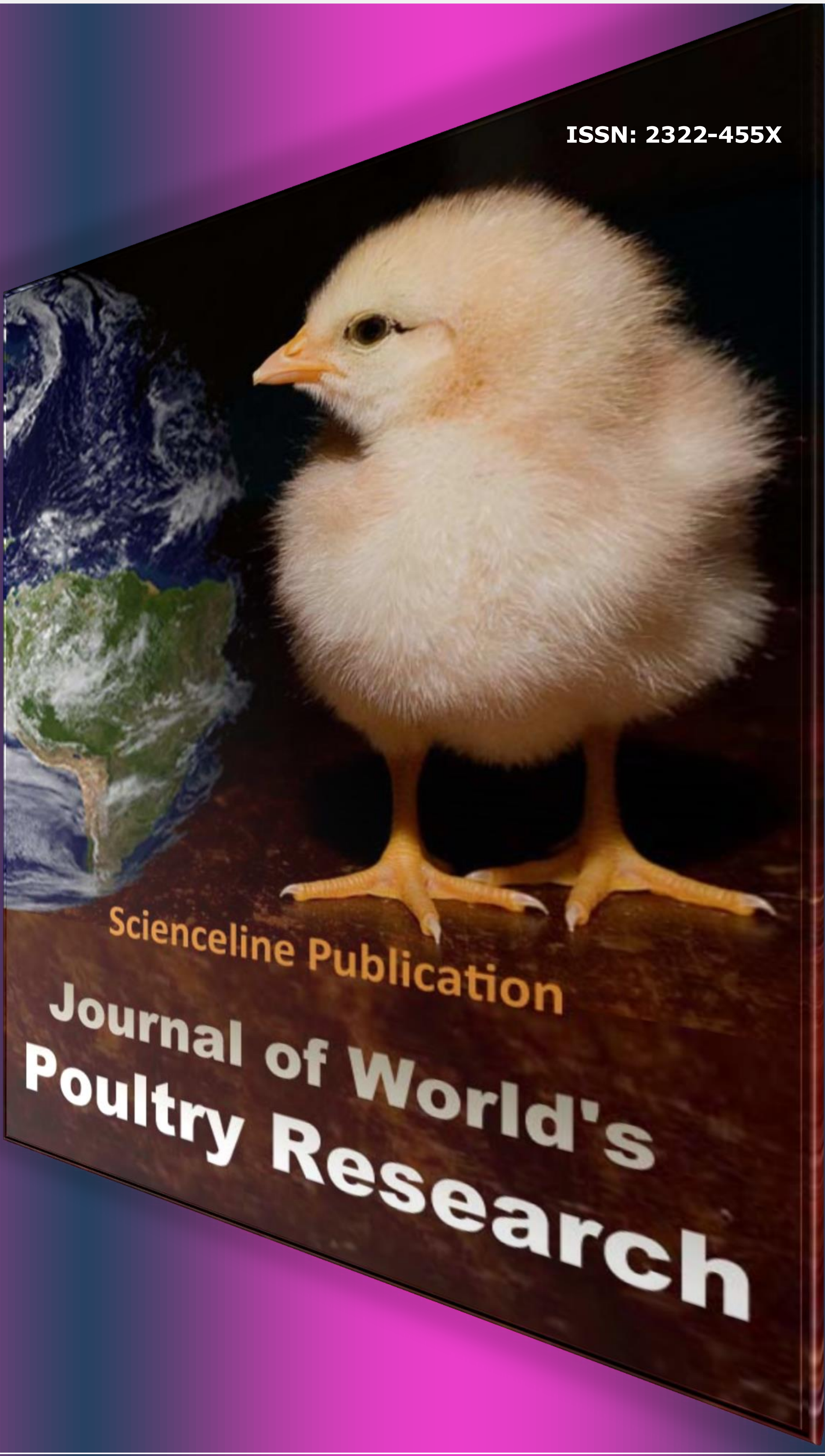
Journal of World's Poultry Research

BOOKLET

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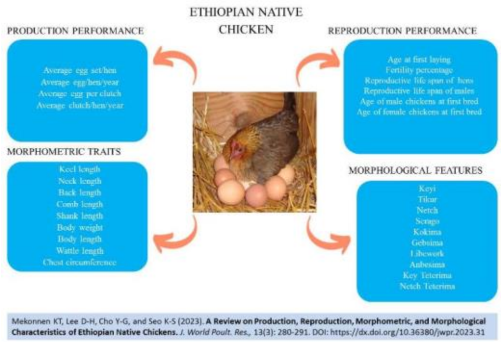
**Review**

### A Review on Production, Reproduction, Morphometric, and Morphological Characteristics of Ethiopian Native Chickens

Mekonnen KT, Lee D-H, Cho Y-G, and Seo K-S.

*J. World Poult. Res.* 13(3): 280-291, 2023; pii: S2322455X2300031-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.31




**ABSTRACT:** Native chickens in Ethiopia are characterized in a fragmented manner for their performance characteristics and genotypes. This review aimed to explore the production and reproduction performance characteristics as well as the morphometric and morphological diversity of Ethiopian native chickens. The investigation was performed on four production performance characteristics, including average egg per clutch, average clutch/hen/year, average egg set/hen, and average egg/hen/year, as well as six reproductive performance characteristics, including age at first laying, age of male chickens at first bred, age at which female chickens are first bred, the reproductive life span of males and females, and fertility percentage in various parts of Ethiopia. Some economically practical morphometric characteristics of native chickens, such as shank length, chest circumference, comb length, body weight, body length, keel length, wattle length, neck length, back length, and morphological diversity, were also summarized. Regarding performance characteristics, there were some variations in eggs' average production performance per clutch (13.56-15.4 eggs) and clutch/hen/year (3.0-4.29) in Ethiopia. The average reproduction performance characteristics of Ethiopian native chickens for age at first laying (6.90-7.13 months), age of male chickens at first bred (5.87-6.15 months), female at first bred (5.20-5.93 months), the reproductive life span of males (3.79 years) and hens (3.56 years), and chicks hatched from set eggs revealed variation across Ethiopia. In various locations of Ethiopia, the average trait values reported for Ethiopian native chickens under the farmer's management differed in terms of morphometric and morphological features. The variation observed in performance characteristics, as well as morphometrics and morphological characteristics for Ethiopian native chicken ecotype population, can help the native breed classification, unique trait conservation, and breed improvement intervention programs.

**Keywords**: Ethiopia, Morphological trait, Morphometric trait, Native chicken, Performance

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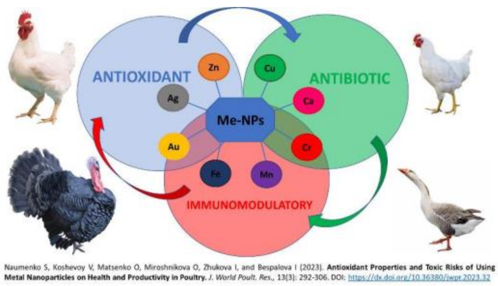
**Review**

### Antioxidant Properties and Toxic Risks of Using Metal Nanoparticles on Health and Productivity in Poultry

Naumenko S, Koshevoy V, Matsenko O, Miroshnikova O, Zhukova I, and Bespalova I.

*J. World Poult. Res.* 13(3): 292-306, 2023; pii: S2322455X2300032-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.32




**ABSTRACT:** Metal nanoparticles (NPs) are introduced into various fields of science, particularly poultry farming. Supplementation of metal salts in nanoform can increase the profitability of poultry farming by enhancing meat and egg production. Although their toxic parameters pose limitations on their use, many studies have evaluated the effects of using metal NPs in modern poultry farming on health, productivity, metabolism, and especially antioxidant properties. In addition, the peculiarities of their toxicokinetic and recommended doses that meet safety criteria in practical activities are highlighted. Zinc oxide NPs are one of the most studied compounds in the poultry industry. Their pronounced antioxidant properties, positive effect on productivity and homeostasis of poultry, egg quality, and immune status have been experimentally confirmed. Copper oxide NPs have similar properties but are limited in usage due to their toxicokinetics. Silver and gold NPs emerge as potential alternatives

to antibiotics and could solve the resistance problem of microorganisms to antibiotics. Other important NPs used in poultry are Iron and Calcium. In their nanoform, these NPs exhibit high bioavailability, which allows for efficient absorption and utilization by poultry. The methods used to synthesize these nanoparticles make it economically viable to incorporate them into poultry diets, reducing overall expenses compared to similar macroergic compounds. Manganese and chromium NPs positively affect sperm survival in turkeys during refrigerated storage and contribute to increasing the resistance of the broiler chickens' body to heat stress and normalizing the metabolism of sex hormones. In conclusion, the application of metal nanoparticles to poultry is a promising research direction, aiming at the development of feed additives, antibiotics, and growth stimulants due to their antioxidant, bactericidal, and immunomodulatory effects.

**Keywords**: Antioxidants, Health, Metal Nanoparticles, Poultry, Productivity, Toxicology

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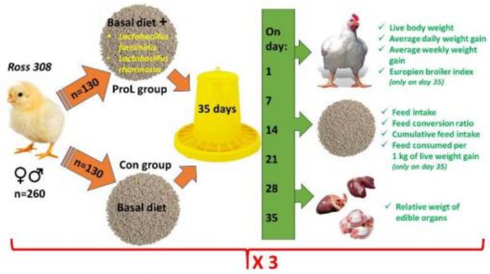
**Research Paper**

### Effects of Dietary Supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on Growth and Production Indicators of Broiler Chickens

Eglite S, Mancevica L, and Ilgaza A.

