nipple drinkers, perches, nesting areas, scratch pad areas, nail shorteners, egg cradle and manure belt. The EC cage provided 750 cm^2/hen. The FR system consisted of pasture (4 m^2/ hen) and indoor areas (m^2/7 hen). The pasture area was protected by wire fences and shelter. The wood shavings litter, rounded feeders and drinkers, perches, and nest boxes were placed in FR system. In all systems, layers were fed with a diet containing 17% CP and 2.750 ME kcal/kg between the 18 and 40 weeks of age, 16% CP and 2.700 ME kcal/kg, 0.7% P and 3% Ca between the 41 and 65 weeks of age (NRC, 1994). Feed and water were offered *adlibitum*. The 16L:8D photoperiod was used at the time of laying.

A total of 180-layer hens, 20 from each genotype (LS, LW and LB) in each rearing system (FR, CC and EC) were used. In order to observe age-related changes in layers' egg production and egg quality throughout the laying period, data were taken at 35, 45, 55, and 65 weeks of age. For each system and genotype group, egg number, egg weight and feed consumption values were collected daily for 1 week in the relevant age periods. Then hen day egg production, egg mass, feed intake and feed conversion ratio (FCR) were calculated as formulas given below;

$$\text{Egg mass} = (\text{Hen day egg production x egg weight})/\ 100$$
$$\text{Feed intake} = (\text{Feed consumption}/\ \text{number of hens})$$
$$\text{Feed conversion ratio} = \text{Feed intake} / \text{Egg mass}$$

A total of 180 eggs were analyzed for egg inner and outer quality traits during study. At each age period, eggs were collected and randomly selected to determine the egg weight, shell weight, yolk weight, albumen weight, shell thickness, shell breaking strength, shape index, albumen index, yolk index, shell ratio, yolk ratio, albumen ratio, yolk color and haugh unit. Before egg quality determination, all eggs were stored for 24 hours, and per egg quality trait was calculated.

The shape index and shell breaking strength were measured using equipment. The albumen was separated from the yolk after the eggs were broken and weighed. After being swilled and dehydrated for 24 hours, eggshells were weighed. Shell thickness was determined at the air cell, sharp end, and equator of egg points using a caliper and the averages of these sites were used. The data for egg weight, yolk weight and shell weight (g) were recorded using a digital scale. The weight of the egg was subtracted from the weight of the yolk and shell to determine the albumen weight. The albumen length, width, and yolk diameter (mm) were measured using a digital caliper (Mitutoyo Corp., Aurora, IL, USA). A tripod micrometer was used to determine the yolk and albumen heights (mm). The yolk color was determined using a Roche yolk color fan scale. The albumen, yolk, and shell ratios, and albumen and yolk index and Haugh unit (Silversides *et al*., 1993) calculated as formulas given below;

$$\text{Yolk ratio } (\%) = (\text{Yolk weight/Egg weight}) \text{ x } 100$$
$$\text{Albumen ratio } (\%) = (\text{Albumen weight/Egg weight}) \text{ x } 100$$
$$\text{Shell ratio } (\%) = (\text{Shell weight/Egg weight}) \text{ x } 100$$
$$\text{Albumen index } (\%) = \left(\frac{\text{Albumen height}}{\frac{\text{Albumen length+Albumen width}}{2}}\right) \text{x} 100$$
$$\text{Yolk index } (\%) = (\text{Yolk height / yolk diameter}) \text{ x } 100$$
$$\text{Haugh Unit } (\%) = 100 \log (\text{Albumen height} + 7.57 - 1.7 \text{xEgg weight}^{\ 0.37})$$

Statistical analysis

The data was analysed by analysis of variance General Linear Models using ANOVA with statistical software Minitab 17. Percentage data were analyzed following an arcsine square root transformation of the data. The age (35, 45, 55 and 65), housing system (FR, CC and EC), and genotype (LS, LW and LB) were the main effects. The model included effects of age, housing system, genotype, and all interactions. Data were presented as mean ± standard error (SE) in all the tables. Differences were considered significant at $P \leq 0.05$ and the statistical difference at $P < 0.10$ was described as a tendency. The statistical model was as follows:

$$Y_{ijk} = \mu + a_i + b_j + c_k + (ab)_{ij} + (ac)_{ik} + (bc)_{jk} + (abc)_{ijk} + \varepsilon_{ijk},$$

where $Y_{ijk} = \mu^{th}$ observation value, μ = expected mean of the population, a_i = i. age effect (i= 35, 45, 55 and 65), b_j = j. housing system effect (j= CC, EC and FR), c_k = k. genotype effect (k= LS, LW and LB), $(ab)_{ij}$ = ij. Age and housing system interaction effect, $(ac)_{ik}$ = ik. Age and genotype interaction effect, $(bc)_{jk}$ = jk. Housing system and genotype interaction effect, $(abc)_{ijk}$ = ijk. Age and housing system and genotype interaction effect, ε_{ijk} = residual error.

Results

The age, housing system, genotype and interactions effects on egg production traits of layers are given in Table 1. The age and genotype of layers affected the hen day egg production, egg mass, feed intake and FCR (P=0.000). The lowest henday egg production, egg mass was found at 65 wks of age. The lowest feed intake was found at 45 and 55 wks of age. The henday egg production and egg mass were higher in the LS and LW genotypes. The lowest feed intake was found in the LB genotype. The FCR was higher in the LB genotype but similar in the LS and LW genotypes. The housing system considerably affected the feed intake of layers (P=0.000). The higher feed intake was found in the FR system but was found similar in the CC and EC system. The effect of housing system on hen day egg production tends to be significant and numerically higher hen day egg production was found in FR system (P=0.056).

The age and system interaction effect on egg mass and FCR was found significant (P=0.035 and P=0.001). The age and genotype interaction effect on egg production traits investigated were found significant (P=0.034; P=0.037; P=0.000 and P=0.041; respectively). The system and genotype interaction effect on hen day egg production and feed intake were found significant (P=0.026 and P=0.000; respectively) (Table 1).

The three-way interaction effect of age, housing system and genotype on egg production traits of layers are given in Table 2. The interaction between age, housing system and genotype was significant for feed intake (P=0.000). The highest feed intake was found in FR reared LS layers at 35 wk of age, and lowest feed intake was found in CC reared LS layers at 45 wk of age (P=0.000). The three-way interaction effect of age, housing system and genotype on hen day egg production, egg mass and FCR of layers was insignificant (P > 0.05).

In summary, as a main factors; age and genotype of layers influenced hen day egg production, egg mass, feed intake and FCR, but housing system affected only feed intake of layers. At 35 wk of age the highest feed intake was found in FR reared LS layers.

The age, housing system, genotype and interaction effects on egg quality of layers are given in Table 3 and Table 4. The layers' age affected the egg weight, albumen, yolk and shell weights, albumen, yolk (P=0.004) and shell (P=0.046) ratios (P=0.000). The highest egg weight, yolk weight, shell weight and yolk ratio were found at 65 wks of age. The lowest albumen weight, albumen ratio was found at 45 and 65 wks of age, respectively. The lowest shell ratio was found at 35 and 55 wks of age. The housing system considerably affected the egg weight (P=0.000), albumen weight (P=0.000), albumen ratio (P=0.005) and yolk ratio of layers (P=0.040). The highest egg weight, albumen weight, albumen ratio and lowest yolk ratio were found in EC system. The genotype of layers affected albumen and shell weight (P=0.004), yolk weight (P=0.000), albumen and yolk ratio (P=0.000), shell ratio (P=0.011). The highest yolk weight and yolk ratio, and lowest albumen weight and ratio was found in LW. The shell weight and shell ratio were higher in the LB genotype but lower in the LS genotype (Table 3).

The age and system interaction effect on egg weight and albumen weight were found significant (P=0.001 and P=0.026). The age and genotype interaction effect on egg weight, albumen weight, yolk weight, shell weight and yolk ratio were found significant (P=0.000; P=0.044; P=0.003; P=0.030 and P=0.050; respectively). The system and genotype interaction effect on egg weight, albumen weight and yolk ratio were found significant (P=0.001; P=0.000 and P=0.030; respectively) (Table 3).

The age of layers affected the SBS, ST (P=0.001), shape index (P=0.003), albumen index, yolk index, haugh unit and yolk color (P=0.000). The highest SBS and yolk color were found at 45 wks of age. The lowest shell thickness was found at 55 wks of age. The lowest albumen index, yolk index, shape index and haugh unit were found at 65 wks of age (Table 4).

The housing system considerably affected the albumen index (P=0.000), SBS (P=0.003), yolk index (P=0.007) and haugh unit (P=0.000). The SBS was found lower in the CC system but higher in the FR system. The haugh unit and albumen index were higher in the CC system, but similar in the FR and EC system. The yolk index was found higher in the EC but was lower in the FR system. The genotype of layers affected SBS (P=0.004), shell thickness (P=0.002), shape index (P=0.000), albumen index (P=0.002), haugh unit (P=0.013) and yolk

Table 1. Effect of age, housing system and genotype on egg production traits of layers

Age, week	Hen day production, %	Egg mass, g	Feed intake, g	FCR, g feed/g egg
35	88.02[a]	53.24[a]	114.21[a]	2.22[b]
45	92.49[a]	54.51[a]	108.11[b]	2.00[c]
55	91.64[a]	54.82[a]	108.90[b]	2.03[c]
65	77.51[b]	48.32[b]	112.70[a]	2.43[a]
SE	1.50	0.90	0.95	0.05
P	0.000	0.000	0.000	0.000
System				
CC	88.24	53.18	108.97[b]	2.12
FR	89.11	53.47	113.64[a]	2.17
EC	84.91	51.52	110.33[b]	2.22
SE	1.30	0.78	0.82	0.04
P	0.056	NS	0.000	NS
Genotype				
LS	94.07[a]	56.16[a]	115.34[a]	2.08[b]
LW	90.55[a]	55.28[a]	110.18[b]	2.05[b]
LB	77.63[b]	46.72[b]	107.42[c]	2.38[a]
SE	1.30	0.78	0.82	0.04
P	0.000	0.000	0.000	0.000
Interactions				
A × S	NS	0.035	NS	0.001
SE	2.59	1.57	1.65	0.08
A × G	0.034	0.037	0.000	0.041
SE	2.59	1.57	1.65	0.08
S × G	0.026	NS	0.000	NS
SE	2.25	1.36	1.43	0.07
A × S × G	NS	NS	0.001	NS
SE	4.79	2.72	2.86	0.15

[a-c] values within columns with different superscripts are significantly different (P < 0.05). NS: Not significant
A: Age; S: Housing System; G: Genotype; CC: conventional cage, FR: Free range, EC: Enriched cage;
LS: Lohmann Sandy, LW: Lohmann White, LB: Lohmann Brown

Table 2. The three-way interaction effect of age, housing system and genotype on egg production traits of layers

Trait	Age, week	Housing System										
		CC			FR			EC				
		LS	LW	LB	LS	LW	LB	LS	LW	LB	SE	P
Hen day, %	35	94.29	97.14	71.43	95.89	91.43	80.00	97.74	92.86	71.43	4.49	NS
	45	93.30	96.43	94.90	95.71	96.99	83.57	97.14	92.48	81.90		
	55	95.24	95.71	95.92	97.14	93.98	82.71	97.32	91.73	75.00		
	65	83.93	75.71	64.84	93.98	81.95	75.94	87.14	80.16	53.97		
Egg mass, g	35	56.98	59.82	42.22	55.71	55.10	46.84	60.29	57.82	44.38	2.72	NS
	45	54.34	58.94	54.32	55.33	58.03	47.91	57.56	55.00	49.24		
	55	55.95	57.73	57.19	58.29	57.42	49.01	57.70	53.90	46.23		
	65	52.58	48.29	39.81	58.53	51.59	47.90	50.76	49.78	35.70		
Feed intake, g	35	117.65[a-f]	117.23[a-f]	104.47[e-g]	131.84[a]	103.44[fg]	119.69[a-e]	121.03[a-c]	109.49[b-g]	103.08[fg]	2.86	0.001
	45	101.30[g]	105.78[c-g]	105.62[c-g]	121.54[ab]	112.18[b-g]	105.76[c-g]	108.51[b-g]	104.86[e-g]	107.45[b-g]		
	55	109.33[b-g]	107.90[b-g]	104.93[d-g]	114.21[b-g]	108.75[b-g]	106.38[b-g]	109.58[b-g]	106.76[b-g]	112.31[b-g]		
	65	114.49[b-g]	115.44[b-g]	103.60[fg]	120.65[a-d]	114.92[b-g]	104.35[e-g]	113.97[b-g]	115.44[b-g]	111.48[b-g]		
FCR,g feed/g egg	35	2.14	1.98	2.52	2.39	1.93	2.80	2.00	1.90	2.32	0.15	NS
	45	1.87	1.80	1.95	2.22	1.94	2.21	1.92	1.92	2.21		
	55	1.98	1.91	1.84	1.96	1.90	2.19	1.96	2.07	2.44		
	65	2.25	2.51	2.64	2.07	2.26	2.22	2.25	2.45	3.18		

[a-o] values within columns and lines with different superscripts are significantly different (P < 0.05). NS: Not significant
A: Age; S: Housing System; G: Genotype; CC: conventional cage, FR: Free range, EC: Enriched cage; LS: Lohmann Sandy, LW: Lohmann White, LB: Lohmann Brown

Table 3. Effect of age, housing system and genotype on egg quality traits of layers

Age	Egg weight, g	Albumen weight, g	Yolk weight, g	Shell weight, g	Albumen ratio, %	Yolk ratio, %	Shell ratio, %
35	60.10^{b}	38.31^{a}	15.89^{b}	5.90^{b}	63.71^{a}	26.46^{b}	9.82^{b}
45	58.20^{c}	36.71^{b}	15.58^{b}	5.90^{b}	63.07^{ab}	26.76^{b}	10.15^{a}
55	59.95^{b}	38.00^{a}	16.04^{b}	5.91^{b}	63.35^{a}	26.77^{b}	9.87^{b}
65	62.11^{a}	38.66^{a}	17.13^{a}	6.31^{a}	62.22^{b}	27.61^{a}	10.16^{a}
SE	0.27	0.25	0.15	0.06	0.25	0.23	0.10
P	0.000	0.000	0.000	0.000	0.000	0.004	0.046
System							
CC	58.91^{c}	36.98^{b}	15.97	5.95	62.79^{b}	27.09^{a}	10.10
FR	59.79^{b}	37.54^{b}	16.22	6.01	62.80^{b}	27.13^{a}	10.06
EC	61.57^{a}	39.23^{a}	16.29	6.05	63.68^{a}	26.48^{b}	9.83
SE	0.24	0.22	0.13	0.06	0.21	0.19	0.09
P	0.000	0.000	NS	NS	0.005	0.040	NS
Genotype							
LS	59.93	37.89^{ab}	16.15^{b}	5.88^{b}	63.22^{a}	26.95^{b}	9.82^{b}
LW	59.99	37.41^{b}	16.61^{a}	5.97^{ab}	62.37^{b}	27.67^{a}	9.95^{ab}
LB	60.35	38.46^{a}	15.72^{c}	6.16^{a}	63.68^{a}	26.09^{c}	10.21^{a}
SE	0.24	0.22	0.13	0.06	0.21	0.19	0.09
P	NS	0.004	0.000	0.004	0.000	0.000	0.011
Interactions							
A × S	0.001	0.026	NS	NS	NS	NS	NS
SE	0.48	0.44	0.25	0.12	0.43	0.39	0.18
A × G	0.000	0.044	0.003	0.030	NS	0.050	NS
SE	0.48	0.44	0.25	0.12	0.43	0.39	0.18
S × G	0.001	0.000	NS	0.066	0.063	0.030	NS
SE	0.41	0.38	0.22	0.10	0.37	0.34	0.16
A × S × G	0.073	NS	NS	NS	NS	NS	NS
SE	0.83	0.76	0.44	0.20	0.75	0.69	0.32

a-c values within columns with different superscripts are significantly different (P < 0.05). NS: Not significant