*J. World Poult. Res.* 13(3): 307-316, 2023; pii: S2322455X2300033-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.33



Eglite S, Mancevica L, and Ilgaza A (2023). **Effects of Dietary Supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on Growth and Production Indicators of Broiler Chickens.** *J. World Poult. Res.*, 13(3): 307-316. DOI: https://dx.doi.org/10.36380/jwpr.2023.33

**ABSTRACT:** In response to the 2006 EU ban on the use of antibiotics as growth promoters, researchers have sought alternatives, leading to a focus on the beneficial effects of probiotics on chickens. The aim of this study was to evaluate the effect of the probiotic mixture containing *Lactobacillus* (*L.*) *farciminis* CNCM-I-3699 and *Lactobacillus rhamnosus* CNCM-I-3698 on the growth, production indicators, and edible organs of broiler chickens. Three trials were conducted, each consisting of 260 newly hatched Ross 308 broiler chicks (males and females) from a commercial hatchery, randomly allocated into control (n = 130) and probiotic-supplemented groups (n = 130). The dietary treatments were basal diet for the control group and basal diet + the mixture of *L. farciminis* CNCM-I-3699 ($2.10^{10}$ GU/g) and *L. rhamnosus* CNCM-I-3698 ($2.10^{10}$ GU/g) at a rate of 4g/10kg of diet for the probiotic supplemented group. Broilers were raised until day 35 of age, and their body weight and feed intake were recorded on days 1, 7, 14, 21, 28, and 35. All broiler chickens were weighed on the first day. The investigated parameters included average weight gain, feed conversion ratio, cumulative feed intake, and the European Broiler Index. Daily mortality was recorded. The average organ's relative weight was calculated for each group on days 1, 7, 14, 21, 28, and 35. Although both groups yielded positive results regarding growth and production indicators, no significant differences were observed between the two groups, suggesting that probiotics may not provide expected outcomes when appropriate conditions and age-related requirements are met. The probiotic-supplemented group exhibited significantly accelerated growth in the heart and liver. However, relative organ weights did not differ significantly between the groups.
**Keywords:** Body weight, Edible organs, Poultry, Probiotic, Productivity

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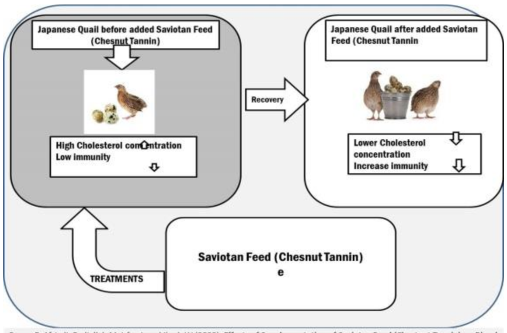
**Research Paper**

### Effects of Supplementation of Saviotan Feed (*Chestnut Tannin*) on Blood Parameters and Yolk Cholesterol Concentration in Japanese Quails (*Coturnix japonica*)

Erwan E, Afriadi, Rodiallah M, Irfan I, and Ibrah W.

*J. World Poult. Res.* 13(3): 317-322, 2023; pii: S2322455X2300034-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.34



Erwan E, Afriadi, Rodiallah M, Irfan I, and Ibrah W (2023). **Effects of Supplementation of Saviotan Feed (Chestnut Tannin) on Blood Parameters and Yolk Cholesterol Concentration in Japanese Quails (*Coturnix japonica*).** *J. World Poult. Res.*, 13(3): 317-322. DOI: https://dx.doi.org/10.36380/jwpr.2023.34

**ABSTRACT:** Tannins are secondary metabolites and active compounds widely present in plants. Tannins have several properties, such as astringent, antiparasitic, anti-diarrheal, anti-bacterial, and antioxidant. Hence, plants containing tannins are a major study subject for a natural alternative to in-feed antibiotics or antioxidants. The functions of tannin extracted from chestnut wood, namely Saviotan Feed (SF) in poultry, especially in quails, have not yet been fully understood. The current study aimed to examine the effect of SF supplementation on some plasma metabolites, including glucose (GLU), triglyceride (TG), total cholesterol (TCHO) concentration, and yolk cholesterol in quails (*Coturnix japonica*). A total of 100 unsexed quails were divided into 4 groups, with 25 quails in each group. These quails were then placed into 20 experimental pens, with 5 quails per pen. Each treatment was replicated 5 times, and the quails were fed a commercial rations diet supplemented with different SF doses of 0% (control), 0.1%, 0.2%, and 0.3%. Quails were provided with SF supplementation from 14 to 56 days of age. A sample of 40 plasma and eggs were randomly collected and analyzed for GLU, TG, and TCHO. The results indicated no significant effects of SF on plasma GLU and TG concentration, but a significant effect was found regarding TCHO. Moreover, supplementation of SF from 0.1 to 0.3% significantly decreased TCHO concentration in the yolk. In conclusion, it has been determined that supplementation of SF may play a significant role in decreasing TCHO in yolk eggs in quails.

**Keywords:** Chestnut tannins, Cholesterol, Glucose, Japanese quail, Plasma metabolites, Triglycerides

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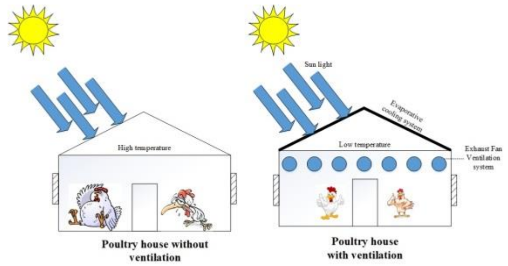
**Research Paper**

## Design and Analysis of Ventilation System for Closed Poultry House in Tropical Climate Conditions

Saner KA and Shekhawat SP.

*J. World Poult. Res.* 13(3): 323-331, 2023; pii: S2322455X2300035-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.35




**ABSTRACT:** The climate significantly impacts the temperature in different parts of the world. A moderate environment makes it simple to construct a chicken farm. Nevertheless, raising the birds in tropical places where typical temperatures can exceed 40-45°C is difficult because they can only survive at temperatures between 30°C and 35°C. As a result, the current study aimed to design a chicken house with a ventilation and cooling system to prevent excessive heat. The effectiveness of ventilation systems in maintaining liveable and constant conditions at the chicken house was assessed using computational fluid dynamics modeling to mimic internal and external airflows. In this study, a water evaporator-based cooling system and an exhaust fan-based ventilation system were built within a poultry house. ANSYS CFD was utilized to create the design and examine the flow of the model. The findings of each model were generated individually, and these results were compared to those of the other models to determine which model could decrease the temperature within the chicken coop. The proposed model's maximum temperature was around 30-32°C. A poultry house can be constructed using this idea to maintain chickens at a suitable temperature range of 30-32°C.

**Keywords**: Computational fluid dynamic, Evaporator, Exhaust fan, Poultry house, Ventilation

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

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**Research Paper**

## Effects of Partnership Patterns on Broiler Chickens' Performance in the Agribusiness System of Indonesia

Febrianto N, Akhiroh P, Helmi M, and Hartono B.

*J. World Poult. Res.* 13(3): 332-341, 2023; pii: S2322455X2300036-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.36


**ABSTRACT:** The partnership system is standard broiler cooperation in Indonesia. This system influenced agribusiness performance. Hence, the current study aimed to analyze the broiler agribusiness system in Kediri Regency, Indonesia, addressing three main areas, including partnership patterns, production performance, and financial performance. In this study, data was gathered from participants using a cross-sectional survey approach, capturing information from individuals at a specific moment in time. The research was performed from July to September 2022, utilizing both primary and secondary data. Primary data was obtained through direct observations and interviews with relevant stakeholders, while secondary data was sourced from various databases, such as the Indonesian Statistical Bureau and the Agriculture Ministry of Indonesia. Both types of data were subjected to quantitative descriptive analysis. The results indicated that the broiler partnership pattern consisted of three subsystems, including the chicken production facility providers (day-old chicks, feed, and medicines), the farming unit responsible for production process management (housing, feeding, drinking, and biosecurity), and the marketing subsystem focusing on chicken prices). The farmers in the farming unit showed effective production performance with a feed conversion ratio of 1.69, an index performance of

307, and an average body weight of 2.03 kg/head. Moreover, the farmers demonstrated a profitable financial performance with the revenue-cost ratio exceeding 1, reaching 1.07.

**Keywords**: Agribusiness, Broiler, Income, Partnership, Profitable, Revenue

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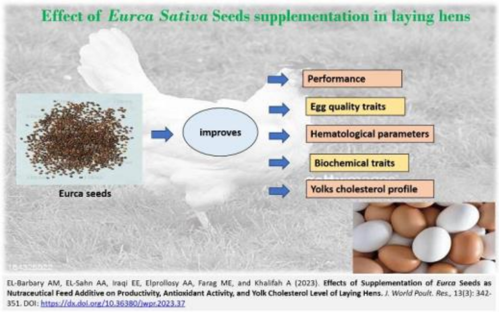
**Research Paper**

### Effects of Supplementation of Eurca Seeds as Nutraceutical Feed Additive on Productivity, Antioxidant Activity, and Yolk Cholesterol Level of Laying Hens

EL-Barbary AM, EL-Sahn AA, Iraqi EE, Elprollosy AA, Farag ME, and Khalifah A.

*J. World Poult. Res.* 13(3): 342-351, 2023; pii: S2322455X2300037-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.37




**ABSTRACT:** *Eruca sativa* (*ES*) seeds are annual herbs belonging to the *Brassicaceae* family, widely grown in Mediterranean countries, such as Egypt, Italy, and Greece. The *ES* is rich in macronutrient components and phytochemical content, exhibiting potent antioxidant properties and functional properties for vital processes such as digestion and absorption of nutrients .Therefore, this research was conducted to evaluate the effects of dietary *ES* supplementation on laying performance ,some blood parameters, and egg yolk cholesterol. A total of 300 Silver Sabahia strain hens, aged 26 weeks, were randomly distributed among four groups of five replicates, each replicate consisting of 15 hens .Chickens in group 1 served as a control and were fed the basal diet .Those in groups 2, 3, and 4 were fed basal diet supplemented with 1, 2 ,and 3% *ES*s, respectively. Productive performance traits, egg quality traits, hematological parameters, blood parameters, and yolk cholesterol profiles were performed throughout the study. The study lasted for 13 weeks (until week 39 of chickens' age). Results indicated that 3% *ES* supplementation had higher results on egg mass ,(%35.68) egg production (21.13%), and improved feed conversion ratio by ,%30.37 compared to all groups. Furthermore ,*ES*s supplementation positively affects the shell thickness and yolk color score compared to the control. Compared to the control, the highest significant blood hemoglobin and lymphocytes were recorded in the groups supplemented with %2 and 3% of *ES*s. The *ES* inclusion at a higher level (3% (in the diet of laying hens led to significantly enhanced serum high-density lipoprotein and total antioxidant capacity, while reducing cholesterol, low-density lipoprotein, and malondialdehyde levels ,compared to the control diet. Serum calcium, tri-iodothyronine, and alkaline phosphatase levels increased significantly in response to 3% *ES* treatment, while liver enzymes decreased significantly compared to the control diet. Notably, the addition of 2% and 3% *ES*s to the hens' ration resulted in reduced egg cholesterol content, which is desirable for consumers seeking healthier dietary choices. Finally ,adding 3% *ES*s to hens' diet improves productive performance, egg quality traits, hematological parameters, blood parameters, and yolk cholesterol profile.

**Keywords:** Blood parameters, Egg production, *Eruca* seed, Nutraceutical additive, Yolk cholesterol

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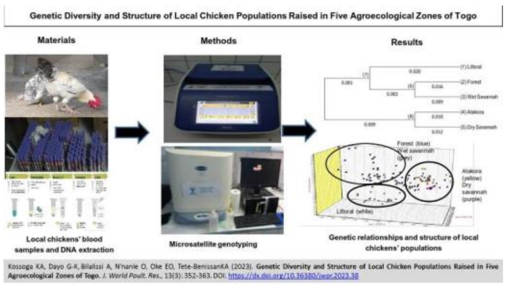
**Research Paper**

### Genetic Diversity and Structure of Local Chicken Populations Raised in Five Agroecological Zones of Togo

Kossoga KA, Dayo G-K, Bilalissi A, N'nanle O, Oke EO, Tete-Benissan KA.

*J. World Poult. Res.* 13(3): 352-363, 2023; pii: S2322455X2300038-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.38




**ABSTRACT:** Local chickens are the most commonly raised poultry breed in rural areas of Togo, where they help in alleviating poverty and food insecurity in households. The current study aimed to ensure the sustainable management of this genetic resource by evaluating the genetic diversity, phylogenetic relationships, and population structure of local chicken populations from five agroecological zones (Dry Savannah, Atakora, Forest, Wet Savannah, and Littoral) in Togo. Genotyping was carried out using 15 microsatellite markers on 30 unrelated individuals per agroecological zone. Genetic diversity was assessed by estimating the number of alleles per locus, observed heterozygosity, unbiased expected heterozygosity, and the polymorphic information content (PIC). The genetic structure of the populations was analyzed using a Bayesian-based approach. The results revealed a high genetic diversity but weak population structuring among local chickens. Moreover, 98 alleles were detected in all population groups, varying from 3 to 12 per locus, with an average of 6.53 ± 2.67 alleles per locus. The PIC values varied from 0.436 to 0.690, with an average of 0.550 ± 0.087. The mean number of alleles per population across all markers ranged from 4.4 ± 1.4 (Dry Savannah) to 5.4 ± 2.0 (Forest). The unbiased expected heterozygosity was high and varied from 0.58 ± 0.07 (Atakora) to 0.65 ± 0.11 (Forest), while that observed varied between 0.46 ± 0.09 (Dry Savannah) and 0.57 ± 0.14 (Forest). All populations deviated significantly from the Hardy-Weinberg equilibrium. Across populations, FIT, FIS, and FST fixation indices were 0.150, 0.132, and 0.021, respectively. The genetic distances were low and varied from 0.022 (between Atakora and Dry Savannah) to 0.045 (between Atakora and Forest). These results could be used in potential genetic improvement programs or the preservation of local chickens in Togo.

**Keywords:** Genetic diversity, Local chickens, Microsatellite markers, Heterozygosity, Togo

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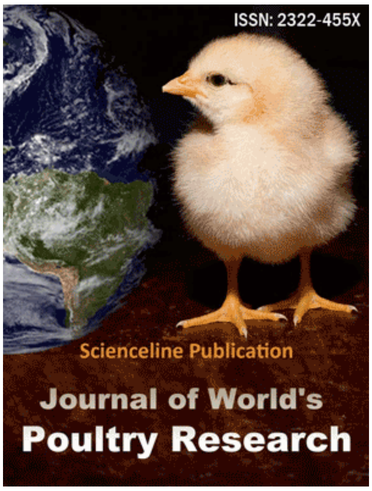


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DOI: https://dx.doi.org/10.36380/jwpr.2023.31

# A Review on Production, Reproduction, Morphometric, and Morphological Characteristics of Ethiopian Native Chickens

Kefala Taye Mekonnen[1,2], Dong-Hui Lee[2], Young-Gyu Cho[2], and Kang-Seok Seo[2,]*

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**ABSTRACT**

Native chickens in Ethiopia are characterized in a fragmented manner for their performance characteristics and genotypes. This review aimed to explore the production and reproduction performance characteristics as well as the morphometric and morphological diversity of Ethiopian native chickens. The investigation was performed on four production performance characteristics, including average egg per clutch, average clutch/hen/year, average egg set/hen, and average egg/hen/year, as well as six reproductive performance characteristics, including age at first laying, age of male chickens at first bred, age at which female chickens are first bred, the reproductive life span of males and females, and fertility percentage in various parts of Ethiopia. Some economically practical morphometric characteristics of native chickens, such as shank length, chest circumference, comb length, body weight, body length, keel length, wattle length, neck length, back length, and morphological diversity, were also summarized. Regarding performance characteristics, there were some variations in eggs' average production performance per clutch (13.56-15.4 eggs) and clutch/hen/year (3.0-4.29) in Ethiopia. The average reproduction performance characteristics of Ethiopian native chickens for age at first laying (6.90-7.13 months), age of male chickens at first bred (5.87-6.15 months), female at first bred (5.20-5.93 months), the reproductive life span of males (3.79 years) and hens (3.56 years), and chicks hatched from set eggs revealed variation across Ethiopia. In various locations of Ethiopia, the average trait values reported for Ethiopian native chickens under the farmer's management differed in terms of morphometric and morphological features. The variation observed in performance characteristics, as well as morphometrics and morphological characteristics for Ethiopian native chicken ecotype population, can help the native breed classification, unique trait conservation, and breed improvement intervention programs.


**Keywords:** Ethiopia, Morphological trait, Morphometric trait, Native chicken, Performance

## INTRODUCTION

Ethiopia is thought to have the largest livestock population in Africa, with a diverse range of animals, including poultry. Among these, Ethiopia's total number of chickens is estimated to be 57 million heads (CSA, 2021). The country's wide range of agro-climatic conditions results in one of the most diverse biological hotspots on the entire globe (Tegegne et al., 2010). The country's diverse agroecology and agronomic practices, combined with its large livestock population, particularly poultry, could contribute significantly to boosting the sector (Melesse, 2000).

Poultry is an essential part of the agricultural system in Ethiopia, where they are reared in all production systems (Alemu and Tadelle, 1997; Melesse, 2000; Demeke, 2004). Native chickens provide a significant portion of the chicken meat (99.2%) and eggs (98.5%) consumed in the country (Tadelle, 1996). According to Guèye (1998), the native chicken constitutes a sizable proportion of the flock in many African countries. These chickens are given fundamental care, with approximately

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5-20 chickens per household and insufficient feeding, housing, and health care management (Guèye, 1998). These flocks are typically replenished with improved chickens supplied by governmental and non-governmental organizations (Demeke, 2008). As a result, the information gathered on native ecotypes must be documented and left intact so genetic materials are not lost to oblivion (Dessie et al., 2012). The identified genotypes must be conserved and studied for their production and reproduction abilities, followed by multiplication (Dessie et al., 2012). Knowledge and understanding of chickens' unique characteristics are critical in designing and implementing indigenous chicken-based development programs that can benefit rural societies in the long run.

Morphometric characteristics can be classified as either qualitative or quantitative. Qualitative morphometric characteristics are observable characteristics that can be described by color and categories. In contrast, quantitative morphometric characteristics are methods for extracting measurable characteristics from shapes. These characteristics are typically used as descriptors of type and function for various livestock, including chickens. Although there are no phenotypic standards for Ethiopian native chickens, they were classified based on their colors and the location where they were characterized. However, those native chickens are non-descriptive in morphometric and morphological characteristics and vary in production and reproduction performance. Thus, this review was done using various published journals on Ethiopian native chickens that were used to systematically characterize their production and reproduction performance characteristics and morphometric and morphological diversity characteristics in their ecotypes, where they are found initially, considering different parts of Ethiopia with different agroecology. This study also examined various documents and research reports from other African countries, as well as the livestock report from the central statistical agency in general and poultry in particular to gain insight into the different types of chickens in Ethiopia, grouped by breed and type of poultry. Therefore, this review provided an overview of organized information and efforts to describe production and reproduction performance characteristics, morphometric and morphological trait diversity, and the genetic resources of native chickens in Ethiopia.

## Ethiopian poultry production, reproduction performance, and morphometric and morphological characteristics

The following sections present and discuss the findings and discussions on the production and reproduction performance characteristics and the morphometric and morphological diversity of Ethiopian native chickens. It also presents their evolution, population, distribution, ecotypes, and special features reported by researchers and scientists from various parts of Ethiopia, considering all agroecological types.