A: Age; S: Housing System; G: Genotype; CC: conventional cage, FR: Free range, EC: Enriched cage

LS: Lohmann Sandy, LW: Lohmann White, LB: Lohmann Brown; SBS: Shell breaking strength, ST: Shell thickness

Table 4. Effect of age, housing system and genotype on egg quality traits of layers

Age	SBS,kg/cm²	ST,mm	Shape index, %	Albumen index, %	Yolk index, %	Haugh unit	Yolk color
35	1.79^{b}	0.406^{ab}	76.46^{a}	8.57^{a}	40.66^{a}	78.93^{b}	12.05^{b}
45	2.36^{a}	0.411^{a}	76.24^{a}	9.01^{a}	40.06^{ab}	83.78^{a}	12.40^{a}
55	1.55^{b}	0.394^{b}	75.53^{ab}	8.86^{a}	39.22^{bc}	83.26^{a}	11.80^{b}
65	1.46^{b}	0.410^{a}	75.06^{b}	7.39^{b}	38.59^{c}	74.65^{c}	11.82^{b}
SE	0.13	0.003	0.29	0.18	0.30	0.89	0.08
P	0.000	0.001	0.003	0.000	0.000	0.000	0.000
System							
CC	1.54^{b}	0.408	76.10	9.10^{a}	39.78^{ab}	82.79^{a}	12.08
FR	2.10^{a}	0.405	75.93	8.11^{b}	38.98^{b}	78.93^{b}	11.98
EC	1.72^{ab}	0.401	75.44	8.17^{b}	40.13^{a}	78.76^{b}	11.98
SE	0.11	0.002	0.25	0.16	0.26	0.77	0.07
P	0.003	NS	NS	0.000	0.007	0.000	NS
Genotype							
LS	1.62^{b}	0.398^{b}	76.45^{a}	8.05^{b}	39.58	78.67^{b}	12.08^{a}
LW	1.64^{b}	0.404^{ab}	74.23^{b}	8.88^{a}	39.33	81.91^{a}	11.83^{b}
LB	2.11^{a}	0.413^{a}	76.79^{a}	8.44^{ab}	39.98	79.90^{ab}	12.13^{a}
SE	0.11	0.002	0.25	0.16	0.26	0.77	0.07
P	0.004	0.002	0.000	0.002	NS	0.013	0.010
Interactions							
A × S	NS	NS	NS	0.004	NS	0.001	NS
SE	0.23	0.005	0.51	0.32	0.51	1.54	0.14
A × G	NS	NS	NS	0.069	0.039	NS	0.000
SE	0.23	0.005	0.51	0.32	0.51	1.54	0.14
S × G	NS	0.071	NS	NS	NS	NS	0.000
SE	0.20	0.005	0.44	0.28	0.45	1.34	0.12
A × S × G	NS	NS	NS	0.078	0.042	0.041	0.009
SE	0.40	0.010	0.88	0.56	0.89	2.67	0.25

a-c values within columns with different superscripts are significantly different (P < 0.05). NS: Not significant

A: Age; S: Housing System; G: Genotype; CC: conventional cage, FR: Free range, EC: Enriched cage

LS: Lohmann Sandy, LW: Lohmann White, LB: Lohmann Brown; SBS: Shell breaking strength, ST: Shell thickness

color (P=0.010). The SBS was higher in LB but was similar in LS and LW genotypes. The ST was found higher in the LB but was lower in the LS genotype. The shape index and yolk color were lower in the LW genotype but similar in the LS and LB genotypes. The haugh unit and albumen index were found higher in the LW, but lower in the LS genotype (Table 4).

The age and system interaction effect on albumen index and haugh unit were found significant (*P*=0.004 and P=0.001). The age and genotype interaction effect on yolk index and yolk color were found significant (P=0.039 and *P*=0.000). The system and genotype interaction effect on yolk color was found significant (*P*=0.000) (Table 4).

The three-way interaction effect of age, housing system and genotype on egg quality traits of layers are given in Table 5 and Table 6. The interaction between age, housing system and genotype was significant for yolk index (P=0.042), haugh unit (P=0.041) and yolk color (P=0.009). The highest yolk index was found in CC reared LW layers at 45 wk of age, and lowest was found in CC and FR reared LW layers at 65 wk of age. The highest haugh unit was found in CC reared LS layers at 45 wk of age, and lowest was found in EC reared LB layers at 65 wk of age. The highest yolk color was found in CC reared LB layers, and EC reared LS and LB layers at 45 wk of age, and lowest yolk color was found in FR reared LS layers at 55 wk of age and in EC reared LB layers at 65 wk of age. The three-way interaction effect of age, housing system and genotype on egg weight and albumen index of layers was tend to be significant (P=0.073 and P=0.078). The three-way interaction effect of age, housing system and genotype on albumen weight and ratio, yolk weight and ratio, shell weight and ratio, SBS, ST and shape index of layers was insignificant (P > 0.05).

In summary, as a main factors; age of layers influenced all investigated egg quality parameters, and housing system influenced egg weight, albumen weight and ratio, yolk ratio, shell breaking strength, index of albumen and yolk, and haugh unit. But genotype of layers affected all the egg quality parameters investigated, except for egg weight and yolk index. At 45 weeks of age, CC-raised LW layers had the highest yolk index, LS layers had the highest haugh unit and LB layers had the highest yolk color, and also EC- raised LS and LB layers had the highest yolk color.

Discussion

Enhancing the living conditions for laying hens has become a major concern for the layer industry. In the study, layer's age influenced hen day egg production, egg mass, feed intake and FCR. The henday egg production and egg mass was the lowest at 65 wks of age. Şekeroğlu *et al.* (2014) reported that hen age affected FCR and egg production rate, Yılmaz Dikmen *et al.* (2016) reported that age of hens affected hen day egg production, feed intake, egg mass and FCR of layers. Thus, Yılmaz Dikmen *et al.* (2016) reported that lowest henday egg production was at 60 wk of age. Indeed, a decrease in egg production with increasing age is an expected situation, also, since egg mass is determined with egg production, it was found to be lower with advancing age. But, in the study the lowest feed intake was at 45 and 55 wks of age of layers. During study, the season corresponded to summer and autumn in these age periods. The high air temperature in these periods might cause low feed consumption. In fact, Yakubu *et al*. (2007) investigated genotype and housing system (battery cage system and deep litter system) effect on the performance of Bovans Brown and Lohmann Brown layers in the wet and hot-dry seasons and found that hen-housed egg production, feed intake, and egg weight of layers improved in the wet season compared to the hot-dry season.

Studies have indicated that egg production in traditional cage, enhanced cage, barn and aviary system was similar (Neijat *et al*., 2011; Ahammed *et al.*, 2014). Some research found that conventional cage systems produced more eggs than aviary, floor systems, or free-range systems (Leyendecker *et al*., 2001; Erek and Matur, 2024). However, in the study, housing system tends to be affected hen day egg production and in terms of numbers higher hen day egg production was found in FR system. Support to this Yılmaz Dikmen *et al*. (2016) found that hen day egg production was comparable in the CC and EC systems, but higher in the FR system. According to certain research, rearing systems have an impact on egg mass (Onbaşılar *et al*., 2015; Erek and Matur, 2024). But in the study, housing systems were not affected by egg mass of layers. However, the housing system influenced feed intake of layers. The higher feed intake was in the FR system. These results were comparable to those of Yılmaz Dikmen *et al*. (2016) and Ahammed *et al*. (2014). It can be thought that layers reared in the free-range system were consumed more feed because they were more mobile than layers in the cage system. In addition, layers in the free-range system were consumed more feed in cold weather seasons, because they were more exposed to seasonal temperature changes. As a matter of fact, it was determined that feed intake was similar in CC and EC systems within the same poultry house. Despite this, housing systems were not found to have any effect on FCR of layers. But research suggests that different rearing systems have varying effects on FCR of layers (Ahammed *et al*., 2014; Onbaşılar *et al*., 2015; Yılmaz Dikmen *et al*., 2016).

The genotype affects the performance of layers, Rakonjac *et al*. (2021) whom investigated the effect of rearing systems (floor and organic) and genotypes, reported that Isa Brown hens had better egg production, egg mass, feed intake and FCR than New Hampshire hens. Also, Tutkun *et al.* (2018) whom compared the performance of free range reared Lohmann Brown and Atak-S, reported that egg production was similar between the genotypes, but there were differences in feed consumption and feed efficiency between the layers. In the study, hen day egg production, egg mass

Table 5. The three-way interaction effect of age, housing system and genotype on egg quality traits of layers

Trait	Age, week	Housing System									SE	P
		CC			FR			EC				
		LS	LW	LB	LS	LW	LB	LS	LW	LB		
Egg weight, g	35	59.82	56.25	57.15	59.54	59.13	61.72	61.57	61.28	64.43	0.83	0.073
	45	57.51	60.11	55.64	57.46	59.24	56.32	58.08	59.60	59.86		
	55	59.58	59.52	60.27	60.02	59.83	59.05	60.31	60.06	60.95		
	65	59.42	61.17	60.45	62.08	61.69	61.42	63.75	62.05	66.96		
Albumen weight, g	35	38.17	35.78	35.66	37.61	37.60	38.95	39.62	39.00	42.37	0.76	NS
	45	36.00	37.41	35.44	35.80	36.87	36.19	36.69	37.55	38.42		
	55	38.20	36.59	38.09	37.88	37.37	38.05	39.11	37.29	39.38		
	65	37.64	37.29	37.53	38.26	37.89	38.04	39.65	38.26	43.37		
Yolk weight, g	35	15.74	14.88	15.61	16.21	15.96	16.57	15.97	16.22	15.84	0.44	NS
	45	15.91	16.34	14.55	15.67	16.47	14.21	15.53	16.15	15.38		
	55	15.52	16.88	16.32	16.30	16.70	14.88	15.21	16.99	15.53		
	65	15.96	17.43	16.47	17.70	17.31	16.66	18.04	17.94	16.62		
Shell weight, g	35	5.90	5.59	5.87	5.71	5.56	6.20	5.97	6.05	6.22	0.20	NS
	45	5.58	6.35	5.64	5.99	5.89	5.91	5.85	5.89	6.05		
	55	5.85	6.04	5.85	5.83	5.74	6.10	5.98	5.78	6.04		
	65	5.81	6.44	6.44	6.11	6.47	6.69	6.01	5.84	6.96		
Albumen ratio, %	35	63.81	63.60	62.39	63.17	63.59	63.09	654.34	63.62	65.75	0.75	NS
	45	62.59	62.26	63.68	62.30	62.24	64.23	63.17	63.01	64.18		
	55	64.10	61.47	63.16	63.11	62.42	64.40	64.83	62.07	64.60		
	65	63.35	60.97	62.11	61.62	61.45	61.93	62.20	61.68	64.67		
Yolk ratio, %	35	26.31	26.43	27.30	27.22	26.99	26.86	25.95	26.46	24.58	0.69	NS
	45	27.68	27.16	26.16	27.27	27.81	25.26	26.74	27.10	25.71		
	55	26.05	28.38	27.10	27.17	27.96	25.24	25.24	28.30	25.49		
	65	26.86	28.48	27.22	28.52	28.05	27.16	28.36	28.89	24.98		
Shell ratio, %	35	9.87	9.96	10.30	9.59	9.41	10.04	9.70	9.90	9.66	0.32	NS
	45	9.72	10.56	10.14	10.42	9.94	10.49	10.08	9.88	10.10		
	55	9.83	10.14	9.73	9.71	9.60	10.35	9.91	9.62	9.90		
	65	9.78	10.53	10.65	9.84	10.49	10.89	9.42	9.42	10.33		

A: Age; S: Housing System; G: Genotype; CC: conventional cage, FR: Free range, EC: Enriched cage; LS: Lohmann Sandy, LW: Lohmann White, LB: Lohmann Brown NS: Not significant.

Table 6. The three-way interaction effect of age, housing system and genotype on egg quality traits of layers

Trait	Age, week	Housing System									SE	P
		CC			FR			EC				
		LS	LW	LB	LS	LW	LB	LS	LW	LB		
SBS, kg/cm^2	35	1.60	1.52	2.21	1.63	1.75	1.87	1.58	1.91	2.06	0.40	NS
	45	1.26	3.14	2.16	2.59	2.25	2.83	2.41	2.06	2.52		
	55	1.55	0.77	1.24	2.13	1.76	2.20	1.65	0.75	1.88		
	65	0.40	1.20	1.47	1.97	1.54	2.71	0.68	1.05	2.15		
ST, mm	35	0.400	0.412	0.427	0.396	0.397	0.407	0.400	0.402	0.412	0.010	NS
	45	0.394	0.432	0.404	0.415	0.406	0.416	0.419	0.405	0.409		
	55	0.400	0.400	0.394	0.383	0.389	0.409	0.388	0.384	0.400		
	65	0.390	0.426	0.424	0.406	0.410	0.431	0.386	0.390	0.425		
Shape index, %	35	77.40	76.20	77.20	77.60	74.60	77.60	75.80	73.80	78.00	0.88	NS
	45	76.20	74.80	76.80	76.60	74.20	78.00	77.40	75.20	77.00		
	55	76.40	73.20	76.80	75.80	74.00	76.80	75.80	74.60	76.40		
	65	78.00	74.20	76.00	77.80	73.00	75.20	72.60	73.00	75.75		
Albumen index, %	35	7.43	9.70	9.74	7.36	9.31	8.18	8.31	8.66	8.43	0.56	0.078
	45	9.12	11.59	8.35	7.91	7.86	8.52	9.81	9.40	8.53		
	55	9.54	10.73	9.80	8.13	9.69	8.89	7.12	7.18	8.03		
	65	7.27	7.26	8.67	7.26	7.06	7.12	7.39	7.45	7.06		
Yolk index, %	35	40.66^{a-c}	39.26^{a-c}	40.26^{a-c}	41.34^{a-c}	40.86^{a-c}	39.90^{a-c}	41.61^{a-c}	39.62^{a-c}	42.41^{ab}	0.89	0.042
	45	39.25^{a-c}	43.05^{a}	39.23^{a-c}	38.09^{a-c}	39.15^{a-c}	38.61^{a-c}	40.63^{a-c}	40.81^{a-c}	41.69^{a-c}		
	55	38.78^{a-c}	38.29^{a-c}	41.55^{a-c}	39.46^{a-c}	37.94^{bc}	39.73^{a-c}	38.75^{a-c}	39.19^{a-c}	39.29^{a-c}		
	65	39.18^{a-c}	37.06^{c}	40.79^{a-c}	37.56^{bc}	37.26^{c}	37.90^{bc}	39.59^{a-c}	39.48^{a-c}	38.44^{a-c}		
Haugh unit	35	74.25^{d-g}	83.87^{a-f}	81.71^{a-g}	74.16^{d-g}	82.51^{a-g}	76.23^{b-g}	78.92^{a-g}	80.06^{a-g}	78.74^{a-g}	2.67	0.041
	45	92.52^{a}	81.70^{a-g}	78.78^{a-g}	78.70^{a-g}	82.92^{a-g}	88.30^{a-d}	85.41^{a-g}	85.41^{a-g}	82.49^{a-g}		
	55	89.78^{a-c}	90.54^{ab}	87.50^{a-e}	80.76^{a-g}	86.73^{a-f}	83.27^{a-g}	74.53^{d-g}	78.30^{a-g}	78.00^{a-g}		
	65	72.55^{fg}	73.55^{d-g}	82.26^{a-g}	74.96^{d-g}	75.39^{c-g}	72.76^{e-g}	73.79^{d-g}	75.35^{c-g}	71.25^{g}		
Yolk color	35	12.40^{ab}	12.25^{a-c}	12.00^{a-c}	12.40^{a-c}	11.40^{bc}	12.00^{a-c}	12.40^{ab}	11.60^{a-c}	12.00^{a-c}	0.25	0.009
	45	12.00^{a-c}	12.00^{a-c}	12.80^{a}	12.20^{a-c}	12.40^{ab}	12.60^{ab}	12.80^{a}	12.00^{a-c}	12.80^{a}		
	55	11.80^{a-c}	11.60^{a-c}	12.60^{ab}	11.00^{c}	11.60^{a-c}	12.40^{ab}	11.80^{a-c}	11.80^{a-c}	11.60^{a-c}		
	65	12.20^{a-c}	11.80^{a-c}	11.60^{a-c}	11.40^{bc}	12.20^{a-c}	12.20^{a-c}	12.60^{ab}	11.40^{bc}	11.00^{c}		

$^{a-g}$ values within columns and lines with different superscripts are significantly different (P < 0.05). NS: Not significant; SBS: Shell breaking strength, ST: Shell thickness

A: Age; S: Housing System; G: Genotype; CC: conventional cage, FR: Free range, EC: Enriched cage; LS: Lohmann Sandy, LW: Lohmann White, LB: Lohmann Brown

feed intake and FCR were affected by the layer's genotype. The LS and LW genotypes had higher henday egg production and egg mass than LB genotype. But LB genotype had lowest feed intake and higher FCR. Similar to our findings Aygün *et al*. (2025) who investigated performance of different genotype layers reared in free-range system, reported that Lohmann Sandy layers laid more eggs than the Lohmann Brown layers.

The significant interactions between genotype and housing system, genotype and season, housing system and season on layers' performance was reported by Yakubu *et al*. (2007) whom investigated genotype and housing system (battery cage system and deep litter system) effect on Bovans Brown and Lohmann Brown layer's performance at the wet and hot dry seasons. Thus, in the study, there were age and genotype interaction effect on investigated all egg production traits. There were age and housing system interaction effect on egg mass and FCR, also it was supported by Yılmaz Dikmen *et al*. (2016). There were housing system and genotype interaction effect on hen day egg production and feed intake of layers. Similarly, Rakonjac *et al*. (2021) reported that there was interaction between the rearing systems and genotypes for egg production, feed consumption, moreover for egg mass and FCR. Also, in the study, age, housing system, and genotype did not interact with hen day egg production, egg mass, and FCR. However, there was age, housing system and genotype interaction effect on feed intake; FR-raised LS layers had the maximum feed intake at 35 weeks of age, while CC-raised LS layers had the lowest feed intake at 45 weeks. These results demonstrate that LS genotypes respond to freedom of mobility and environmental weather conditions, consuming more feed in a rearing system allow both indoor and outdoor access than in cage systems at early and mid-flock ages. The quality of eggs has a great impact on consumer egg purchases, specially egg weight (Aygün and Narinç, 2024). The internal and exterior quality of eggs are affected by a variety of genetic and environmental factors. In various researches suggests that flock age have varying effects on egg quality parameters, thus some was reported that egg weight (Tůmová *et al*., 2017; Tainika *et al*., 2024), weight of yolk and albumen, and ratio of yolk increased with flock age (Suk and Park, 2001), but some was reported that albumen ratio (Rizzi *et al*., 2005), egg shell quality (Tainika *et al*., 2024), shape index (Van Den Brand *et al*., 2004, Tainika *et al*., 2024), yolk index, albumen height, haugh unit (Tainika *et al*., 2024) decreased with increased flock age, but some was reported that egg weight (Zemková *et al*., 2007), shape index, yolk index (Alkan, 2023), egg shell traits (Yannakopoulos *et al*., 1994) did not affect by flock age. In the study, layer's age influenced egg weight, weight of albumen, yolk and shell, ratio of albumen, yolk and shell, SBS, ST, shape index, albumen index, yolk index, haugh unit and yolk color. The highest egg weight, yolk weight, shell weight and yolk ratio were found at 65 wks of age. Our findings were in accordance with (Yılmaz Dikmen *et al*., 2017).

The lowest albumen weight was at 45 wks of age, and albumen ratio, albumen index, and haugh unit were at 65 wks of age. These finding agree with (Yılmaz Dikmen *et al*., 2017) who found that some albumen traits decrease in late flock age. Also, Riczu *et al*. (2004) suggested that shell quality traits decreased with flock age, while only eggshell weight increased. But, in the study, the lowest shell thickness was at 55 weeks of age, the shell ratio was at 35 and 55 weeks of age, and the highest SBS was at 45 weeks of age. In the study, the highest yolk color was at 45 weeks of age, and the lowest shape and yolk index were at 65 weeks of age. It is thought that the differences in egg quality traits with age may be due to the seasonal changes in production systems.

In the study, housing system influenced egg and albumen weight, ratio of albumen and yolk, SBS, index of albumen and yolk, and haugh unit of layers. Several research (Zemková *et al*., 2007; Yılmaz Dikmen *et al*., 2017) demonstrated that eggs were heavier in litter and FR systems than in cages. Also, some research demonstrated that eggs heavier in deep litter system than different vegetated FR system (Tainika *et al*., 2024). In other research, egg weight was higher in cage systems than in floor or FR systems (Leyendecker *et al*., 2001; Erek and Matur, 2024). Similarly, in the study, the highest egg weight was found in EC system. Yılmaz Dikmen *et al*. (2017) found that eggshell weight, yolk weight, albumen weight, albumen index, and Haugh unit were greater in the FR system compared to cage systems. However, in the study the highest albumen weight, albumen ratio, yolk index and lowest yolk ratio were in EC system. Also, according to earlier reports, conventional cages had a greater Haugh unit value than other systems (Ahammed *et al*., 2014; Samiullah *et al*., 2014). Similarly, in the study, albumen index and haugh unit were higher in the CC system but were similar in the FR and EC system. Some research suggests that different rearing systems have varying effects on shell traits of egg, thus Samiullah *et al*. (2014) demonstrated that shell weight, shell ratio, and shell thickness of eggs were heavier in traditional cage systems than in free range systems. In contrast, Erek and Matur (2024) demonstrated that shell weight was heavier in furnished cages than free range system. Tainika *et al*. (2024) reported that higher shell-breaking strength and thickness in free access to vegetated environments outdoor compared to deep litter system. But some studies reported no significant differences between housing systems in terms of shell thickness (Van Den Brand *et al*., 2004), shell breaking strength, and shell ratio (Yılmaz Dikmen *et al*., 2017). However, in the study higher SBS was in FR system, but it was lower in the CC system, and no differences were found between housing systems for shell weight, shell ratio and shell thickness. The housing system effect on yolk color was reported by several studies; thus, Samiullah *et al*. (2014) reported that dark color yolk was in the cage system, but Şekeroğlu *et al*. (2010) and Yılmaz Dikmen *et al*. (2017) reported that yolk color was not affected by the rearing system.

Thus, in the study there were no differences between housing systems for yolk color. During the study, the outdoor vegetation of the free-range system was exposed to seasonal changes, and it can be thought that the lack of difference in yolk color in housing systems is due to the use of the same feed in all systems. In various researches suggests that different rearing systems have varying effects on egg shape index, thus Şekeroğlu *et al*. (2010) reported that egg shape index was higher in cage systems than others, but Yılmaz Dikmen *et al*. (2017) reported that egg shape index was higher in free range systems than others, but Stojčić *et al*. (2012) and Ahammed *et al*. (2014) reported that egg shape index did not affect by housing system. Similarly, in the study there were no differences between housing systems for shape index. It is thought that the differences between our research findings and the findings of other studies may be due to the differences in the genotypes used and exposure to different seasonal changes in open production production systems.

The weights and ratios of the eggshell, albumen, and yolk vary according to the commercial genotype that produces the egg (Johnston, 2007). Rakonjac *et al*. (2021) investigated the effect of rearing systems (floor vs organic) and genotypes (Isa Brown vs New Hampshire) on egg quality, reported that egg weight, Haugh unit, albumen height, proportions of albumen, yolk and shell, shell thickness and breaking strength, and egg shape index affected by genotype of layers. Tainika *et al*. (2024) investigated the effect of rearing systems (free access to vegetated environments outdoor vs deep litter system) and genotypes (Lohmann Sandy vs Lohmann LSL Classic) on egg quality, reported that shell thickness, Haugh unit, shape index, albumen height and index, yolk index and color affected by genotype of layers. Thus, in the study, albumen, shell and yolk weight, albumen, yolk and shell ratio, SBS, shell thickness, shape index, albumen index, haugh unit and yolk color were affected by the layer's genotype. Aygün *et al*. (2025) reported that Lohmann Brown genotype had darker yolk color than Lohmann Sandy and ATAK-S genotypes. However, in the study, yolk color was lighter in the LW genotype but similar in the LS and LB genotypes. The SBS was found higher in LB but was similar in LS and LW genotypes. In contrast to our findings Aygün *et al*. (2025) who investigated performance of different genotype layers reared in free-range system, reported that Lohmann Sandy genotype demonstrated stronger resilience to egg breakage than Lohmann Brown and ATAK-S genotypes. And Tainika *et al*. (2024), reported that there was no difference in shell breaking strength of layer genotypes (Lohmann Sandy vs Lohmann LSL Classic). Also, in the study ST was found higher in the LB genotype but was lower in the LS genotype. In contrast to our findings Tainika *et al*. (2024), found that Lohmann Sandy eggs had higher shell thickness than Lohmann LSL Classic. In addition, Tainika *et al*. (2024), reported that Lohmann Sandy eggs had higher shape index, yolk index, and yolk color, but Lohmann LSL Classic eggs had greater albumen height, albumen index, Haugh unit. The highest albumen quality in free range reared Lohmann Brown genotype was reported by Aygün *et al*. (2025). But in the study, a higher albumen index, haugh unit and lower albumen weight and ratio, and shape index was found in the LW genotype. The highest yolk weight and yolk ratio was in LW genotype. The shell weight and shell ratio were higher in the LB genotype but lower in the LS genotype. However, Tutkun *et al*. (2018) whom compared to the egg quality traits of free range reared Lohmann Brown and Atak-S genotypes, reported that egg quality traits were similar between the genotypes. It is thought that the differences between our research findings and the results of other researches might be due to the differences in the genotypes used in the studies. The rearing system and flock age interactions effect on egg weight, albumen height and eggshell content was reported by (Van Den Brand *et al*., 2004) and egg weight, shell thickness, shell weight, shell ratio, haugh unit, albumen height and yolk color was reported by (Samiullah *et al*., 2014). Thus, in the study, there were interactions between age and housing system on egg weight, albumen weight, albumen index and haugh unit. Similarly, Yılmaz Dikmen *et al*. (2017) reported that there were housing system and hen age interaction effect on these egg quality traits. In the study, there were interactions between age and genotype on egg weight, albumen weight, yolk weight and ratio, shell weight, index and color of yolk. Also, interactions between rearing system and genotype of layers for egg weight, the proportions of albumen and shell, albumen height, haugh unit, shell thickness, and shell breaking strength was reported by (Rakonjac *et al*., 2021). Thus, in the study there were housing system and genotype interaction was on egg weight, albumen weight, yolk ratio and yolk color. The interactions between flock age, strain, and rearing systems on yolk and albumen weight, albumen height, and yolk color was reported by (Singh *et al*., 2009). The interactions between age, housing system and hen genotype on yolk index and yolk color was reported by (Tainika *et al*., 2024). Similarly, in the study, there was interaction between age, housing system and genotype on yolk index, haugh unit and yolk color. At 45 weeks of age, CC-raised LW layers had the greatest yolk index, LS layers had the largest haugh unit and LB layers had the highest yolk color, and also EC-raised LS and LB layers had the highest yolk color. But, at 65 weeks of age CC-and FR-raised LW layers had the lowest yolk index, EC-raised LB layers had the lowest haugh unit and EC- reared LB layers had the lowest yolk color, whereas at 55 wk of age FR- raised LS layers had the lowest yolk color. However, there was no any interaction between age, housing system and genotype on some other egg quality traits. Support to our findings, no interaction effect between age, housing system and hen on egg weight, shape index, shell breaking strength, albumen ratio, yolk ratio, shell ratio, shell breaking strength was reported by (Sokolowicz *et al*., 2018) and egg weight, shell breaking strength, shape index and albumen index reported by (Tainika *et al*., 2024).

Conclusion

The age and genotype of layers influenced hen day egg production, egg mass, feed intake and FCR, but housing system affected only feed intake of layers. The age of layers influenced all investigated egg quality parameters. And housing system influenced egg weight, albumen weight and ratio, yolk ratio, shell breaking strength, index of albumen and yolk, and haugh unit. But genotype of layers affected all the egg quality parameters investigated, except for egg weight and yolk index. It can be concluded that feed intake of layers, yolk index, haugh unit and yolk color of eggs are affected by the age and genotype of layers reared in different housing systems. We believe that results of this study might contribute to researchers and breeders looking for commercial genotypes for rearing in various housing systems.

Ethical Statement

This study was approved by the Bursa Uludağ University Animal Experiments Local Ethics Committee (Approval no: 2023-05/01).

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PROOF
RESEARCHPAPER

POULTRY STUDIES

Seasonal Impacts on Thermal Comfort and Growth Performance of Broilers in Commercial Conditions

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Abstract

This study was conducted on 12 broiler flocks (Ross 308) reared during the spring and autumn seasons on commercial farms in Bursa and its districts, in a contracted production model under an integrator firm. Broiler performance parameters, including final body weight, cumulative feed and water intake, and feed conversion ratio (FCR), along with environmental parameters such as temperature, relative humidity (RH), and CO_2 concentration, were monitored over a 42-day growing period. The Temperature-Humidity Index (THI) was calculated using average temperature and RH data. While body weight and feed intake remained consistent across seasons, FCR was more efficient in spring (1.38 vs. 1.53, $P<0.05$). The back-side temperature was higher in the spring than in the autumn (28.8ºC vs. 27.9ºC, $P<0.001$). The back size THI with a value of 80.1 was found to be higher in the spring compared to the autumn (78.4, $P<0.01$). The CO_2 concentration was found to be higher in the autumn (1801.1 ppm) than the spring (1415.1 ppm, $P<0.001$). These findings clearly showed that the season affects economic performance through FCR. Investigating the potential positive or negative effects of the seasons could provide crucial insights for developing new strategies to optimize flock management and improve welfare standards in broiler production.