### The evolution of chicken domestication

Today's chicken (*Gallus gallus domesticus*) is classified with its primary origin being the Red Junglefowl. Domestication probably occurred 7,000-10,000 years ago in Southeast Asia and Oceania. Archaeological evidence indicates that the first instance of chicken domestication dates back to as early as 3250 BC in the Indus River Valley, located in modern-day Pakistan. The wildfowl species that contributed to the development of the modern-day domesticated chicken, *Gallus varius*, include the Red Jungle Fowl, Grey Jungle Fowl, Ceylon Fowl, and Green Fowl. These species, among others, are examples of the Gallus family that conceivably played a role in domestication. Comparative analysis of morphological characteristics, such as comb and feather characteristics, has revealed striking resemblances between the Red Jungle Fowl and domesticated chickens. According to genetics experts, the archetypal ancestor of the domesticated chicken is commonly recognized as the Red Jungle Fowl, which can still be found in the wilds of Asia (Crawford, 1990; Horst, 1991). Therefore, it cannot be disputed that the origin of domesticated fowl is rooted in Asia, and the chicken's worldwide spread and distribution can be traced back to the region.

As described by Crawford (1984; 1990), the domestication of chickens around the world went through four stages of evolution. In the first phase of evolution, the utilization of animals for religious, cultural, and traditional purposes led to the selection of color and distinct morphological characteristics in chickens. The second phase involved the dissemination of chickens from their original centers of domestication to various regions, countries, and continents, leading to genetic changes through processes such as genetic drift, migration, and natural selection that facilitated adaptation to new environmental circumstances. The third phase was exemplified by the nineteenth-century phenomenon known as "hen crazy". Most existing breeds and varieties are the result of human intervention. The fourth phase occurred in the twenty-first century when the cultural phenomenon known as "hen crazy" gave rise to today's massive chicken meat and egg industry (Crawford, 1990). The industry has moved quickly to incorporate cutting-edge technological, genetic, and breeding advances. A small number of breeds, varieties, and strains are now responsible for the vast majority of food production.

**An overview of the poultry population in Ethiopia**

All domesticated birds raised for human consumption (meat and eggs), including chickens, turkeys, ducks, geese, ostriches, guinea fowl, doves, and pigeons, are considered poultry. However, the phrase only applies to chickens in Ethiopia. Other species of birds, including ostriches, ducks, guinea fowls, doves, and pigeons found in their natural habitats are wild birds that have not been domesticated to produce meat and eggs (Molla, 2010).

Based on data from the Central Statistical Agency of Ethiopia (CSA, 2021), the country's total poultry population is approximately 57 million, including cocks, cockerels, pullets, laying hens, non-laying hens, and chicks. Indigenous poultry breeds account for the majority at 78.85%, while hybrid and exotic breeds make up 12.02% and 9.11% of the total poultry population, respectively (CSA, 2021) Among the different poultry types, laying hens comprise the largest share at 34.26%, followed by chicks at 32.86%. The number of pullets is estimated to be 6.47 million, while cocks and cockerels are also separately estimated at 6.38 million and 3.27 million, respectively. Non-laying hens constitute a relatively small portion of the total poultry population, accounting for around 4.59% or 2.61 million chickens. The data obtained (CSA, 2021) also reveals that the indigenous, hybrid, and exotic poultry breeds account for 78.85%, 12.02%, and 9.11% of total poultry, respectively. Table 1 provides a comprehensive summary of the estimated number and percentage of poultry by type and breed (indigenous, exotic, and hybrid/cross-breed) that offers an insightful overview of the poultry population in Ethiopia.

**Table 1.** Estimated number and percentage of poultry by type and breed in Ethiopia

| Poultry type | All | | Indigenous | | Exotic | | Hybrid | |
|---|---|---|---|---|---|---|---|---|
| | Number | Percentage | Number | Percentage | Number | Percentage | Number | Percentage |
| Cocks | 6,380,732 | 11.19 | 5,160,983 | 9.06 | 398,452 | 0.70 | 821,296 | 1.44 |
| Cockerels | 3,268,614 | 5.74 | 2,364,747 | 4.15 | 316,885 | 0.55 | 586,982 | 1.03 |
| Pullets | 6,474,755 | 11.36 | 4,688,266 | 8.23 | 675,687 | 1.19 | 1,110,802 | 1.95 |
| Laying hens | 2,614,965 | 4.59 | 2,117,083 | 3.71 | 205,449 | 0.36 | 292,433 | 0.51 |
| chicks | 18,729,950 | 32.86 | 16,322,355 | 28.64 | 1,244,426 | 2.18 | 1,163,169 | 2.04 |
| Non-laying | 19,523,972 | 34.26 | 14,287,489 | 25.07 | 2,353,446 | 4.13 | 2,883,037 | 5.06 |
| Total | 56,992,987 | 100 | 44,940,924 | 78.86 | 5,194,345 | 9.11 | 6,857,718 | 12.03 |

Source: CSA (2021)

**Table 2.** Some native chicken production performance comparison in Ethiopia, Ghana and Tanzania

| Parameters | Average (Number) | Sites | References |
|---|---|---|---|
| Average egg per clutch | 13.56-15.4 | Ethiopia (Southeast, Metekel) | Negassa et al. (2014), Zewdu et al. (2013) |
| Average clutch per hen per year | 3.0-4.29 | Ethiopia, Ghana | Zewdu et al. (2013), Hagan et al. (2013) |
| Average egg set per hen | 11.3-10.3 | Ghana, Tanzania | Hagan et al. (2013), Guni et al. (2013) |
| Average egg per hen per year | 45.2-59.51 | Tanzania, Ethiopia | Guni et al. (2013), Zewdu et al. (2013) |

**Table 3.** Estimated number of hen egg production per year of native or local chickens in some African countries

| Country | Number of hen egg production per year | Reference |
|---|---|---|
| Ethiopia | > 80 | Dessie and Ogle (2001), Zewdu et al. (2013) |
| Morocco | 60- 80 | El Houadfi (1990), Benabdeljelil and Arfaoui (2001) |
| Senegal | 50 – 60 | Boye (1990), Missohou et al. (2002) |
| Somalia | 100 -144 | Ahmed (1990) |
| Namibia | 100-150 | van Niekerk (1998) |
| Togo | 80 -150 | Aklobessi (1990) |

**Ethiopia native chicken ecotypes**

Native chickens in many developing countries may include mixed (unspecified) breeds or ecotypes resulting from panmictic breeding (Mushi et al., 2005). Several ecotypes of chickens have been identified and characterized in Ethiopia, including *Tilili*, *Horro*, *Jarso*, and *Tepi*, as reported by Dessie et al. (2003). Additionally, *Gelila*, *Debre Elias*, *Melo-Hamusit*, *Guangua*, and *Mecha* were identified and characterized by Mogesse (2007), while *Farta*, *Konso*, *Mandura*, and *Sheka* were identified and reported by Dana et al. (2011), and *Hemete*, *Kukuate*, and *Yeberha Tsehaye* were reported by Getu (2014). Besbes (2009) found that improved genotypes were distributed in many countries to improve the livelihoods of beneficiaries. As indicated by Hassen et al. (2009), indigenous chickens in many parts of Africa have high genetic variability between and within ecotypes and populations, implying that genetic improvement of these chickens through selective breeding is possible.

**Ethiopian native chickens' performance**

***Native chicken production performance***

Backyard-reared chickens are generally low in productivity, producing (annually) around 40-60 small-sized eggs and varying degrees of hatchability, with low chick survival rate (Dana et al., 2010; Melesse and Negesse, 2011). According to some studies, approximately 40-60% of chicks that hatched, die within the first 8 weeks of life owing to various vaccine-preventable diseases and predators (Demeke, 2007; Molla, 2010; Moges et al., 2010). According to FAO (2010), there are no significant differences in the backyard chicken production system in five different zones of Ethiopia. Backyard-based chicken production needs less space and lower initial investment cost, compared to other livestock and thus plays an essential role in improving the livelihoods of resource-constrained families (Leta and Endalew, 2010). According to Moges et al. (2010), half of the eggs produced by laying hens must be incubated to replace those that have perished. The brooding period for these hens is relatively longer, and multiple cycles are required to make up for unsuccessful brooding attempts.

The smallholder system's productivity of village chickens is relatively inefficient, compared to on-station performance, characterized by low productive performance and high reproductive wastage, as indicated by some authors (Dessie and Ogle, 2001; Pedersen, 2002). Furthermore, Moges et al. (2010) assert that the production potential of native chickens is significantly constrained by their smaller egg size, reduced annual egg output, and lower body weight relative to exotic breeds. The available feed to the chickens also influences productivity, as quality and quantity vary erratically across seasons. On the other hand, these chickens can utilize locally available feed, including household waste, and they do not compete with humans for grain (Sonaiya, 1990).

Table **2** indicates the summary information of some native chicken production performance comparison in three African countries, namely Ethiopia, Ghana, and Tanzania.

Compared to their exotic counterparts, native hens have a strong maternal instinct and high broodiness (Dana et al., 2011). Research conducted by Dessie et al. (2003) revealed that the mean egg-laying performances of hens for their top three clutches were 17.0, 20.9, and 24.8, respectively. The Ethiopian Ministry of Agriculture (1980) also reported that under village conditions, native chickens produce 30 to 40 eggs, which can be doubled to 80 eggs per year with improved management such as feeding, watering, housing, and healthcare. The relatively lower productive performance of native chickens compared to the White Leghorn breed is attributed to their thicker eggshells. However, their fertility was higher when compared to the exotic chickens (Alemu and Tadelle, 1997).

Table 3 provides an overview of the estimated annual egg production for various indigenous chicken breeds from different African countries in comparison with that of native chickens in Ethiopia, presenting a comparative analysis of egg output per year among different groups. According to the summary review results, the average performance of egg production per hen per year in some African countries ranges from 20 to 150 eggs per year, which could be attributed to breed performance and management practices. Although the performance of Ethiopian native chickens per hen per year is comparable, the majority of them are scavenging chickens that are also poorly managed by the farmer community. Aside from improving breed performance, better management practices can increase the number of eggs harvested per year from individual chickens.