Introduction

The investigation of thermal comfort and growth performance of broilers has garnered significant attention in the poultry industry, reflecting the critical role these factors play in optimizing production efficiency and animal welfare. Thermal comfort is a critical factor influencing the growth performance, welfare, and overall productivity of broilers in commercial poultry production. Broilers are particularly sensitive to environmental temperatures, and maintaining optimal thermal conditions is essential for their growth, feed efficiency, and health (Lara and Rostagno, 2013). The thermal comfort zone, typically between 18-22°C, supports optimal metabolic activity and growth rates, while deviations can lead to heat or cold stress, impacting growth performance (Sahin *et al.,* 2018). Heat stress, often encountered in commercial operations, disrupts metabolic processes, reduces feed intake, and impairs weight gain, leading to significant economic losses (Azad *et al.,* 2010).

The growth performance of broilers is highly dependent on maintaining a stable thermal environment. Proper management strategies, such as adequate ventilation, temperature control, and humidity regulation, are essential to optimize broiler performance and welfare (Nawab *et al*., 2018). A previous research shows that temperature management directly affects physiological responses, nutrient metabolism, and immune function, highlighting the importance of environmental control in broiler production systems (Lin *et al*., 2006). Therefore, understanding and managing

thermal comfort are crucial for enhancing growth performance and ensuring sustainable and efficient poultry production. The thermal comfort and growth performance of broilers are critical factors influencing poultry production, particularly under commercial conditions where seasonal variations can significantly impact these parameters. Thermal comfort refers to the state where the environmental conditions allow birds to maintain their body temperature without excessive metabolic energy expenditure, which is crucial for optimal growth and welfare (Dedousi *et al.,* 2023; Franco and Alencar, 2022). As broilers are particularly susceptible to heat stress, understanding the interplay between seasonal changes and thermal comfort is essential for enhancing their performance and overall health (Purswell *et al*., 2012; Akter *et al.,* 2022).

A Previous research indicates that environmental factors such as temperature, humidity, and air velocity play a pivotal role in determining the thermal comfort of broilers (Akter *et al.*, 2022; Teles *et al.,* 2020). For instance, the Temperature-Humidity Index (THI) is commonly used to assess the comfort level of poultry, with higher THI values correlating with increased heat stress and reduced growth performance (Dedousi *et al.,* 2023; Purswell *et al.,* 2012). Studies have shown that broilers exposed to high THI conditions exhibit decreased feed intake and growth rates, highlighting the importance of maintaining optimal environmental conditions throughout different seasons (Dedousi *et al.,* 2023; Purswell *et al*., 2012). Furthermore, the design and management of poultry housing, including ventilation systems and insulation, are critical in mitigating the adverse effects of seasonal temperature fluctuations (Curi *et al*., 2017; Abreu *et al.*, 2011). The literature also emphasizes the importance of environmental management in maintaining thermal comfort for broilers. A Previous research has highlighted the effectiveness of climate control systems in poultry houses, which are crucial for sustaining optimal thermal conditions and minimizing stress during extreme weather (Teles *et al.,* 2020; Vieira *et al.,* 2010). Furthermore, studies have demonstrated that maintaining appropriate humidity levels and air circulation can significantly enhance the thermal comfort of broilers, leading to improved growth outcomes (Dedousi *et al.*, 2023). Moreover, dietary interventions have been explored as a means to enhance broiler resilience to thermal stress. For example, incorporating specific feed additives or adjusting protein levels in diets can improve growth performance and thermal acclimatization in heat-stressed broilers (Popoola *et al.,* 2020; Popoola *et al.,* 2021). The nutritional strategies employed during critical growth phases can significantly influence the birds' ability to cope with seasonal temperature extremes, thereby optimizing their overall productivity (Dedousi *et al.,* 2023).

The aim of this study was to assess the performance and environmental conditions of commercial broiler farms located in Bursa region. This study monitored a total of 12 broiler flocks during the Spring (March, April, May) and Autumn (September, October, November) seasons of 2021-2022, with 6 broiler flocks observed per season. Key performance metrics, such as final body weight, feed intake, water intake, and feed conversion ratio (FCR) were evaluated.

Material and Method

The current study was carried out on commercial broiler farms located in Bursa and its districts, following a contracted production model under an integrator firm. A total of 12 broiler flocks (Ross 308) were monitored in the same farm with two broiler houses (H1 and H2, Figure 1) during spring (March, April, May) and autumn (September, October, November) seasons in 2021-2022 (n: 6 broiler flocks/season). Poultry houses are fully environmentally controlled and PET panels are used for ventilation along with tunnel-type fans, ensuring negative pressure ventilation in the broiler houses. The characteristics of the broiler houses where the study was conducted are given in Table 1.

All flocks were kept in littered houses covered with rice hulls and equipped with automatic ventilation, heating, drinkers, and feeders. The production data including number of birds, live bird per m^2 and live weight per m^2 was given in Table 2. The management practices, including feeding (65-70 birds per pan feeder) and drinking (8 drinkers per 1000 birds) vaccination (Newcastle and infectious bronchitis on day 10, Gumboro on day 15, Newcastle on day 21), ventilation, stocking density, lighting (intermittent lighting schedule with 4 hours dark period) and health protection, were applied according to the management guide provided by breeding company (Aviagen, 2020). Each flock was fed commercial diets with similar composition, as feed formulation was standardized by the company.

Figure 1. The location of the broiler houses (Bursa, Turkey)

Tablo 1. General characteristics of the broiler houses

House	Breeding Type	Number of birds	Direction	House width (m)	House Length (m)
H1	Broiler	46000	N-S	32	80
H2	Broiler	46000	N-S	32	80

Tablo 2. Production information

House	Number of birds	Live bird per m^2	Live weight per m^2
Autumn 1	44000	17.19	49.45
Autumn 2	42080	16.44	46.73
Autumn 3	45950	17.95	50.83
Spring 1	42080	16.41	42.57
Spring 2	44000	17.19	54.19
Spring 3	44000	17.19	47.71

The birds were provided with commercial diets ad libitum: starter (between 1-10 days, 23% CP and 3000 kcal ME/kg), grower (between 11-21 days, 22% CP and 3100 kcal ME/kg), and finisher (between 21-42 days, 20.5% CP and 3200 kcal ME/kg).

Performance data included the final body weight, total feed intake, water intake and feed conversion rate (FCR). The final body weight was calculated as the averages of 10% of the flocks with random samples. All flocks were monitored using a computerized controlling system for feed and water consumption, with feed and water consumption were calculated as a ratio between feed or water consumption and the number of birds. FCR was expressed as the ratio between cumulative feed intake and body weight gain.

The temperature and relative humidity of the houses were recorded hourly and then presented as averages for each growing cycle using an automatic data logger (Testo 174H Data Logger, Pennsylvania, USA) throughout the growing period. Based on the measurements, Temperature-humidity Indices (THI) were calculated for each hour according to the equation described by Berman et al. (2016):

$$THI=3.43+1.058 \times T- 0.293 \times RH + 0.0164 \times T\times RH+35.7$$

where; T is temperature and RH is the relative humidity. The outdoor temperature data of the research area was obtained from the website "Weatherspark" (Anonymous, 2024). Figure 2 shows the outdoor temperature data at the location of the broiler houses.

The CO_2 concentrations were monitored hourly using an INNOVA 1314i photoacoustic multi-gas monitor (LumaSense Technologies A/S, Ballerup, Denmark). The data was given as daily average value of CO_2 concentration for each house.

A completely randomized block design was used to evaluate the effects of season in broiler production. The effects of season (autumn vs. spring) on thermal comfort and growth performance were analyzed with the t-test procedure in the statistical analysis software Minitab 17. Analyses of percentage data were conducted after arcsine square root transformation of the data. Differences were considered statistically significant at $P \leq 0.05$.

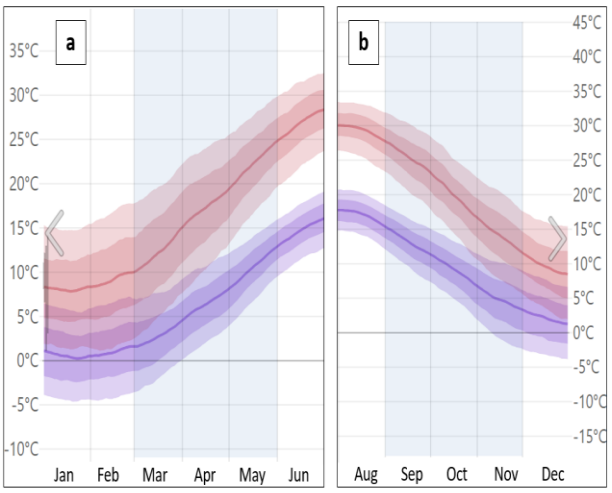

Figure 2. Outdoor temperature data at the location of broiler houses during spring and autumn seasons

Results

The temperature, RH and THI of front and back side of houses is shown in the Table 3. The findings indicate that while the temperature on the front side of the houses remained consistent across seasons, the back side exhibited a significant increase in temperature during spring compared to autumn (28.8ºC vs. 27.9ºC, $P<0.001$). This difference may be attributed to variations in solar exposure and ventilation dynamics, which could affect the microclimate within poultry houses (Onagbesan *et al.*, 2023). The higher temperatures in spring could lead to increased metabolic rates in broilers, potentially impacting their growth performance and FCR value (Wasti *et al.,* 2020). Relative humidity levels were found to be similar between the seasonal groups on both the front and back sides of the houses ($P>0.05$). This stability in humidity is crucial as

excessive humidity can exacerbate heat stress in broilers, leading to decreased feed intake and growth performance (Qiu *et al.,* 2023).

The THI, which is a critical indicator of heat stress, was significantly higher in the back side during spring (80.1%) compared to autumn (78.4%, P<0.01). Elevated THI levels can negatively affect broiler welfare and performance, as they indicate a higher risk of heat stress, which has been shown to impair growth rates and feed efficiency (Liu *et al*., 2020). In the study, a small increase in THI for spring was observed, but this did not cause an effect for performance parameters. Quintana-Ospina *et al*. (2023) found any significant variation by changes in THI commercial tropical conditions.

CO_2 concentrations were notably higher in the autumn (1801.1 ppm) than in the spring (1415.1 ppm, P<0.001). This finding suggests that ventilation practices may differ between seasons, potentially leading to increased accumulation of CO_2 in the autumn months. Elevated CO_2 levels can have detrimental effects on broiler health and performance, as they may lead to respiratory issues and reduced oxygen availability, ultimately affecting growth and feed conversion ratios (Varol *et al.,* 2018). Moreover, the relationship between CO_2 levels and overall air quality in broiler houses underscores the importance of effective ventilation systems to maintain optimal environmental conditions for poultry (Khan *et al.,* 2023).

The seasonal variations in temperature, humidity, THI, and CO_2 concentrations within broiler houses highlight the need for careful management of environmental conditions to optimize broiler performance. The significant differences observed in temperature and CO_2 levels between seasons suggest that poultry producers should implement strategies to enhance ventilation and mitigate heat stress, particularly during warmer months. Future research should focus on developing adaptive management practices that can effectively address the challenges posed by seasonal changes in broiler housing environments. The effects of season on broiler performance parameters is shown on Table 4. The findings indicate that broilers raised in both autumn and spring exhibited similar final body weights at 42 days of age, with values of 2850.7 g and 2841.0 g, respectively (P=0.96). This suggests that seasonal variations in temperature and humidity may not significantly impact the overall growth potential of broilers during these periods, aligning with previous research that indicates environmental factors like seasonality can influence poultry performance but may not always lead to drastic differences in weight gain (Singh *et al*., 2018).

The current finding is consistent with previous studies reporting any significant differences for body weights of broilers reared in winter or summer period (Nembilwi *et al.,* 2002; Thirumalesh *et al.,* 2012). Although the study was conducted in different seasons, the similarity in final body weight between autumn and spring seasons could be explained by the effective environmental control in the poultry house. Nowadays, the use of environmentally controlled and full automated poultry houses in broiler production could minimize the negative effects of the season on performance. Current findings demonstrated that the management conditions, especially ventilation and cooling systems, had effective for maintaining a suitable environment during spring by providing good indoor air and litter quality. The mean value of feed and water intake were found to be similar between the seasons (P>0.05). However, the feed intake tended to decline in spring period compared to the autumn, with a difference of 352.7 g in the experiment. The decrease in feed intake observed during the spring season caused a significant improvement in FCR.

Table 3. The temperature, relative humidity, and THI of front and back side of the broiler houses

Season	Temperature (°C)		RH (%)		THI		CO_2
	Front	Back	Front	Back	Front	Back	
Spring	28.0	28.8	63.3	59.7	79.1	80.1	1415.1
Autumn	28.2	27.9	62.9	59.3	79.4	78.4	1801.1
SEM	0.07	0.08	0.27	0.28	0.17	0.18	33.4
P value	<0.07	<0.0001**	<0.215	<0.368	<0.19	<0.0001**	<0.0001**

*: P<0.05, **: P<0.01

Table 4. The effect of season on broiler performance parameters

Performance parameter	Season		SEM	P-value
	Autumn	Spring		
Final body weight (FBW) (g/bird)	2850.7	2841.0	121.8	0.96
Total feed intake (TFI) (g/bird)	5181.4	4828.7	263.6	0.44
Water intake (L/bird)	6.58	6.46	1.42	0.96
FCR (feed:gain)	1.53	1.38	0.02	0.03*

*: P<0.05, **: P<0.01

In previous studies, heat stress during spring and summer seasons could cause heat stress and subsequently harmful effects on feed intake (Liu *et al.,* 2020; Youssef *et al.,* 2021). It is thought that the variability in the effects of the season on broiler performance parameters among studies might be related to many management conditions and feeding principles, such as the season, housing conditions, flock health, and feed composition.

In contrast, the FCR was significantly better in broilers raised in spring compared to those in autumn (1.38 vs. 1.53, P=0.03). This finding highlights the importance of seasonal conditions on feed efficiency, suggesting that broilers in spring may experience more favorable metabolic conditions or feed quality that enhances their ability to convert feed into body weight effectively. Research has indicated that environmental stressors, such as heat during summer months, can negatively impact FCR by increasing maintenance energy requirements and reducing feed efficiency (Suryadi *et al*., 2021). The observed differences in FCR between seasons may also reflect variations in nutrient availability or the physiological responses of broilers to seasonal changes, which can influence their growth efficiency (Aggrey *et al.*, 2010).

Conclusion

This study evaluated the performance and environmental conditions of commercial broiler farms in Bursa, monitoring 12 broiler flocks during spring and autumn. Performance parameters such as body weight, feed and water intake, and FCR were assessed alongside environmental factors like temperature, humidity, THI and CO_2 levels. The findings indicate that while growth performance remained similar between seasons, feed conversion ratios were better in spring, likely due to more favorable conditions. The study highlights the importance of managing environmental variables like temperature, CO_2 levels, and ventilation to optimize broiler performance across seasons.

Ethical Statement

The ethical approval is not required for this study. This is a study field performed by standard free-range process. Any animal was suffered during this study.

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PROOF

REVİEW

Blue-Breasted Quail (*Synoicus chinensis*): Characteristics, Breeding Techniques and Research

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Abstract

The blue-breasted quail is a socially monogamous, sexually dimorphic and precocial species pan-Asian, classified in the order Galliformes and family Phasianidae. There are different names for the blue-breasted quail in the scientific literature, so it is easily be confused that these names refer to the same species, and recently the scientific name *Synoicus chinensis* has been used. The main advantages include easy rearing in small areas, small body size, hardiness, high egg-laying performance, short generation interval and sex determination after the fourth week of life. In addition, blue-breasted quail are smaller than Japanese quail and still retain species-specific behavioural traits even after domestication. The blue-breasted quail was proposed as a model animal nearly three decades ago and has been used for more than two decades, mainly in zoology, animal genetics, heredity and immunology research. It is also worth noting that the blue-breasted quail is a widespread used parthenogenesis model. This study compiles scientific research on the blue-breasted quail and presents this information under physiological, morphological and behavioural characteristics, including information on the breeding techniques of the species. The studies show that there is growing recognition of the significance of blue-breasted quail in research laboratories and that their position is likely to be strengthened in the future.

Introduction

Quails are divided phylogenetically into two groups, The Old-World quail belongs to the Phasianidae (including several genera representing Old World species) and distributes in the Palaearctic region (Europe, North Africa and Asia), while the New World quail classified as the Odontophoridae is restricted to North and South America (Shibusawa *et al*. 2004; Lukanov, 2019). Among Galliformes, 38 species are named "quails"; 14 species are classified as Phasianidae, comprising 5 genera, and 24 as Odontophoridae, comprising 7 genera (Nishibori *et al.*, 2002). Synoicus chinensis (blue-breasted quail) is classically classified in the order Galliformes and family Phasianidae on the basis of morphological characteristics and cytogenetic data and biochemical evidence, as are chicken and Japanese quail (Shibusawa *et al*. 2004; Nishibori *et al.,* 2002). The blue-breasted quail is the smallest species of the order Galliformes (Tsudzuki, 1994) and has even been described as dwarf (Prinzinger *et al*., 1993) and miniature (Landry, 2015).

The blue-breasted quail is a geographic specie of pan-Asian (Roberts and Baudinette, 1984), originating from India, Southeast Asia and the southeastern region of China (Pis and Luśnia, 2005; Nakamura *et al.,* 2019'a). Despite the fact that this quail is thought to have always been widespread in Asia, it has not been recorded in Sumatra in recent times, and there are 40 % fewer areas recorded in the sub-region than before 1970 (Mcgowan

and Gillman, 1997). On the Australian mainland, the species is restricted to the mesic coastal belt around eastern Australia and the coastal Northern Territory (Pearson, 1994). Blue-breasted quail is among the non-migratory species living in these regions (Saini *et al*, 2019). With the exception of the Japanese quail (*Coturnix coturnix japonica*), it is the most widely raised quail species and is relatively easy to obtain from commercial suppliers in Europe (Pis and Luśnia, 2005).

The blue-breasted quail is also called "button quail" in the USA, "King quail" in Australia and "Chinese painted quail" in Europe. Other common names are Indian blue quail, Asian blue-breasted quail and blue quail (Ono *et al*., 2005). The three Latin binominal names of this species are chronologically *Excalfactoria chinensis* (Tsudzuki, 1995'a), *Coturnix chinensis* (Shibusawa *et al*. 2004) and *Synoicus chinensis* (Adkins-Regan, 2016; Zi *et al.*, 2023).

Its major advantages include easy rearing in small areas, small body size, durability, high spawning performance, short generation interval and sex discrimination after the fourth week (Wei *et al.,* 2011a; Nishibori *et al*., 2002; Tsudzuki, 1994; Kageyama *et al*., 2018; Nakamura *et al*., 2019b). In addition, blue-breasted quail are smaller than Japanese quail (Sarkadi *et al*., 2013) and have retained species-specific behavioural traits even after domestication (Hickman, 1984), but have not been selected for any traits (Pearson *et al*., 1998). Hence, the benefits of using blue-breasted quail as an avian model for research are the reduction of costs, area and labour required for their care (Nakamura *et al.,* 2019a), as well as their suitability for improvement due to the variation of their biological traits. Monogamous housing leads to more systematic searching and less aggressive behaviour (MacDonald, 2010). Due to these advantages, blue-breasted quail have been used for over 20 years in numerous disciplines including developmental biology, genetics, reproduction, behaviour and immunology (Nakamura et al., 2019b). Besides, because of its small size and variety of feather colours, the blue-breasted quail is occasionally bred as a pet (Kageyama *et al*., 2018).

Although the Japanese quail is the most studied domestic quail in scientific research, the blue-breasted quail was proposed as a potential model animal about three decades ago (Tsudzuki, 1994). Subsequently, the complete mitochondrial (mt) genome of the blue-breasted quail was sequenced for phylogenetic analysis, confirming its close relation to the Japanese quail (Nishibori *et al.*, 2002). A phylogenetic analysis of avian and some mammalian β-defensin-9 (BDF9) sequences showed that blue-breasted quail AvBD9 clusters with chicken and duck AvBD9s (97% homology) (Wang *et al.,* 2010). Another study found that blue-breasted quail and chicken share highly repetitive microchromosome-specific sequences from a common ancestor, forming key components of chromosomal heterochromatin in Galliformes (Yamada *et al*., 2002).

The aim of this review is to compile information on the characteristics and breeding techniques of blue-breasted quail and to analyse the status of blue-breasted quail in scientific research.

Morphological and physiological characters

The morphological characteristics of birds offer valuable insights into their physiology and behaviour (Vatsalya and Arora, 2011).The blue-breasted quail is a sexually dichromatic bird (Hickman, 1984) and with the exception of monochromatic white and some tuxedo plumage mutations, sex can be determined at 4 (Wei *et al*., 2011a; Parker *et al*., 2014; Parker *et al.,* 2017) and/or 5 weeks of age by the presence or absence of male secondary sexual characteristics in feather pattern and colour (Andersson *et al*., 2004; Landry, 2015).

As shown in Figure 1, the wild-type plumage of the male, from which the species takes one of its names, is quite striking and has a slate blue-grey breast, a dark rust to chestnut-red abdomen with a slate blue-grey throat marking bordered by a black and white throat marking bordered by a black stripe called a badge (Uller *et al*., 2005), while the wild female has dulled and rusty brown plumage. The whole pattern is in an anti-predatory plumage structure designed to be least noticeable when viewed from above and is surprisingly effective at concealing the bird. Black beak, yellow to orange leg and short dark brown tail are common to both males and females (Harrison, 1965). Nevertheless, the colour of the leg and toe are rubbery yellow in egg-laying females and more orange in males.

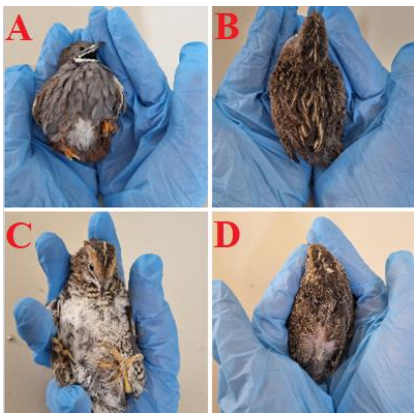

Figure 1. Wild feather color of male and female blue-breasted quails (A and B: top and bottom views of male; C and D: top and bottom views of female)

The blue-breasted quail is characterized by social monogamy and strong pair bonds, with ongoing research examining the role of male coloration in pair behaviour (Adkins-Regan, 2016). While no paternal care has been documented, badge size -a secondary sexual trait- has been positively correlated with male mating success, either due to female mate preference

or male coercion. Additionally, females mating with males possessing larger badges produced significantly larger eggs, suggesting badge size may serve as an indicator of genetic quality or confer material benefits such as courtship feeding (tidbitting) or protection (Uller *et al*., 2005). Furthermore, an investigation into the relationship between adult sexual characteristics and juvenile immune function found no association between badge size and testis traits. However, a significant negative correlation was observed between juvenile immune response and adult badge size (r=-0.54, P=0.0014), indicating potential trade-offs between ornamentation and immune function (Uller *et al.,* 2006). Linear growth in blue-breasted quail to adulthood occurs between 4 and 5 weeks of age, with the growth period divided into two stages: weeks 1-4 and weeks 5-8 (Wei *et al.*, 2011a). At 8 weeks, the increase in body weight in females was described to be due to the rapid development of the ovaries and oviducts in preparation for the onset of ovulation (Wei *et al*., 2011a). Adult blue-breasted quail, on the other hand, show a marked reversed sexual dimorphism in body size (females are slightly larger than males) (Zi *et al.,* 2023). The gonadal development of blue-breasted quail at 7-8 days does not allow for 100% accurate sex determination (Bautista *et al*., 2021). However, the melting curve analysis-based real-time PCR method, applied to feather samples for the first time, proved reliable for sex determination (Chen *et al*., 2012). The morphological and developmental traits of the blue-breasted quail are summarized in Table 1.

The thermal neutral zone for adult, blue-breasted quail (47.2±1.2 g) is between 28-35°C, with an average body temperature of 41.7°C. Body temperature increases slightly above 20°C, with variability at lower temperatures (Roberts and Baudinette, 1986). At temperatures up to 40°C, quails can dissipate 75% of heat via evaporation, compared to 20% at thermal neutrality (Roberts and Baudinette, 1986). Breathing frequency in the thermal neutral zone is 50-60/min, with no sex differences in metabolic or respiratory rates (Prinzinger *et al*., 1993). No mortality occurred in quails exposed to 20-50°C, with a maximum body temperature of 46°C at 40.9°C (Roberts and Baudinette, 1988). Skin evaporation surpassed respiratory evaporation in 0-3-day-old, 24-28-day-old, and adult quail at 25-35°C, with higher rates in young quail (Roberts and Baudinette, 1988). Despite males having lower body mass (44.5-45.8 g) compared to females (58.7-63.8 g), no significant difference was found in body temperature (40.7±0.18°C) (Bautista *et al.,* 2021).

Blue-breasted quail chicks were partially endothermic between days 2-10, maintaining high body temperature at ∼13 g body mass, and exhibited shallow nocturnal torpor with >40% decrease in metabolism for 4-5 hours on days 14 and 17 (Aharon-Rotman *et al*., 2020). Poikilothermia in 3 g chicks transitioned to homeothermia by 25 g at 28 days (Bernstein, 1973), with a key development period between days 10-16 (Pis and Luśnia, 2005). Hatchling oxygen consumption was 214.8 ± 36.0 mL O2 day-1 (4.03 g) (Pearson, 1999), with brooded chicks maintaining constant consumption at 10-35°C, while un-brooded chicks consumed twice as much oxygen at temperatures below 30°C (Pearson, 1994). Weight-specific oxygen consumption was higher in 24–28-day-old quail than in younger or older birds at 25°C, with increased consumption at 35°C during puberty (Bernstein, 1971).

The metabolic and respiratory physiology of blue-breasted quail exposed to low (800 PAH ng/g food) and high (2,400 PAH ng/g food) crude oil doses was evaluated. Results indicated that neither sex nor exposure levels influenced resting O_2 consumption or CO_2 production, which were both significantly correlated with body mass, but not body temperature. Resting O_2 consumption ranged from 50–60 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$, while CO_2 production varied between 28.9 and 41.5 mL/kg/min (Bautista *et al*., 2021). Moreover, minute ventilation and breathing frequency showed no significant differences between the experimental groups or sexes (Bautista *et al*., 2021).

Following the first week post-hatching, the heart rate (fH) of blue-breasted quail was maintained at elevated levels (550–650 beats per minute), subsequently decreasing with age and increased body mass. Notably, the maximum heart rate observed in quail chicks exhibited a more pronounced post-hatching increase in fH compared to larger precocial species, such as chickens. This observed difference can be attributed to the heightened thermoregulatory demands associated with the relatively smaller body mass of quails (Pearson *et al*., 1998).

The study on the water requirements of blue-breasted quails indicated that they need at least 2.3 ml of water per day, which corresponds to 5.8±1.0% of their body weight, with an ad-libitum water consumption rate of 9.8 (BW day^{-1} %) (Roberts and Baudinette, 1984). The study examining the effects of leptin on risk-taking and feeding behavior in blue-breasted quail found that risk-taking behavior, measured by the time to start feeding, was influenced by social factors and the bird's weight, but not leptin treatment. Focal birds with leptin-treated mates took longer to begin feeding than those with control mates Additionally, leptin-treated focal birds spent less time feeding than controls (Lõhmus and Sundström, 2004). In another study, leptin-treated birds showed decreased body weight and feeding activity, while males became more active and molted more. Leptin treatment also reduced plasma cholesterol, maintained low plasma triglycerides, and had no effect on glucose levels. Furthermore, leptin-treated males stayed closer to females, and females mated with leptin-treated males took longer to lay their first eggs (Lõhmus *et al*., 2006)

Both photoperiod and social interaction can influence maternal hormone levels, impacting steroid allocation to offspring and their growth and behavior. Testosterone injection had no effect on offspring size or immune response, but testosterone-treated offspring exhibited impaired immune function compared to controls, suggesting an immunological cost of steroid allocation that may outweigh post-hatching benefits (Andersson *et al.,* 2004).

Blue-breasted quail are precocial birds, where brood patches are not necessary for incubation or brooding. Brooding behavior in adults is triggered by chicks seeking warmth, prompting adults to squat and fluff their feathers (Pearson, 1994). Additionally, the precocial nature of quails, unlike large mammals, eliminates potential indirect effects of parental behavior on offspring immunity (Saini *et al.,* 2019). In blue-breasted quail, the fertility period following male removal was found to be 9 days, with approximately 75 sperm-egg penetrations required to achieve over 95% fertility. This species exhibits a shorter fertility period compared to other galliforms and demands a higher number of sperm-egg interactions for optimal fertility. Such a requirement may lead to an excessive release of spermatozoa from the sperm-storage tubules at each ovulation, ultimately resulting in inefficient sperm storage and potentially diminishing the long-term effectiveness of sperm reserves (Ramachandran *et al.,* 2019a).

The diploid chromosome number of the blue-breasted quail is 2n = 78-80. Its G-banded karyotype closely resembles that of the Japanese quail, with notable differences in the centromere position on chromosome 1 and the banding pattern of chromosome 2. The C-banded chromosomes of the blue-breasted quail are also similar to those of the Japanese quail (Shibusawa *et al.,* 2004).

Kálmán and Sebők (2023) found that entopallial astrocytes in blue-breasted quail, chicken, Japanese quail, pigeon, and duck exhibited high levels of GFAP (glial fibrillary acidic protein) immunoreactivity, whereas the telencephalon, nidopallium, and lateral striatum displayed comparatively low levels.