***Average egg per clutch***

Clutch size is the number of eggs laid in a single nest. The average egg per clutch is the number of eggs laid in a single nest when different chickens of similar ecotypes or breeds are considered. In the western region of Ethiopia, the average number of clutches per year is 2.67, with an average of 10.07 eggs per clutch and a single clutch duration of 27.9 days. According to a study by Alewi et al. (2015), the average number of eggs per hen per clutch in Bure, Fogera, and Dale districts of Ethiopia was 15.7, 13.2, and 14.9 eggs, respectively.

***Average clutch of a hen per year***

According to Mogesse (2007), an assessment of local hens in Northwest Ethiopia for their production performance average clutch per hen per year shows that these local chickens are expected to produce 2 to 3 clutches per year. Based on the assumption of three clutches per year for each individual, the hen would have to be out of production for approximately 168 days each year in their reproductive life. Meanwhile, in Southern Ethiopia, hens produce an average of 4.6 clutches per year, with each clutch consisting of about 15.4 eggs, as reported by Alemu (2020). In the same study, they reported that the average duration of egg-laying periods among local, hybrid, and exotic breeds of hens was 21, 38, and 159 days, respectively. Another study by Alewi et al. (2015) indicated that local hens in Metekel, Northwest Ethiopia, typically produce an average of 13.6 eggs per hen per clutch, and they have about 4.3 clutch periods per year under farmer management conditions. According to a study conducted by Banerjee (2012), the observed variations in the number of clutches per hen per year may be due to the genotype by environmental interaction effects. In addition to genetic factors, the poor management of traditional household poultry production systems may also contribute to the low productivity of native breeds. It is worth noting that these factors can significantly influence the reproductive performance of avian populations and have significant implications for conservation effort.

***Average egg of a hen per annum***

According to Tesfay et al. (2018), native household poultry in Ethiopia typically lays approximately 36 eggs in three clutches per year, with each clutch consisting of 12-13 eggs and lasting about 16 days. Meanwhile, Litigebew et al. (2021) reported an average of 3.2 clutches per year for each indigenous hen in Northern Ethiopia, with a mean clutch length of 21.6 days. Cross-bred hens had an average of 3.1 clutches per year, ranging from 18 to 40 days, while exotic breeds had an average of 3.2 clutches per year, with a mean clutch length of 44.4 days. In Northern Ethiopia, Getu et al. (2014) found that under small-scale management, local chickens produced an average of 54.3 eggs per year, with an egg weight of 42.2 g. The total number of eggs produced per hen per year in the Bure, Fogera, and Dale regions of Ethiopia was reported as 60, 53, and 55, respectively. However, according to Tadesse et al. (2015), indigenous poultry in Northwest Ethiopia yielded an average of 59.5 eggs per year under household management conditions. Metanne and Afardual (2015) reported that a significant number of average eggs per hen per annum was 78 eggs for Moroccan hens, with a general mean egg size of 44.1 grams. Meanwhile, Getu and Birhan (2014) observed that the household management system typically resulted in low productivity of indigenous chickens due to high chick mortality rates prior to hatching. This lack of controlled breeding methods and management, along with uncontrolled breeding between different ecotypes of indigenous poultry, likely contributes to the variable performance of native breeds.

**Table 4.** Different reproduction performances of native chicken ecotypes conveyed in different parts of Ethiopia

| Parameters | Average | Sites | References |
|---|---|---|---|
| Age at first laying (month) | 6.9-7.13 | BG, B | Sisay (2017), Moges et al. (2010) |
| Age of cockerels at first mating (month) | 5.87-6.15 | SW, B | Negasi and Melaku (2016) |
| Age of pullets at first mating (month) | 5.20-5.93 | BG, M | Sisay (2017), Zewdu et al. (2013) |
| The reproductive life span of males (year) | 3.79 | M | Zewdu et al. (2013) |
| The reproductive life span of hens (year) | 3.56 | M | Zewdu et al. (2013) |
| Fertility (%) | 75-78.6 | B, WG | Moges et al. (2010), Mogesse (2007) |
| Hatchability (%) | 59.6-82.83 | B, EG | Moges et al. (2010), Yitbarek and Zewudu (2014) |
| Brooding length (month) | 3.5 | EG | Yitbarek and Zewudu (2014) |

BG: Benishangul-Gumuz, SW: South Wollo, M: Metekel, B: Bure, EG: Eastern Gojjam, WG: West Gojjam

**Table 5.** Linear body measurements of male and female indigenous chickens from different parts of Ethiopia

| Parameters | Female | Male | Sites | References |
|---|---|---|---|---|
| SL | 6.53 ± 0.13 | 7.42 ± 0.27 | Arsi, Oromia | Negassa et al. (2014) |
| CHC | 25.06 ± 0.06 | 24.98 ± 0.13 | North Shewa | Yisma and Kebede (2015) |
| CL | 2.48 ± 0.73 | 4.82 ± 1.70 | Arsi, Oromia | Negassa et al. (2014) |
| BW | 1.37 ± 0.02 | 1.63 ± 0.03 | North Gonder | Getu et al. (2014) |
| BDL | 22.65 ± 1.40 | 24.11 ± 1.11 | Arsi, Oromia | Negassa et al. (2014) |
| KL | 8.29 ± 0.02 | 8.34 ± 0.05 | North Shewa | Yisma and Kebede (2015) |
| WL | 1.48 ± 0.03 | 3.97 ± 0.10 | South Wollo | Negasi and Melaku (2016) |
| NL | 10.8 ± 0.05 | 11.1 ± 0.12 | North Shewa | Yisma and Kebede (2015) |
| BKL | 17.0 ± 0.05 | 17.3 ± 0.13 | North Shewa | Yisma and Kebede (2015) |

CHC: Chest circumference (cm), BKL: Back length (cm), SL: Shank length (cm), NL: Neck length (cm), KL: keel length (cm), CL: Comb length (cm), BDL: Body length (cm), WL: Wattle length (cm), and BW: Body weight (kg)

### Native chicken reproductive performance

#### *Age of sexual maturity and first mating*

The information provided in Table 4 summarizes various studies conducted on the age at sexual maturity of male and female native chickens, including the number of eggs per clutch, the number of clutches per year, and egg production per hen per year in different regions of Ethiopia. Genetic and non-genetic factors may influence the observed differences. Guni et al. (2013) found differences in the age at first egg for pullets due to genetic and non-genetic factors. Native female chickens reach sexual maturity at 27.2 weeks or 6.8 months. Another report by Owoya et al. (2018) indicated that the average age for native chickens to reach sexual maturity is 23.48 weeks and 23.6 weeks, respectively. In contrast, Moges et al. (2010) reported the average age of initial mating for male and female chickens to be 24.6 and 27.5 weeks, respectively, in the Burie district of Ethiopia. In Beneshangul-Gumuz, western Ethiopia, male chicks reach initial mating age at 24 weeks (Sisay, 2017).

The average age of initial mating for native male chickens in the Metekel zone of Northwest Ethiopia was reported to be 20.8 weeks (Alewi et al., 2015). Similarly, Kamel (2016) found that the initial mating age for the crosses of Fayoumi and Naked-neck and the Rhode Island Red and indigenous white poultry was 26.1 and 26.4 weeks, respectively.

#### *Age at first laying*

According to Sisay (2017), native chickens in the western Amhara region of Ethiopia start laying eggs at 26 weeks of age. Furthermore, in Beneshangul-Gumuz, western Ethiopia, the average ages for female chickens' first mating and egg-laying are 23.7 and 28.5 weeks, respectively. According to Litigebew et al. (2021), native local breeds of chicken between 24 and 28 weeks of age laid their first eggs at an average age of 27.2 weeks compared to hybrids and exotics of the same age laid their first eggs at an average of 25.7 and 25.4 weeks, respectively. The findings of the research suggested that cross-bred and exotic chicken breeds have a shorter onset time for laying eggs compared to indigenous chicken ecotypes/breeds and can initiate the process at younger ages. According to reports from other parts of Ethiopia, the age of the first clutch of chickens is shortened as the breed's genotype is upgraded from a local low-yielding to an exotic high-yielding one.

#### *Reproductive life span*

According to Kibret (2008), the reproductive life span of native chickens is longer than that of exotic breeds. However, the author also noted that in terms of long-term reproductive performance, including life span, fertility, hatchability, and egg production, exotic breeds have a better performance than native breeds. The reproduction potential of native chickens is harmed because they mature later than exotic chickens (Pedersen, 2002). This condition could be attributed to selection goals, with native chickens primarily chosen for their adaptive characteristics from a socio-cultural angle. In contrast, exotic chickens were chosen for their production and reproductive abilities. According to Zewdu et al. (2013), the reproductive life spans of males and females in the Metekel zone of Northwest Ethiopia were 3.79 and 3.56 years, respectively.

#### *Hatchability percentage*

Hatchability percentage is the proportion of eggs that survive incubation and hatch into chicks. Hatchability is a crucial economic factor in the poultry industry because it significantly impacts chicken output Malik et al. (2015). From the early years to now, eggs from native chickens were hatched by placing them under broody hens in Ethiopia. Moges et al. (2010) reported a natural hatchability percentage of 82.83% for native chickens from the Bure district of northern Ethiopia. The natural hatching percentage of local chickens in Ethiopia under the backyard management system is higher than the hatching percentage of local chickens (73.6%) under backyard management conditions in Pakistan (Farooq et al., 2003). This difference in hatchability could be attributed to differences in the genotype and husbandry practices of chickens in different parts of the world. In addition, as indicated by Kirunda and Muwereza (2011) and Yemane et al. (2013), the hatchability can be affected by a factor such as laying season, disease, nutrition, age, egg quality, genetic factors, hygiene, and the condition of incubation. Furthermore, the variation in hatchability may also arise from the incapacity of broody hens to generate sufficient heat when attempting to incubate a number of eggs that surpass their ability to accommodate beneath their wings.