Plumage color variations

Blue-breasted quail are primarily bred for their feather color, a key economic factor for breeders (Van der Zwan *et al.,* 2019), and are also used in research due to their genetic mutations (Kageyama *et al*., 2018). In addition to the wild feather form, various color mutations are bred in captivity, with the most common being the silver mutation. Other color variations include shades of white (non-albino), brown, extended brown and red-breasted, and mottled colours: silver-red-breasted, cinnamon-red-breasted, blue-face-cinnamon, golden-pearl, silver-pearl, cinnamon-pearl, fallow, ivory, ivory-pearl, slate, smoky, splash and tuxedo (Landry, 2015).

The first feather color mutation identified in blue-breasted quail is a light grey (silver) plumage, controlled by an autosomal recessive gene (*lg*), resulting in a diluted grey color instead of the wild-type dark plumage (Tsudzuki, 1995a). The second mutation, brown, is also autosomal recessive (*br*) and does not allelically relate to (Landry, 2015), and contains the p. Pro292Leu mutation, likely affecting feather pattern and color distribution (Araguas *et al*., 2018). Newly hatched chicks with light grey plumage exhibit a greyish-creamy yellow base color with the same stripe pattern as the wild type, though the surface is significantly diluted (Tsudzuki, 1995a). In brown-feathered chicks, the base color is a greenish creamy yellow with a bright brown tint, and the stripes are lighter brown compared to the wild type (Tsudzuki, 1995b). Red-breasted chicks, identifiable by their yellow striped dorsal markings and light yellow abdomen, differ from others after hatching (Landry, 2015). Upon reaching maturity, female red-breasted quails are lighter brown, while males develop a completely blackened face and an elongated red patch on the ventral side (Araguas *et al*., 2018).

Egg and hatchling characters

Precocial species, such as blue-breasted quail, which are feathered, mobile, and capable of thermoregulation immediately after hatching (Aharon-Rotman *et al*., 2020), tend to lay larger eggs relative to the female's body mass. This is to support a higher degree of physiological maturity at hatching compared to less active, altricial species. Furthermore, these species invest more energy into their eggs, with a greater proportion of yolk (the primary energy source) and less water (Pearson, 1999). The ratio of egg mass to female body mass varies, ranging from approximately 8.7% (5.33 g per 61.51 g) (Pis and Luśnia, 2005) to around 10% (5.96 g per 55.89 g) (Tsudzuki, 1994). It has also been observed that the primary components of albumin are similar across chicken, Japanese quail, and blue-breasted quail (Ono *et al*., 2005). Additionally, energy values for the egg, yolk-free hatchling, spare yolk, yolk-free hatchling yield, spare yolk ratio, and the cost of development from egg to hatchling were reported as 35.75, 16.74, 6, 49.5, 16.6, and 13.86 kJ, respectively (Pearson, 1999). As shown in Table 2, the egg and hatchling characteristics of the blue-breasted quail are detailed.

Behaviour

Over four decades ago, the blue-breasted quail was identified as a promising candidate for use as a standard animal model in various behavioral observations and experiments, particularly in ethology laboratories (Schleidt *et al*., 1984). Subsequently, it was recognized as a valuable species for studying avian mating behaviors, owing to its distinctive and easily measurable actions (Adkins-Regan, 2016). An ethogram was published that documented and illustrated the sixty most frequently observed and visually distinguishable behaviors in blue-breasted quail. These behaviors were categorized into six behavioral complexes: individual maintenance (36 behaviors), interindividual mating and courtship (8 behaviors), interindividual incubation (5 behaviors), interindividual parental (1 behavior), interindividual agonistic (7 behaviors), and interindividual miscellaneous (3 behaviors) (Schleidt *et al.,* 1984).

Table 1: Morphological and developmental traits of blue-breasted quail

Attribute	Age/Day and sex	Value (unit)/Situation	References
Chick weight	1-3 days M, F	3,48±0,343 g	Pis and Luśnia (2005)
	7-8 days M, F	8.1g	Bautista *et al*. (2021)
	7-10 days M, F	8,51±1,011 g	Pis and Luśnia (2005)
	16-19 days M, F	21,14±1,438 g	
	22 days M	27,58 g	
	22 days F	30,34 g	
Organ mass ratios	7-8 days M, F	Ratio of organ weight to body weight	Bautista *et al.* (2021)
Heart		1.2±0.04%	
Liver		4.3±0.4%	
Lungs		0.7±0.04%	
Gut		5.7±0.9%	
Eyes		2.5±0.2%	
Brain		3.3±0.2%	
Ceca		1.0±0.04%	
Kidneys		1.3±0.2%	
Sex determination	22 days	Body mass differences	Pis and Luśnia (2005)
	25 days	Exhibition the initial secondary sexual plumage	Zi *et al.* (2023)
	28-35 days	Presence or absence of male secondary sexual characteristics	Andersson *et al*. (2004)
	28 days		Tsudzuki (1994)
Adult age	65 days, M, F		Zi *et al*. (2023)
Adult weight	56-63 days	50 g	Ono *et al*. (2005)
	59 days, M	46.99 g	Pis and Luśnia (2005)
	59 days F	61.51 g	
	M, F	49.7±0.09 g	Roberts and Baudinette (1984)
	M, F	44.9±2.1 g	Roberts and Baudinette (1986)
	M, F	49.6±1.7 g	Roberts and Baudinette (1988)
	M	45–50 g	Andersson *et al.,* (2004)
	F	50–60 g	
	63 days, M, F	45.7±1.4 g	Askew and Marsh, (2001)
First laying time	Mostly 56-63, but also at 47 days, F	Age at first lay an egg	Tsudzuki, (1994)
	80 days, F		Zi *et al.*, (2023)
Body weight	112 days, M	47.66±0.52	Tsudzuki, (1994)
	112 days, F	55.89±0.75 g (without egg in uterus) 61.89±0.77 g (with egg in uterus)	Tsudzuki, (1994)
Organ mass ratios	Adult	Ratio of organ weight to body weight	Bautista *et al*., (2021)
Liver	M and F, respectively	1.84±0.10 and 3.05±0.42%	
Gut		1.77±0.09 and 2.38±0.16%	
Ceca		0.40±0.03 and 0.43±0.02%	
Kidneys		0.67±0.04 and 0.90±0.08%	
Brain		1.11±0.04 and 0.84±0.11%	
Heart		0.74±0.05 and 0.9±0.05%	
Lungs		0.87±0.11 and 0.8±0.07%	
Eyes		0.53±0.05 and 0.97±0.12%	
Kidney weight	Adult M, F	0.42±0.03 g	Roberts and Baudinette (1984)
Wing Length	Adult M, F	9.6±0.1 cm	Askew *et al.*, (2001)
Wing Span		22.0±0.3 cm	
Wing Area		97.7±1.5 cm^2	
Pectoralis mass ratio		15.0±0.6 %	
Testicular mass	Adult M	1.11±0.05 g	Uller *et al.,* (2005)
Testicular length		14±0.30 mm	

M: male and F: female

Table 2. Egg and hatchling characteristics of blue-breasted quail

Characteristic	Value(unit)/attribute	Reference
Fresh egg mass	5.7±0.5 g (n=577, range:4.0–7.5 g)	Zi *et al*., (2023)
Egg weight	5.96±0.04 g	Tsudzuki, (1994)
	5-6 g	Pearson *et al*., (1998)
	4.86 g	Pearson, (1999)
	5.33±0.458 g	Pis and Luśnia, (2005)
Egg length (major axis)	25.6±0.01 mm	Tsudzuki, (1994)
	25.0±0.012 mm	Bautista *et al*., (2021)
Egg width (minor axis)	20.5±0.01 mm	Tsudzuki, (1994)
	19.0±0.011 mm	Bautista *et al*., (2021)
Egg volume	5.2±0.6 cm^3	Zi *et al*., (2023)
	5.09±0.5 mL	Lewis and Montevecchi, (1999)
Eggshell color variations	Greyish beige, reddish beige, greenish beige, bluish beige	Tsudzuki, (1994)
Albumen mass	2.54 g (58.2% of unshell eggs)	Pearson, (1999)
Yolk mass	1.79 g (41.8% of unshell eggs)	
Eggshell mass	0.51 g	
Water content in eggshell	37.3%	
Water content in albumen	88.9%	
Water content in yolk	49.5%	
Water content in whole egg	72.6%	
Dry matter in eggshell	0.31 g	
Dry matter in albumen	0.28 g	
Dry matter in yolk	0.90 g	
Energy content in egg	35.75 kJ (8.54 kcal; 5.46 g egg)	
Hatchling weight	3.5–5 g	Pearson *et al*., (1998)
	3.84 g	Pearson, (1999)
	3.59±0.11 g	Andersson *et al*., (2004)
	3.48 g	Pis and Luśnia, (2005)
	4.19±0.15 g	Nakamura *et a*l., (2019a)
Hatchling yield	72.6%	Pearson, (1999)
Hatchling tarsus length	10.30±0.11 mm	Andersson *et al*., (2004)
Hatchling beak length	2.57±0.21 mm	Nakamura *et al*., (2019a)
Hatchling third toe length	10.73±0.45 mm	
One day-old hatchling weight	3.80 ± 0.40 g	Wei *et al*., (2011a)
Yolk-free hatchling weight	2.83 g	Pearson, (1999)
Spare yolk weight	0.42 g	
Yolk-free hatchling yield	58.6%	
Spare yolk ratio	8.5%	

Blue-breasted quail are highly social, often engaging in group activities such as eating, hiding, preening, and sleeping simultaneously. Despite their small size, they can exhibit aggression, particularly among males. As a result, males are typically housed separately or with one or more females to minimize conflict (Lõhmus and Sundström, 2004). Pairs that had to be separated due to intra-pair antagonism were excluded from analyses before the experiment's completion (Uller *et al*., 2005). While previously unmated males and females were initially avoidant or aggressive, they quickly displayed allopreening and huddling behaviors. Males that had previously mated showed aggression toward females other than their mates, while unmated males exhibited mating behavior towards familiar females. Notably, pairs were able to recognize and remember their former cohabitation partners for at least ten weeks post-separation (Adkins-Regan, 2016). Furthermore, both sexes of blue-breasted quail demonstrate clear pecking orders when introduced to a new environment, with the pecking order correlating strongly with their arrival order. When multiple quail are transferred together, they typically do not exhibit mutual hostility and share the environment peacefully. Residents tend to show negative behaviors, such as pecking, towards outsiders. However, outsiders were more likely to join a new group when fewer females were present in the resident cohort, when they were younger than the residents, or when they engaged in pecking towards the residents with less intensity (Zi *et al.,* 2023).

The maximum instantaneous and cycle-averaged skeletal muscle powers measured in the pectoralis muscle of blue-breasted quail, operating at a midcycle frequency of 23 Hz, were reported to be approximately 1200 W kg^{-1} and 400 W kg^{-1}, respectively (Askew and Marsh, 2001; Askew and Marsh, 2002). Quail exhibit typical flight behavior characterized by a rapid take-off followed by brief, intermittent flapping flights. After several flights, the birds quickly become exhausted and flightless, landing and seeking escape (Askew and Marsh, 2002). This adaptation, which facilitates high power production, may lead to muscles that are unable to sustain power due to rapid fatigue. Thus, explosive

flight in blue-breasted quail is an initial, rapid anti-predatory response (Askew et al., 2023). It was reported that only half of the blue-breasted quail subjected to the learning experiment successfully adapted to social isolation in the experimental cage and acquired the ability to open the feeder lid required for the tests, maintaining high response accuracy for 15 days. However, the quail forgot how to perform the task 45 days after the learning exercise. In contrast, stereotypic pacing and frequent calling were observed in quail that did not adapt to social isolation (Ueno and Suzuki, 2014).

It was noted that when quails fed a low-fibre diet were given a high-fibre diet, they exhibited feed sorting behaviors, which were believed to be triggered by a physiological challenge. This behavior enabled them to maintain their body condition (mass, abdominal fat mass) without any changes in the sizes of intestinal organs or gastrolith mass (Stewart and Munn, 2014).

Parthenogenesis research

The incidence of parthenogenesis (embryonic development of an unfertilized egg) in blue-breasted quail was first shown to decrease as egg production and clutch size increased, with the highest occurrence in the first egg of a clutch, which dropped by approximately half by the second egg (Parker and McDaniel, 2009). The average parthenogenesis incidence was reported to be 4.8%, with a parthenogenetic germinal disc size of 3.7 mm (Parker and McDaniel, 2009). In a subsequent study on genetic selection for parthenogenesis across five generations, it was found that selection for parthenogenesis increased both the incidence of parthenogenesis and embryonic size, but reduced egg production and the position of eggs within the clutch as the selection generations advanced (Parker *et al.,* 2010). Notably, parthenogenesis incidence nearly tripled in the fourth generation (from 4.6% to 12.5%) compared to the base generation, with hens exhibiting parthenogenesis showing a fourfold increase in the proportion of eggs with embryonic development (Parker *et al*., 2010). Additionally, a study investigating the relationship between incubational egg weight loss, eggshell quality, and parthenogenesis revealed that egg weight loss was negatively correlated with the incidence of parthenogenesis, parthenogen size, and egg storage length (r = −0.56, −0.56, and −0.24, respectively), while it was positively correlated with clutch sequence position (r = 0.32), suggesting that eggshell quality significantly affects parthenogenesis incidence in blue-breasted quail eggs (Wells *et al*., 2012). A study examining the relationship between pre-mating parthenogenesis and post-mating embryonic development and hatchability in blue-breasted quail divided females into seven groups based on their parthenogenesis incidence (0%, 10%, 20%, 30%, 40%, 50%, and more than 50%). It was found that as the incidence of parthenogenesis increased, hatchability of set eggs, hatchability of fertile eggs, and late embryonic mortality significantly decreased, while early embryonic mortality increased (Parker *et al*., 2012). Additionally, a study investigating the effects of parental and seven generations of selection on hatching outcomes, based on parthenogenesis incidence in both maternal and paternal lineages, reported that Generation 1 had the highest percentage of eggs hatched for both set eggs and fertile eggs. In contrast, the percentage of eggs hatched decreased linearly as the selection generation increased (Parker *et al.,* 2014). The same study found a linear increase in both the percentage of eggs showing possible parthenogenesis and early embryonic mortality as the selection generation advanced, while Generation 2 had the highest percentage of infertile eggs.