#### *Brooding length*

In the poultry industry, the phrase "chicken brooding length" refers to young chicks (0-8 weeks old) that require additional warmth to maintain their average body temperature. Hassen et al. (2009) found that native hens in Northern Ethiopia have a brooding length of 56 days when raised under scavenging conditions. Conversely, Yitbarek and Zewudu (2014) reported a more extended brooding length (3.5 months) in the Eastern Gojjam region of Ethiopia, indicating a higher level of variability in brooding length in Ethiopia.

### Morphometric and morphological characterization of Ethiopian native chickens

#### *Morphometric characteristics of Ethiopian native chickens*

According to the Food and Agriculture Organization (FAO, 2012), phenotypic characterization of livestock, which entails identifying diverse breed populations and characterizing their features and production conditions, is a word widely used to describe the process of studying chickens. The term "Indigenous breed" is used to refer to chickens that are raised under a complex system, scavenge in the wild, lack a distinct description, have multiple purposes, and are found in large numbers, as stated by Horst (1989). Indigenous chickens are known to possess variable morphological characteristics and genes with adaptive values to their environment and diseases. They have a variety of morphological qualities as well as genetic characteristics that aid in adaptation to various habitats and disease resistance. Certain local breeds, for example, may have developed inherent resistance to common chicken diseases prevalent in their region, allowing them to survive under certain conditions. Furthermore, morphological characteristics such as feather color, body size, and beak shape can change dramatically amongst indigenous chicken populations, indicating adaptations to local climates, predator avoidance, or other environmental factors. According to Horst (1989), indigenous chickens can also serve as a gene pool, especially for genes linked to adaptive values in tropical environments. The diversity of phenotypes observed in Ethiopian indigenous chickens is also a clear indication of their high genetic variability (Aklilu, 2013). Various researchers have reported some linear body measurements of indigenous chickens from Ethiopia, which are summarized in Table 5.

#### *Morphological characteristics of Ethiopian native chickens*

In Ethiopia, the plumage color of a chicken holds significant socio-cultural and religious value. According to Dessie and Ogle (2001), the red and white cock is sacrificed to invoke good rainfall and bountiful harvest, while the red and black spotted (Gurraacha) cock is offered during the New Year's celebration. Similarly, the white and black spotted (Gebsima) cock is sacrificed to avert evil and calamities, and the red pullet is offered as a sacrifice for deceased ancestors following animistic beliefs. The differences in plumage type are also related to adaptive features, with frizzled and naked-neck birds better adapted to tropical climates (Melesse and Negesse, 2011). Variations in morphology may impact the market value of chickens (Mengesha, 2012). The information provided in **Error! Reference source not found.** summarizes native Ethiopian chickens have distinct morphological characteristics and are found in various ecotypes of the country.

As mentioned by Nesheim et al. (1979), the size and color of a chicken's comb and wattles are closely linked to gonad development and the secretion of sex hormones. In hot tropical regions, the morphological characteristics of large combs, wattles, and long legs play a crucial role in dissipating heat, as emphasized by Horst (1989). Although they are not classified as major genes, these characteristics are the outcome of a combination of multiple genes and their intricate interplay. Therefore, when striving to breed high-performance local chicken species suitable for hot tropical climates, it is imperative to incorporate the coding genes responsible for these characteristics, as suggested by

Horst (1989).

**Table 6.** Some distinct morphological characteristics of Ethiopian native chickens' ecotype

| Ecotype | Distinct morphological feature | Sites | References |
|---|---|---|---|
| Mecha | Plain and crest head shape, pea comp | West Gojjam | Mogesse (2007) |
| Farta | Crest head shape, pea comp type | South Gondar | Mogesse (2007) |
| Sheka | Flathead, pea comb, yellow shank color | SNNP region | Dana (2011) |
| Horro | Flathead shape, pea comb, yellow shank color | East Welega | Mogesse (2007) |
| Jarso | Red plumage color, no black eye color | East Hararghe | Aklilu et al. (2013) |
| Tepi | Naked neck, black eye, single combed | Tepi | Dessie Alemayhu (2003) |
| Tilili | Pea comb, lack of shank feather | West Gojjam | Mogesse (2007) |
| NN | Aggressive, absent of feather at neck | Quara | Getu et al. (2014) |
| Gasgie | Long-necked and red color | Alefa | Getu et al. (2014) |
| Sheka | Flathead, pea comb, yellow shank color | SNNP region | Dana (2011) |

NN: Necked neck, SNNP: Southern Nations, Nationalities, and Peoples' Region


**Figure 1**. Morphological characteristics of some Ethiopian native chickens. A: Male and female chickens of Horro (Dana et al., 2010), B: Cock with red (Kei) plumage, southeastern Oromia, Ethiopia (Negassa et al., 2014), C: Wosera (yellowish brown) hen with a single comb and yellow shank of southeastern Oromia (Negassa et al., 2014), D: Sheka male chickens (Dana et al., 2010), E: Tikur (Black plumage color hen), a single comb and black shank, Southeastern Oromia, Ethiopia (Negassa et al., 2014) and F: A single comb 'Gebsima' male of the Farta (Dana et al., 2010).

According to Alemu and Tadelle (1997), local chickens in Ethiopia exhibit significant variation in their physical attributes, such as body size, conformation, and plumage color. The native chicken breeds are distinguished by different names like Netch (pure white), Tikur (black), Keyi (deep red), Gebsima (mix of grayish shades), Anbesima (multicolored), Serago (white with red stripes), Libework (white with golden breast color), Key teterima (red with white stripes), Netch teterima (white with black or red stripes), Tikur teterima (black with white stripes), Kokima (red-brownish). In addition to plumage color, Kibret (2008) states that when naming native chicken ecotypes, people consider body shape, kind of feathering, and further phenotypic characteristics. Figure 1 displays a list of some morphological features of Ethiopian native chickens, along with their corresponding descriptions.

## CONCLUSION

This review has identified significant differences in the production and reproductive performance as well as morphometric and morphological characteristics among the different ecotypes of native chicken populations in Ethiopia, which are managed by hundreds of millions of rural smallholder farmers. Although the significant variability of Ethiopian native chicken across different agroecological zones of Ethiopia is reported by many researchers, each native chicken ecotype in Ethiopia is named after its color and place of discovery. Moreover, no distinct breed is reported. To preserve the genetic diversity of these native chicken population, a well-organized and comprehensive research approach is required. This should involve characterizing native chicken ecotypes in all Ethiopian agroecologies, under the same season, to reduce non-genetic variation in production and reproduction performance characteristics, as well as determining morphometric and morphological variation with the support of molecular characterization. The results of the present study can be used to categorize the different ecotypes among distinct breeds before they are entirely mixed with exotic or cross-bred chickens.

## DECLARATIONS

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**Availability of data and materials**

For this study, no new data were created or analyzed, instead, published research, articles, and other verified materials from multiple sources were used. As a result, the data supporting the context of this study are entirely extracted from the resources cited in the reference section of this research, and data sharing is not applicable to this article.

**Authors' contributions**

Kefala Taye Mekonnen and Kang-Seok Seo conceptualized the idea and Kefala Taye Mekonnen, Dong-Hui Lee, and Young-Gyu Cho wrote the first draft of manuscript. Kefala Taye Mekonnen, Dong-Hui Lee, Young-Gyu Cho, and Kang-Seok Seo reviewed and edited the writing and all authors have read and approved the final manuscript.

**Ethical consideration**

The authors have ensured that the research adheres to ethical principles such as avoiding plagiarism, obtaining consent to publish, avoiding misconduct, preventing data fabrication or falsification, refraining from double publication or submission, and avoiding redundancy.

**Competing interests**

The author declares that there is no competing interest with any financial organization regarding the materials discussed in the manuscript.

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# Antioxidant Properties and Toxic Risks of Using Metal Nanoparticles on Health and Productivity in Poultry

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**ABSTRACT**

Metal nanoparticles (NPs) are introduced into various fields of science, particularly poultry farming. Supplementation of metal salts in nanoform can increase the profitability of poultry farming by enhancing meat and egg production. Although their toxic parameters pose limitations on their use, many studies have evaluated the effects of using metal NPs in modern poultry farming on health, productivity, metabolism, and especially antioxidant properties. In addition, the peculiarities of their toxicokinetic and recommended doses that meet safety criteria in practical activities are highlighted. Zinc oxide NPs are one of the most studied compounds in the poultry industry. Their pronounced antioxidant properties, positive effect on productivity and homeostasis of poultry, egg quality, and immune status have been experimentally confirmed. Copper oxide NPs have similar properties but are limited in usage due to their toxicokinetics. Silver and gold NPs emerge as potential alternatives to antibiotics and could solve the resistance problem of microorganisms to antibiotics. Other important NPs used in poultry are Iron and Calcium. In their nanoform, these NPs exhibit high bioavailability, which allows for efficient absorption and utilization by poultry. The methods used to synthesize these nanoparticles make it economically viable to incorporate them into poultry diets, reducing overall expenses compared to similar macroergic compounds. Manganese and chromium NPs positively affect sperm survival in turkeys during refrigerated storage and contribute to increasing the resistance of the broiler chickens' body to heat stress and normalizing the metabolism of sex hormones. In conclusion, the application of metal nanoparticles to poultry is a promising research direction, aiming at the development of feed additives, antibiotics, and growth stimulants due to their antioxidant, bactericidal, and immunomodulatory effects.

**Keywords:** Antioxidants, Health, Metal Nanoparticles, Poultry, Productivity, Toxicology

## INTRODUCTION

The poultry industry is a part of the agri-food sector, playing a vital role in ensuring food security (Ahmad et al., 2022). Advancements in technology have led to the development of innovative feed additives based on nanomaterials, as well as the introduction of nano vaccines and diagnostics to increase the efficiency of the final poultry product (Skliarov et al., 2021a). Modern science, specifically nanobiotechnology, is actively engaged in improving diagnostic and therapeutic measures and addressing the solution of nutritional problems in animal husbandry and poultry farming using nanotechnological means (Patra and Lalhriatpuii, 2020).

Nanoparticles (NPs) are becoming widely used in poultry farming due to their advantageous characteristics, such as low toxicity, high bioavailability, significant surface area compared to macroergs, and prolonged action (Rana, 2021). Various mineral NPs have become a significant focus of research in animals, including poultry. It should be noted that mineral NPs are characterized by small sizes and exhibit a wide range of properties due to increased digestibility in the gastrointestinal tract and the effect on target tissues of the body (Patra and Lalhriatpuii, 2020).

Nanoparticles have a positive effect on the health, productivity, quality, and safety of their products due to the regulation of hematopoietic vascular, digestive, and excretory. One of the most significant benefits is their ability to protect against free radical oxidation and their immunomodulatory properties (Michalak et al., 2022). Although there are numerous publications on the effects of NPs on animals and poultry, research specifically focused on the impact of trace elements in nanoform on farm animals remains limited. However, several studies have pointed out the positive changes (feed digestibility and live weight gain) occur due to the use of trace elements in feeding various types of poultry. Of particular importance is the use of silver (Ag), copper (Cu), and zinc (Zn) NPs as an alternative to antibiotics against the main pathogens of poultry infectious diseases, such as *Salmonella* and *Campylobacter* (Hassan et al., 2020). In addition, the use of NPs with an enzyme-like effect (for example, similar to glutathione peroxidase) will contribute to preventing infertility and improving the qualitative indicators of various species of poultry sperm (Koshevoy et al., 2021).

It should be noted that the results of some studies have indicated that metal NPs *in vivo* and *in vitro* experiments can negatively affect the intestinal function and health of poultry (Kolba et al., 2020). Another application of NPs in poultry lies in developing nano vaccines to replace traditional viral vector vaccines. This highlights the significant antiviral potential of NPs, which can be attributed to their unique physical properties and pharmacokinetics (Fawzy et al., 2021). Metal NPs play a notable role in stimulating the immune system, especially its humoral link. It should be noted that the use of NPs as immunomodulators will prevent an increase in the resistance of poultry to infectious diseases (Ren et al., 2021).

The authors of the current study opine that a promising direction of research for modern poultry farming involves using NPs of metal oxides and salts with antioxidant properties and low toxicity as well as developing safe regulations for their use. Therefore, the aim of this study was to explore the toxic risks and antioxidant properties of metal NPs, and their impact on health and productivity when applied to poultry.

### Metal nanoparticles in poultry farming

Regarding the use of metal NPs in poultry farming, many studies indicate that nanometals exhibit increased bioavailability compared to their macroergic counterparts. Of particular significance are their unique antioxidant properties, which are not commonly found in traditional forms of metals (Koshevoy et al., 2022).

Metal NPs are widely used in treating, diagnosing, and preventing diseases of various etiologies (Koshevoy and Naumenko, 2023). It is known that the deficiency of some essential metals leads to the occurrence of many pathological disorders. It is shown that most diseases are accompanied by significant changes in the balance of metals and trace elements, and the introduction into the body leads to therapeutic results (Skliarov et al., 2021b).

It has been established that NPs have a high biological activity, and it is necessary to take into account the factors (form-factor, geometry, solubility, dimensions) the design of which, in turn, will allow to adjust the activity of NP metals if necessary. For the synthesis and application of NPs, it is necessary to use a comprehensive, safe, and effective approach to assess possible health and environmental risks. The unique parameters (physical and chemical) of metal NPs allow for obtaining special modulations of absorption mechanisms, which will solve the problem of mineral antagonism in the poultry intestine (Patra and Lalhriatpuii, 2020). The NPs can improve the immune response of the chicken's body and digestion due to the normalization of the digestibility of nutrients from feed, contributing to effective feeding and decreased rate of early embryonic mortality (Givisiez et al., 2020).

Their bioavailability usually decreases when passing through the gastrointestinal barriers, the intestinal mucosa, and the liver (Skliarov et al., 2021c). Depending on the size, NPs can transit through the digestive tract without being absorbed by the body, or penetrate through the intestine and enter the organs and tissues with the bloodstream. The addition of metal NPs to the diet of broilers improved the hatchability of chickens and their growth, optimized immune responses, and reduced oxidative stress due to NPs effects on the antioxidant defense system (Michalak et al., 2022).

### Statistical analysis

In conducting a comprehensive review of the scientific literature to assess the toxic risks associated with using metal NPs for poultry, as well as evaluating their antioxidant properties, the authors of this study analyzed the results presented in 70 articles sourced from the scientific databases Scopus and PubMed. The selection of these articles focused primarily on publications from the last 10 years, utilizing specific metal NPs, poultry, antioxidants, health, and productivity. Following the compilation of relevant data, the results were processed and organized based on the metal-forming principle.

**Zinc nanoparticles**

Zinc oxide nanoparticles (ZnO-NPs) are considered safe and stable antimicrobial agents that can inactivate bacteria through several potential mechanisms of action (Hakeem et al., 2020). In addition, ZnO-NPs serve as an alternative source of the mineral zinc in poultry diets. Depending on the dosage, form factor, and size, they can increase productivity, normalize hormonal balance, and exert an antioxidant effect. The toxicological parameters of zinc and its compounds can cause an oxidative imbalance, and initiate oxidative modifications of lipids and proteins, leading to damage to cell membranes and mitochondria, as well as negative effects on DNA. The toxic effect of NPs depends on the dose, size, and shape, and is sometimes more toxic than similar macro compounds.

According to Mahmoud et al. (2020), the use of ZnO-NP in low doses accelerates the weight gain of the bird and improves the digestibility of all dietary components, without compromising their resistance to diseases. Different doses of ZnO-NP could affect the change in the relative weight of the stomach, compared to the intact bird. At the same time, a low dose (10 ppm) of ZnO-NP increased the weight of the spleen and the weight of the bursa (in all groups of poultry), compared to the control group. It should be noted that a high dose (40 ppm) of ZnO-NP contributes to the increase in pH, reduced meat color, and improved broilers' resistance to Newcastle disease (Mahmoud et al., 2020).

The use of dietary supplements containing ZnO-NPs at doses ranging from 20 to 60 mg/kg is beneficial for broiler chickens, especially under heat-stress conditions. These supplements have demonstrated significant improvements in body weight gain, feed digestibility, and carcass characteristics of the birds. Notably, ZnO-NP supplementation enhances the functions of the digestive tract and the excretory system. The administration of ZnO-NPs at these doses does not lead to a toxic effect on serum levels of trace elements and thyroid hormones compared to intact controls (Abdel-Wareth et al., 2022).

The antioxidant properties of these NPs depend on their quantity in the diets of broilers, leading to a compensatory effect on the redox status of the serum of birds and their tissues. In high doses (40-60 mg/kg body weight), ZnO-NPs cause intensification of lipoperoxidation (an increase in the content of thiobarbiturate-active products) in the femoral muscles of broiler chickens 7 days after death, indicating that ZnO-NPs induces a system of antioxidant protection in muscle tissues. The use of ZnO-NP reveals antioxidant properties and an anti-stress effect, evidenced by a decrease in the level of the stress hormone (corticosterone) in blood when the bird is kept in conditions with increased environmental temperature. At the same time, ZnO-NPs can mitigate the negative effects of thermal stress, as a result of which further research is necessary for introduction into poultry diets (Ramiah et al., 2019).

Zhao et al. (2014) found that total antioxidant capacity (TAC) in the serum and liver tissue of broilers was significantly higher in the ZnO-NPs (20 mg/kg) group at all time points, as compared to the intact control group. Additionally, in the 60 mg/kg and 100 mg/kg ZnO-NPs groups, the TAC was significantly higher in blood serum on days 28 and 35, and in liver tissues on days 21 and 28. Compared to the control, the activity of copper-zinc superoxide dismutase (SOD) was significantly higher in the blood on days 28 and 35, and in the liver tissues after 21 days, specifically in the 60 mg/kg and 100 mg/kg ZnO-NPs groups. The malondialdehyde (MDA) content in blood serum and liver tissues decreased significantly in the 20, 60, and 100 mg/kg ZnO-NPs groups, compared to the control group.

The SOD activity in the liver, pancreas, and blood plasma increased significantly in the chickens treated with ZnO-NPs. The MDA content in eggs was significantly reduced in the ZnO-NPs treated groups. It was found that ZnO-NPs as a dietary supplement could improve the productivity of laying hens, and levels of 40 to 80 mg/kg ZnO-NP are optimal concentrations (Abedini et al., 2018b).

Dietary supplementation to broiler chicks ZnO-NPs increased TAC, and 80 mg/kg ZnO-NPs decreased MDA content in the small intestinal mucosa, compared to the $ZnSO_4$ group. In contrast, dietary ZnO-NPs did not alter the mRNA expression of superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, heme oxygenase-1, and NADPH plus quinone oxidoreductase 1. No significant difference was found in individual mineral concentrations (Mn, Cu, Fe, and Zn) in the liver among the $ZnSO_4$ and ZnO-NPs groups. However, 160 mg/kg ZnO-NPs increased fecal Zn, Fe, and Cu, but did not affect fecal Mn (Zhang et al., 2022). Generally, the use of these NPs as correctors of antioxidant protection and scavengers of oxygen radicals is an effective alternative to macroergic antioxidants. It should be noted that the oral administration of ZnO-NPs does not have a negative effect on the immune status and DNA integrity in broilers. The study by Mahmoud et al. (2021) indicated minor liver histopathological changes and DNA damage. However, the treatment did not significantly affect the levels of IgG, IgM, and interferon-gamma. Results of a study conducted by Ramiah et al. (2020) indicated that dietary ZnO-NPs altered the gene expression of *cholecystokinin* (ileum), heat stress proteins (HSP) 70 (jejunum and ileum), and HSP 90 (duodenum, small intestine, and ileum). The interaction between ZnO-NPs concentration and temperature in the duodenum and stomach influenced ghrelin gene expression.