Furthermore, unlike early embryonic mortality, the parental generation exhibited the highest percentage of embryo mortality at both the middle and late stages of hatching (Parker *et al.,* 2014). Another study revealed that albumin from parthenogenetically developing unfertilized eggs during a 12-day incubation period had lower pH, O_2, and Cl^- concentrations, as well as a lower egg weight loss rate. In contrast, it had higher Ca^{+2}, Na^+, and CO_2 concentrations compared to albumin from non-developing unfertilized eggs (Santa Rosa *et al*., 2016a). The authors also reported that as parthenogenetic size increased, albumin pH, O_2, and Cl^- concentrations decreased, while CO_2 and Ca^{+2} concentrations increased (Santa Rosa *et al*., 2016a). In a subsequent study, it was found that in eggs from quails selected for parthenogenesis and mated after selection, parthenogenesis decreased albumin pH, O_2, and protein concentrations, while increasing Ca^{2+} and CO_2 levels compared to non-developing eggs (Santa Rosa *et al.,* 2016b). For eggs from quail mated after selection for parthenogenesis, albumin pH and O_2 were lower, while CO_2 was higher in eggs containing parthenogenetic or early-dead embryos compared to unfertilized eggs. Additionally, in terms of sperm-egg penetration, eggs classified as infertile or parthenogenetic from quail mated after selection for parthenogenesis had similar sperm-egg penetration holes as those from quail not selected for parthenogenesis, whereas fertilized eggs had only one-sixth the number of sperm-egg penetration holes (Santa Rosa *et al*., 2016b). Eggs from mated quail not selected for parthenogenesis showed 3.5 times more sperm-egg penetration holes compared to eggs from parthenogenesis-selected quail that were then mated. Thus, parthenogenesis in mated quail eggs, similar to parthenogenesis in unfertilized eggs and early embryonic death in fertilized eggs, inhibits fertility and alters albumin properties, although the parental sex responsible remains unclear (Santa Rosa *et al*., 2016b).

The investigation examined the influence of sex on egg weight, albumin pH, hatchability, and sperm-egg penetration in parthenogenetic birds by mating quail lines selected for parthenogenesis with a control line, using a factorial design with four breeding combinations. The results indicated a significant effect of dam on egg set weight, with parthenogenetic dams laying heavier

laying heavier eggs than control dams. Parthenogenetic dams and sires also exhibited lower albumin pH, shorter incubation times, and higher incidences of parthenogenesis compared to the control group. On the other hand, only sire had a significant effect on fertility and sperm-egg penetration. Parthenogenetic sires showed the highest infertility, linked to reduced sperm-egg penetration. The study concluded that both parthenogenetic dams and sires contributed to reduced reproductive performance, with low fertility attributed to the low sperm-egg penetration caused by the parthenogenetic sires (Parker *et al*., 2017). A study examining the contribution of the parthenogenetic trait to changes in egg components such as yolk, albumen, and shell weights in dams, sires, or both, used identical treatment groups and found a significant dam effect. Parthenogenetic line dams exhibited heavier total egg weight, yolk, albumen, and shell weight, a greater albumin percentage, and a higher albumin/yolk ratio compared to control line dams, while yolk percentage was higher in control line dams. This increase in egg and egg component weights due to the parthenogenetic trait was interpreted to result from altered egg passage through the oviduct, with prolonged passage through the magnum and uterus potentially leading to increased albumin and shell weights (Ramachandran *et al*., 2018a). In a separate study investigating the impact of parthenogenesis selection on offspring performance, it was found that parthenogenetic line dams produced heavier offspring at hatch and at four weeks, but also had higher first-week chick mortality compared to control line dams (Ramachandran *et al.,* 2018b). Additionally, in the parental interaction, eggs from parthenogenetic parental matings had the highest number of eggs and the highest proportion of female progeny exhibiting parthenogenesis, while control matings produced the lowest egg weights for the first twenty eggs. The study concluded that both dams and sires selected for parthenogenesis influence progeny performance by contributing to the degree of parthenogenesis exhibited by virgin female progeny. The study also highlighted two key points: accidental selection for the parthenogenetic trait in poultry could negatively impact chick production and performance, and parthenogenesis in blue-breasted quail, like in other birds, is likely an autosomal recessive trait, though further research is needed to confirm this (Ramachandran *et al*., 2018b). The first study exploring the effects of existing virus vaccine strains, pigeon pox and Newcastle disease, on parthenogenesis and their mechanisms of action using the blue-breasted quail as a model, demonstrated that vaccination of virgin chickens with live pigeon pox virus could potentially increase parthenogenesis and parthenogenetic size through direct effects on the embryo. Additionally, live Newcastle disease virus was found to have similar effects to live pigeon pox virus under in vitro conditions (Ramachandran *et al*., 2019).

Veterinary research

Isolates from a blue-breasted quail infected with avian tuberculosis, which showed multiple lesions in the liver, oviduct, and intestine, were inoculated into chickens, resulting in the development of clinical symptoms of avian tuberculosis (Morita *et al.,* 1999). In contrast, reovirus infection observed in budgerigars (*Melopsittacus undulatus*) did not affect blue-breasted quail housed in the same aviary (Perpiñán *et al*., 2010). A persistent ectoparasitic mite (Acariformes: Prostigmata) living inside hollow feathers, piercing the feather wall with its long chelicerae and feeding on the living tissue of the feather follicles (Grossi and Proctor, 2020), was noted in blue-breasted quail (Skoracki and Sikora, 2011).

A newly identified avian beta-defensin (AvBD) orthologue was isolated from lung and bone marrow tissues (Wang *et al.*, 2010), and a novel avian beta-defensin (AvBD10) was discovered in the liver and bone marrow of blue-breasted quail (Ma *et al*., 2011). Blue-breasted quail were shown to be suitable for testing vaccine-induced protection against avian H5N1 viruses. A single dose of NIBRG-14 vaccine induced low titres of antibodies against the homologous strain, with only partial seroconversion to the heterologous strain. A high dose of the A/Swan/Nagybaracska/01/06 (H5N1) strain provided 100% protection (Sarkadi *et al*., 2013).

A study examining the effects of inactivated Newcastle Disease virus vaccine (Nobilis Paramyxo P201) and bacterial vaccine against Salmonella (Nobilis SalenvacT) administered to blue-breasted quail females at the onset of spawning found significant variations in the maternal antibody transfer to offspring over 42 days. Notably, these variations were independent of the females' overall immune response (Coakley *et al*., 2014).

Breeding techniques

Incubation and egg storage

The eggs are laid in afternoon hours at between 15.30-16.00 (Harrison, 1968). Only the female blue breasted quail incubate the eggs and lay around 10-13 eggs per clutch (Harrison, 1968). The average clutch size was 3.3 (ranging from 1-20) in the study using unfertilised eggs (Parker and McDaniel, 2009). Andersson *et al.* (2004) reported that the average clutch size of fertilised eggs laid for two weeks was 12.

As shown in Table 3, various parameters, including temperature, humidity, and turning frequency, are applied at different settings. The classification system developed for Japanese quails (Petek and Dikmen, 2004) was adapted for blue-breasted quails. At the end of 18 days of incubation, unhatched eggs were opened and macroscopically classified into several categories: infertile, early embryonic death (1–6 days of incubation), mid embryonic death (6–12 days of incubation), late embryonic death (13–18 days of incubation), pipped, cracked, or contaminated eggs (Parker *et al.,* 2012).

In this study, early embryonic deaths occurring within the first six days were further classified based on the size of the germinal disc: small early possible parthenogenic deaths (≤7 mm) and large fertilized early deaths (>7 mm) (Parker *et al*., 2012). Another study similarly evaluated hatching outcomes based on this classification (Parker *et al*., 2014). It identified hatching failures as follows: undeveloped unfertilized eggs, unfertilized eggs with parthenogens (≤7 mm), early deaths (1–6 days), mid deaths (6–12 days), and late deaths (12–18 days) (Parker *et al*., 2017). In contrast, a simpler approach was used in another study, where all unhatched eggs were opened after 20 days of incubation, and embryos were classified into early or late stages based on feather visibility (Cai *et al.*, 2019).

It has been demonstrated that blue-breasted quails can be successfully reared under laboratory conditions, exhibiting egg production rates ranging from 60% to 80% (Tsudzuki, 1994). Additionally, it has been reported that these quails can produce approximately 250 eggs annually (Cai *et al.*, 2019). Furthermore, fertility, hatchability, and survival rates to maturity were reported as 91%, 84%, and 78%, respectively (Tsudzuki, 1994). However, a more recent study indicated a lower fertility rate of 42.8% (247 out of 577 eggs) and hatchability of 19.1% (110 out of 577 eggs) (Zi *et al.,* 2023). Moreover, the hatching outcomes were categorized with the following ratios: 83.9% for fertile eggs, 18.6% for early dead embryos, 1.7% for possible parthenogenetic embryos, 1.3% for mid-dead embryos, 9.0% for late dead embryos, 1.3% for pipped embryos, 12.6% for cracked eggs, 0.7% for contaminated eggs, 38.6% for hatch of set eggs, and 46.0% for hatch of fertile eggs (Parker *et al.,* 2014).

As with other galliform species, the embryonic development stages initially identified in blue-breasted quail were based on those described for chicken embryos by Hamburger and Hamilton (1951) and adapted by Perry *et al.* (2022). In incubators set at 37.5°C, no significant morphological changes were observed until the 5-hour mark. At this stage, a belt-like structure, similar to Koller's sickle, became visible, along with a transparent belt. By 6 hours of incubation, the primitive streak began to form, marking the transition to Hamburger-Hamilton (HH) stage 2. After 9.5 hours, a complete primitive groove appeared, indicating HH stage 4. At 24 hours, the embryos showed a closed neural tube and 6 somites, corresponding to stages HH8 and HH9 (Cai *et al.,* 2019). In contrast, Nakamura *et al*. (2019a) established a normal staging table for blue-breasted quail embryos at 37.7°C with 39 incubation stages, with stages 1-16 very similar to those of chicken and Japanese quail. According to this study, the primitive streak was not visible until 9 hours of incubation (stage 1). It became visible between 6 and 11 hours (stage 2), with Hensen's node appearing within the same period (stage 3). Additionally, during stages 7 to 16 (22–55 hours of incubation), somitogenesis occurred, with somite numbers increasing from 1 to 26-27.

Key morphological developments, including limb bud formation, were observed during stages 17-24 (57 hours to 5.5 days of incubation). Feather germ formation and pigmentation occurred during stages 25-33 (6–11 days), while embryo weight and third toe length were used as indicators during stages 34-39 (12–17 days of incubation) (Nakamura *et al.,* 2019a).

Furthermore, according to the NNT (Nakamura, Nakane, and Tsudzuki) developmental stages, most membranous skull bones began ossifying between days 7 and 11 of incubation (NNT stages 27-33). All skull bones became visible by day 11 (NNT stage 33), while ossification continued in the later stages (11–17 days of incubation; NNT stages 33–39). Notably, bones underwent chondrification between days 3 and 5 (NNT stages 19-23) (Nakamura *et al*., 2019b). Additionally, the yolk-free embryo mass increased sigmoidally, and the embryos' oxygen consumption rate rose exponentially during incubation (Pearson, 1999).

In blue-breasted quail, embryonic diapause was induced at 21°C, while hatchability was significantly higher at 16°C. Prolonged egg storage resulted in reduced hatchability and increased embryonic mortality. In contrast, pre-incubation had no significant effect on hatchability (Cai *et al*., 2019). The egg storage conditions in blue-breasted quail are outlined in Table 4.

Lighting and heating

After hatching, the chicks were kept in a brooder for 12 hours before being transferred to cages with a 40 W red spotlight, which created a thermal gradient of 33–42°C under continuous illumination (Andersson et al., 2004). Additionally, chicks were dried in an incubator set to 37,8°C for 12 hours, maintaining this temperature for the first week. Subsequently, the temperature was reduced by approximately 5°C each week, reaching a final ambient temperature of 23,9°C by week 3 (Saini et al., 2019; Sepp et al., 2021). The brooder temperature was maintained at 40±1°C for the first two weeks, then decreased by 2°C every two days until reaching 30°C, which was sustained until the chicks reached 8 weeks of age (Wei *et al*., 2011a; Wei *et al.,* 2011b).

It has been reported that the majority of blue-breasted quail hens lay their eggs under a light exposure of 12 to 14 hours (Tsudzuki, 1994). In contrast, pair-housed quail were initially subjected to a 10:14 h light/dark photoperiod for one week to ensure the birds remained in a non-reproductive state. Subsequently, the photoperiod was increased by 1 minute every 2 days for one week, followed by an increase of 2 minutes every 2 days until reproduction commenced, with a maximum photoperiod of 10.25 hours of light and 13.75 hours of darkness (Parker et al., 2008). However, both chicks and adult quail were exposed to 17 hours of light (Parker and McDaniel, 2009; Parker *et al*., 2010; Parker et al., 2012; Parker *et al.,* 2014; Santa Rosa et al., 2016b; Parker et al., 2017). The temperature of the rearing room was maintained at 25±3°C after 30 days of age (Zi *et al*., 2023).

Poultry Studies, 22(1), 24-39

Table 3. Incubation process of blue-breasted quails

Parameter	Value (unit)/Process	Reference
Incubation period	16 days	Pearson (1999); Cai *et al*. (2019)
	17 days	Ono *et al.* (2005); Nakamura *et al*. (2019a); Cai *et a*l. (2019)
	18 days	Pis and Luśnia (2005); Parker *et al.* (2014); Aharon-Rotman *et al*. (2020)
	Up to 21 days	Harrison (1968); Zi *et al*. (2023)
Externally pipped	Hatches within 12 hours	Pearson (1999)
Temperature and humidity	37.5°C; ~30% RH	Adkins-Regan (2016)
	37.5°C; 50% RH	Parker *et al.* (2012)
	37.5°C; 60% RH	Parker et al. (2017); Ramachandran *et al*. (2019); Bautista *et a*l. (2021)
	37.7±0.2°C; 70% RH	Tsudzuki (1994); Nakamura *et al*. (2019a); Nakamura *et al*. (2019b)
	37.7°C; 50% RH	Aharon-Rotman *et al*. (2020)
	38°C; 50–80% RH	Zi *et al*. (2023)
	38±0.5°C; 55% RH	Pearson *et al*. (1998)
	38.5±0.5°C; 58–59% RH	Pearson (1999)
	37–38°C; 40–50% RH (rising to 70% prior to hatching)	Coakley *et al*. (2014)
	40°C with 95% RH	Bernstein (1973, 1971)
	37.5°C; 55–65% RH	Cai *et al*. (2019)
	37.8°C; 45–65% RH for 13 days; >65% RH for next 11 days	Saini *et al.* (2019)
	38.5°C; increased RH 2 days before hatching	Andersson *et al*. (2004)
Egg Rotation	Stopped on day 13	Bautista *et al*. (2021)
	Stopped on day 15	Saini *et al*. (2019)
	Every hour for the first 14 days at a 45° angle	Cai *et al*. (2019)
	Twice daily (180° manual rotation)	Pearson (1999)
	Twice daily	Andersson *et al*. (2004)
	Several times per day	Bernstein (1973)
	Every 1.5 hours	Zi *et al*. (2023)
	Every hour at a 90° angle	Nakamura *et al.* (2019a)

RH: relative humidity

Table 4. Egg storage conditions in blue-breasted quail

Purpose	Storage Condition	Storage Duration	Reference
Embryo culture study	12-14 °C	Maximum 7 days	Ono *et al*. (2005)
Embryonic development study	15 °C	Maximum 5 days	Nakamura *et al*. (2019a)
Embryonic heart rate study	15 °C	Maximum 5 days	Pearson *et al*. (1998)
Parthenogenesis study	20 °C	Maximum 3 days	Parker and McDaniel (2009); Parker *et al*. (2010); Parker *et al.* (2012); Wells *et al*. (2012); Parker *et al.* (2014)
Fertilized egg and parthenogenesis study	20 °C	Maximum 7 days	Parker *et al*. (2012), Parker *et al*. (2014)
Testosterone manipulation effects on offspring	Room temperature	Maximum 14 days (last 8 eggs)	Andersson *et al.* (2004)
Effect of storage temperature on hatchability and embryonic diapause	16°C or 21°C and 65-75% RH	3, 7, or 14 days	Cai *et al.* (2019)
Effect of pre-incubation	45◦ rotation at 37.5◦C for 6 h and 55-65% RH		

RH: relative humidity, h: hour

In another study, a 12-hour light:12-hour dark photoperiod was used, with lights on from 06:00 to 18:00 following hatching (Aharon-Rotman *et al.,* 2020). As shown in Table 5, the environmental temperature and photoperiod for blue-breasted quail are presented. In the blue-breasted quail, a diurnal species (Aharon-Rotman *et al*., 2020), it has been reported that after 3 weeks of age, exposure to weak artificial blue night light (approximately 0.3 lx; 18 hours light:6 hours dark) for 6 weeks significantly enhanced bactericidal activity, as evidenced by the weekly assessment of plasma bactericidal activity against *Escherichia coli*—a common marker of innate immunity. This effect was observed across quails of various plumage colors, including wild type, silver (light gray), white, black (extended brown), and fawn (cinnamon), with immune responses differing between males and females at distinct developmental stages (Saini *et al.,* 2019). Additionally, the same artificial light exposure led to a reduction in digestive efficiency at two specific time points between weeks 4 and 9 post-hatching, as indicated by steatocrit values from weekly faecal samples. This decline in digestive function coincided with a period of rapid skeletal growth, suggesting that increased energetic demands during growth may compromise digestion. However, it was also noted that growth rate remained unaffected by the artificial light manipulation. It was suggested that ad libitum feeding may mask the adverse physiological effects, as the changes in digestive efficiency were too minor to influence growth or overall condition, and that energy expenditure in the exposed birds was reduced (Sepp *et al*., 2021).

Feeding

In the breeding of blue-breasted quails, feed and fresh water were provided ad libitum, with varying types and compositions of feed used. For example, both chicks and juvenile quails were given commercial Japanese quail diets containing 24% protein (Tsudzuki, 1994), whereas adult quails were fed exclusively commercial poultry feed (Ueno and Suzuki, 2014). Alternatively, quails were also fed a mixture of finch seed (Roberts and Baudinette, 1984), supplemented weekly with greens (Roberts and Baudinette, 1986). Additionally, adult quails were provided a diet containing 2900 kcal/kg of metabolizable energy, 23% protein, and 3.5% calcium supplementation (Cai *et al.,* 2019). The quails were initially fed commercial game bird feed (28% protein, 3% fat) immediately after hatching (Aharon-Rotman *et al*., 2020). Additionally, they were provided Bell turkey and game bird feed, supplemented with vitamins and water, while their diet was further enriched weekly with Tenebrio larvae and lettuce leaves (Bernstein, 1971). In another study, chicks were initially fed a commercial quail starter diet until 4 weeks of age, after which they transitioned to a commercial quail breeder diet (Parker and McDaniel, 2009; Parker *et al.*, 2010; Parker *et al*., 2012; Parker *et al*., 2014; Parker *et al.*, 2017).

In contrast, chicks were initially provided animal feed, including chopped tuber weevil and mealworm larvae, as well as standard chicken feed (26.5% protein content) for approximately the first week. Following this, they were switched to a commercial budgerigar diet (composed of Laplata millet, white millet, Japanese millet, sorghum, canary seed, hemp, and oats), supplemented with poppy seeds, apples, chopped alfalfa, yarrow, cottage cheese, and boiled egg whites (Pis and Luśnia, 2005). In other studies, quails were also fed a mixture of finch seed, egg feed, protein-rich feed, and vitamins and minerals (Lõhmus and Sundström, 2004; Lõhmus *et al.,* 2006). Insect feed was added to their diet using a similar mixture (Andersson *et al.,* 2004). Furthermore, quails were given a mixture of small carrot seed ad libitum, supplemented with mineral cuttlebone, lettuce, fruit, and mealworm larvae (Tenebrio sp.) (Pearson, 1999). Alfalfa sprouts and game bird feed were also included in their diet (Askew and Marsh, 2001).

It was reported that there were no significant differences in feed intake, gut morphology, or gastrolith mass between quail fed low-fiber diets (comprised of a commercial pullet starter crumble with 13% neutral detergent fibre and 4% acid detergent fibre) and high-fiber diets (achieved by diluting the low-fiber control diet with 20% dry wood shavings, resulting in 23% neutral detergent fibre and 14% acid detergent fibre) (Stewart and Munn, 2014). On the other hand, a study comparing quail fed low-fiber (8.5% neutral detergent fibre, i.e., high-quality) and high-fiber (16% neutral detergent fibre, i.e., low-quality) diets, both at identical energy levels, indicated that the available energy in the diet may have a more pronounced effect on inducing phenotypic changes in the gut than the physical impact of dietary fibre on feed intake or muscle compensation for fibrous nutrients (Williamson *et al*., 2014).

In addition to the various diets, the dietary crude protein requirements and the effect of dietary metabolisable energy concentration on these requirements for growing blue-breasted quail were investigated. The study reported that it is not necessary to provide extremely high crude protein diets, such as turkey starter feed containing around 30% crude protein. Instead, good performance can be achieved with diets containing more than 20.4% crude protein and 2,750 kcal/kg metabolisable energy during weeks 1 to 4 (Wei *et al.*, 2011a).

It was indicated that blue-breasted quails, with similar initial weights (mean 10.4 g) at seven days of age, were fed diets with varying crude protein levels of 12.5%, 15%, 17.5%, 20%, 22.5%, and 25% at 2,900 kcal/kg metabolisable energy. At 21 days of age, their weights were 9.3, 13.1, 20.3, 25.8, 27.5, and 29.2 g, respectively (Wei *et al.,* 2011b). Moreover, it was determined that, after meeting maintenance requirements, quails in this period required an extra 0.47 g of protein to gain 1 g of weight and 13 g of protein to accumulate 1 g of body nitrogen.

Table 5. Environmental temperature and photoperiod for blue-breasted quail

Age Weeks)	Photoperiod light/dark	Temperature and Humidity (unit)	Reference
0-2	24h	36±2°C	Tsudzuki, (1994)
2-4	24h	33±2°C	
2+	16 h/8 h	25-27°C, 65-70% RH	Pis and Luśnia, (2005)
4	14 h/10h		Adkins-Regan, (2016)
4-6	24h	28±2°C	Tsudzuki, (1994)
6-8	24h	25±2°C	
8+	14 h/10h	23±2°C	
Adult	16 h/8 h	28°C, 45% RH	Bernstein, (1971)
Adult	16 h/8 h	25-30°C,30-60% RH	Bernstein, (1973)
Adult	12 h/12h	23±1°C	Stewart and Munn, (2014)
Adult	14 h/10h		Schleidt *et al*., (1984)
Adult	14 h/10h	23°C	Lõhmus and Sundström (2004); Lõhmus *et al*. (2006)
Adult	14 h/10h	28°C (day), 22°C (night)	Andersson *et al.,* (2004); Uller *at al*., (2005); Uller *et al.,* (2006)
Adult	14 h/10h	23-25◦C, 40-60% RH	Cai *et al*. (2019)
Adult	14 h/10h	∼24◦C,∼60% RH	Bautista *et al*. (2021)
Adult	12 h/12h	∼25°C	Roberts and Baudinette (1986); Roberts and Baudinette (1988)
Adult	12 h/12h	25°C, 45- 62% RH	Roberts and Baudinette (1984)
3-9	12 h/12h	24°C	Askew and Marsh, (2001)

h: hour, RH: relative humidity

The dietary crude protein level was calculated to be 20.6% or 19.6%, based on estimated daily feed intake, as an additional 412 mg of protein per day was needed for average daily maintenance and 456 mg per day for average daily growth (Wei *et al*., 2011b).

Housing

The relatively small size of blue-breasted quails facilitates their handling and maintenance in cage environments (Lõhmus and Sundström, 2004). Adult quails were individually housed in metal cages (0,3 m²) (Roberts and Baudinette, 1984; Roberts and Baudinette, 1986; Roberts and Baudinette, 1988). Additionally, quails were maintained in pairs or larger groups within terrariums (62x31x31 cm) or cages of varying sizes (335x315x240 cm) (Schleidt *et al*., 1984), as well as in pairs in cages measuring 80x60x50 cm (Andersson *et al.,* 2004; Uller *et al*., 2005; Uller *et al*., 2006). Pairs of one male and one female were housed together in plastic cages (60x48x35 cm) equipped with ventilation and lined with wood shavings to mitigate foot injuries and absorb moisture (Bautista *et al.,* 2021), as well as in smaller cages (37x13x19 cm) (Cai *et al*., 2019). In another study, quails were housed in groups of three (one male with two females) in large plastic boxes (80x60x50 cm) lined with a mixture of fine bird sand, crushed shells, and very fine gravel (Lõhmus and Sundström, 2004; Lõhmus *et al*., 2006). Furthermore, wood chips were utilized as bedding material, and a sand bowl was provided in the cages (Andersson *et al.,* 2004; Uller *et al*., 2005; Uller *et al*., 2006). Pairs were housed in relatively small commercial aviaries (75x28x28 cm, two individuals per cage) (Parker *et al*., 2008), while randomly sexed pairs were housed in cages measuring 38x46x46 cm, with rubber matting on the cage floors to prevent slipping and leg splay (Saini *et al.,* 2019; Sepp *et al.,* 2021). Additionally, quails were housed in pairs and trios in cages (Bernstein, 1971). One-day-old quails were reared in cages (48x33 cm²) with a stocking density of 10 birds until they reached eight weeks of age (Wei *et al*., 2011a). In a separate study, quails were housed in cages of the same dimensions, with five birds per cage, from one to three weeks of age (Wei *et al.,* 2011b). Additionally, chicks were housed individually in wire cages (57 cm long x 32 cm deep x 40 cm high) after reaching four weeks of age (Adkins-Regan, 2016). In contrast, some chicks were raised in larger mixed-sex cages (240x50x37.5 cm) with wood shavings, multiple feeding stations, enclosures, and sand baths, which allowed the birds to engage in their full natural behavioural repertoire, with 14 birds per cage, until sexual maturity (Coakley *et al.,* 2014). Similarly, four or five quails were housed in plastic cages (43.3x80x51 cm), bedded with pine shavings (Aharon-Rotman *et al*., 2020). Although quails were housed individually in smaller cages (21x29x40 cm) during learning experiments (Ueno and Suzuki, 2014), they were kept in steel cages (41x28x38 cm) for a study on physiological and behavioural flexibility in response to dietary changes (Stewart and Munn, 2014). Chicks were initially housed in sibling pairs in cages until sexual maturity, which occurred at approximately five weeks of age. Afterward, they were assigned to pairs of unrelated individuals (one male and one female) (Uller *et al*., 2006). Similarly, quails were mated in wire cages, ensuring that sibling matings were avoided (Tsudzuki, 1994).

Blue-breasted quail research

This study utilized the Web of Science database (2024), a comprehensive commercial product developed by the Institute for Scientific Information, to assess the global volume and scope of blue-breasted quail research. The database was queried using the three scientific names of the blue-breasted quail, and all studies published from 1982 to the present were analyzed. Since 1982, a total of 73 publications have been produced on the blue-breasted quail, with each publication potentially spanning one to four Web of Science categories. Figure 2 aggregates these publications into 10-year periods, excluding the three articles published in 1982 and 1983, which are not included in the figure. Additionally, three publications—two in forestry and one in ornithology—investigating predation rates in experimental nests using blue-breasted quail eggs were not considered. Furthermore, a publication from the Multidisciplinary Sciences category, which used the blue-breasted quail to compare thermoregulatory behavior in reptiles, was not included due to the unavailability of the full text.

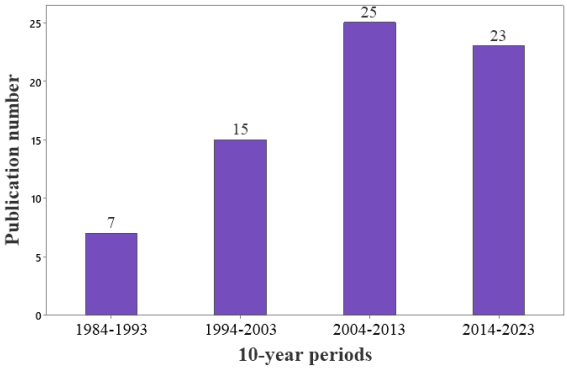

Figure 2. Number of publications on the blue-breasted quail over 10-year periods, as retrieved from the Web of Science database

Figure 2 illustrates that there were 7 publications during the 1984-1993 period. In the subsequent decade (1994-2002), the number of publications nearly doubled to 15. Surprisingly, the number of publications increased further to 25 in the 2004-2013 period, representing a significant rise compared to previous periods. However, in the most recent decade (2014-2023), the number of studies on the blue-breasted quail slightly decreased compared to the previous period.

Figure 3 presents a pie chart showing the distribution of publications by research field across twenty-two categories. As depicted in the chart, the blue-breasted quail was most frequently studied in the field of zoology (29 publications), followed by agriculture, dairy and animal science (14), genetics and heredity (11), veterinary sciences (10), and physiology (9).

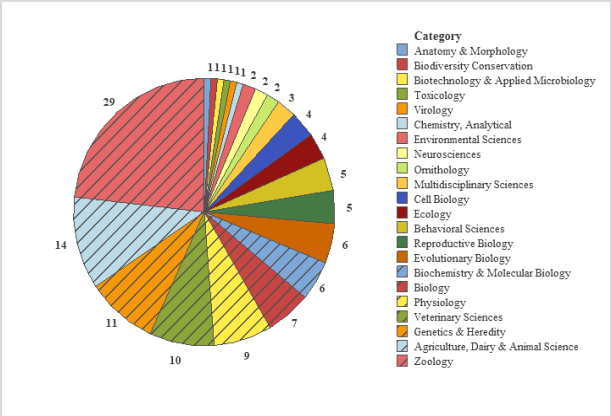

Figure 3. Pie chart of number of Web of Science categories, the number is rising downwards

Conclusion

The morphological, physiological and behavioral characteristics of blue-breasted quail have attracted attention and have been studied for various purposes. The study of quail reared under specific conditions has greatly improved our understanding of numerous physiological systems, their behavior and performance. The use of the blue-breasted quail, ranging from being a pet animal to being studied in complex experimental analyses, makes it valuable in both hobby breeding and scientific studies. The quail's average embryonic developmental period of 17 days, coupled with an 8-week sexual maturation period, results in significantly shorter generation times than other poultry, with the exception of Japanese quail. Furthermore, the body size of the adult quail, which is even smaller than the Japanese quail, greatly reduces the area and cost of rearing. Over the last four decades, the blue-breasted quail has proven to be a truly diverse and productive animal model, but it also has many potential subjects for study in terms of genetic variation, as it has not yet been the subject of intensive selection breeding. It should also be pointed that the blue-breasted quail is a suitable experimental animal for parthenogenesis studies. Studies so far suggest that blue-breasted quail will continue to occupy a small but important place in research laboratories around the world in the future.

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