It was also found that 20 ppm ZnO-NPs increased calcium (Ca), low high-density cholesterol, and decreased urea and triglyceride levels. Moreover, 40 ppm ZnO-NPs increased creatinine. Hematological and immunological parameters showed significant dose-dependent modulation by ZnO-NPs supplementation. The phagocytic activity, phagocytic index, and levels of IgM and IgG were all influenced by the dosage of ZnO-NPs. The best values for these parameters were observed in broilers that received 5 and 10 ppm ZnO-NPs/kg diet, followed by the 20 ppm group. So, to eliminate the risk of heat stress of broilers in the summer season, ZnO-NPs should be added to the diet in a dose of no more than 10 ppm/kg of diet (Dosoky et al., 2022).

For the first time, Zhang et al. (2017) reported that intact NPs had a different effect on the egg yolk proteome, compared to $Zn^{2+}$. A total of 37 proteins were specifically regulated by ZnO-NPs (50 mg/kg), 22 proteins were altered exclusively by $ZnSO_4$ (similar dose), and 17 proteins were regulated by both ZnO-NPs and $ZnSO_4$. In addition, changes in protein levels due to ZnO-NPs in egg yolk may affect the nutritional value of egg yolk and embryonic development. The ZnO-NPs only slowed the laying rate at the beginning of the laying period. The ZnO-NPs did not affect egg protein or water content, slightly reduced egg physical parameters, such as shell thickness and porosity (12-30%) and trace elements (20-35%) after 24 weeks of treatment. However, the lipid content in the yolk significantly decreased under the influence of ZnO-NPs (from 20 to 35%). The mechanism of action of yolk lipid-lowering ZnO-NPs is that they decrease lipid synthesis and increase lipid digestion (Zhao et al., 2016).

Egg laying and egg weight were significantly higher in the ZnO-NPs treated groups. In addition, eggshell thickness and shell strength increased in the ZnO-NPs group, compared to the other groups. Furthermore, the addition of Zn reduced egg loss. There were significant differences between zinc deposition in the tibia, liver, pancreas, eggs, and feces. The addition of ZnO-NPs supplements to the diet of broiler increased SOD activity in the liver, pancreas, and plasma, but decreased MDA content in eggs (Abedini et al., 2018a).

According to the results of Mesak et al. (2018), ZnO-NPs did not affect the locomotor activity of chicks and did not cause anxiolytic or anxiogenic effects on birds in an open-field test. However, based on the lowest cluster score recorded during the social aggregation test, chicks exposed to ZnO-NPs failed to recognize predatory threats, compared to the control group. The findings highlighted the challenges in evaluating the effects of introducing NPs on the behavioral characteristics of birds. While these indicators play a crucial role in regulating higher nervous activity, their complexity makes the assessment process difficult. The higher concentration of Zn in the brains of animals exposed to ZnO-NPs indicates the ability of these NPs to cross the blood-brain barrier even at low concentrations. Between 1 and 42 days of age, broiler chickens fed 100 mg/kg ZnO-NPs showed lower feed intakes and feed conversion ratios than controls. The amount of Zn accumulation in the liver was significantly higher in all treatment groups than in breast and thigh muscle tissues regardless of temperature conditions (both at normal temperature (control group) and under conditions of heat stress (experimental group, Ramiah et al., 2020).

Adding ZnO-NPs to the sperm storage medium can be introduced as an effective method to preserve the quality of rooster sperm during the cooling period. Extender supplementation with 100 μg/ml ZnO-NPs showed higher total motility, progressive motility, activity mitochondria, viability, membrane integrity, and lower lipid peroxidation, compared to other groups during 22 and 45 hours of refrigerated storage. The fertility rate of 22-hour chilled sperm samples was higher in the ZnO-NPs groups, compared to the control group (Khodaei-Motlagh et al., 2022).

A positive effect of metal oxide NPs, such as ZnO-NPs, $ZnO_2$-NPs, and $TiO_2$-NPs, against multi-resistant and/or pan-resistant strains of *staphylococcus (S.) aureus* has been observed. $ZnO_2$-NPs exhibited higher inhibitory activity against *S. aureus* strains, compared to $ZnO_2$-NPs and $TiO_2$-NPs. The anti-inflammatory activity results suggest that $ZnO_2$-NPs are a lead compound for developing an alternative antimicrobial agent against drug-resistant and virulent *S. aureus* isolates (Ali et al., 2021).

### Copper nanoparticles

Copper is an important vital element playing a role in different physiological processes, such as hemoglobin synthesis, hematopoiesis, bone formation, and functions of the nervous system. It also is a part of the enzymes tyrosinase and cytochrome oxidase (El-Sabry et al., 2021).

Copper nanoparticles (CuO-NPs) have a small size and high surface-to-volume ratio, so they are relatively bioavailable. Sharif et al. (2021) demonstrated the potential effects of CuO-NPs on growth, antioxidant status, immune system, nutrient digestibility, and feed conversion ratio in poultry. Using small concentrations of CuO-NPs as a feed supplement does not negatively affect probiotic strains, and important normal microflora of poultry (*Lactobacillus, Enterococcus, Enterobacterium*). The positive effect of Cu is related to the low level of dissociation of NPs since biologically active ions are released more slowly, creating a prolonged effect of exposure (Sizentsov et al., 2018).

A comparative analysis of the influence of Cu sources on the improvement of energy and nitrogen use has indicated the dominance of the influence of CuO-NPs over $CuSO_4$ (Scott et al., 2018). Blood cholesterol, urea, and glucose levels were reduced by CuO-NPs treatment, compared to other groups. With the addition of Cu supplement to the diet, the relative weight of the liver was reduced, and the Bursa of Fabricius was enlarged. The Cu excretion decreased only in chicks (using as a water supplement in a dose of 50 mg/kg CuO-NPs with 20 mg/kg of water). Immune genes were not affected by the treatment. However, the administration of CuO-NPs *in ovo* is more effective for broiler performance than the administration of CuSO4 or using CuO-NPs and/or CuSO4 as a water supplement (Scott et al., 2018).

Morsy et al. (2021) showed a dose-dependent increase in the levels of MDA, Cu content, percentage of DNA fragmentation, and microscopic estimations of different organs of the chickens treated with CuO-NPs. The histopathological changes, decreasing weight gain, food conversion ratio, catalase activity, and antibodies titers of both New Castle and Avian Influenza viruses were observed in birds treated with CuO-NPs with variation in severity.

CuO-NPs used as a feed supplement for poultry, affected the assimilation of mineral elements. Thus, the oral administration of CuO-NPs to chickens in doses of 5, 10, and 15 mg/l leads to its accumulation in the intestinal walls. The highest level of Cu NPs application increased the content of Cu in the blood plasma of birds. An *in vitro* study showed that Cu accumulated in the intestine reduced Ca and Zn absorption but had no effect on iron absorption (Ognik et al., 2016).

CuO-NPs exhibit superior pro-angiogenic capabilities, compared to $CuSO_4$ salt. Its significant effects on the mRNA concentration and on the mRNA gene expression of all pro-angiogenic and proliferative genes at the molecular level have been confirmed by Mroczek-Sosnowska et al. (2015).

According to Sawosz et al. (2018), using CuO-NPs instead of traditional CuSO4 supplements, the amount of Cu provided to the animals is decreased by 75 %, but this reduction does not negatively impact their growth. At the same time, it increasingly reduces the release of Cu into the environment. The CuO-NPs supplement linearly increases body weight, average daily weight gain, and feed intake in broiler chickens. The levels of uric acid, blood glucose, and feed conversion ratio decrease linearly with the addition of CuO-NPs to the diet.

Using CuO-NPs supplement increases weight, length, diameter, weight/length index of the leg, and tibiotarsal index in broiler chickens. It also improves the muscle parameters of broilers, pH, fiber diameter, fiber cross-sectional area, bundle diameter, and bundle cross-sectional area. The concentrations of Cu, iron, Ca, and phosphorus increased in the blood after administration of the CuO-NPs (Abdullah et al., 2022).

The results of Kozłowski et al. (2018) showed that reducing Cu levels from 10 mg/kg to 2 mg/kg in the diet of turkeys did not worsen their growth performance, but weakened the antioxidant protection. Dietary supplementation of Cu at a dose of 20 mg/kg induced an oxidation reaction and inhibited antioxidant protection to a greater extent than at 2 mg/kg. The CuO-NPs dietary supplements also have a more positive effect on carbohydrate metabolism and antioxidant status in turkeys, compared to $CuSO_4$. The analysis of the antioxidant and metabolic status of young turkeys showed that Cu at the dose of 10 mg/kg was an optimal level.

The high level of phagocytic activity, serum lysozyme activity, and activation of immunomodulatory genes, including NF-κβ, PGES, IL-1β, TGF-1β, IFN-γ, *BAX*, and *CASP8* demonstrate a significant enhancement of the immune response in chickens treated by CuO-NPs under the normal temperature (El-Kassas et al., 2018). The responses between two tested broiler strains (Ross 308 and Cobb 500) were different, especially in terms of gene expression. CuO-NPs reduced heat stress-induced inflammatory conditions in broilers. This is evidenced by low gene expression, the absence of degenerative changes in the spleen, and an altered ratio of heterophils and lymphocytes. Supplementing chickens' feed diets with CuO-NPs is recommended, particularly when feed is supplemented with 50% of the required Cu amount. This addition is especially beneficial at normal housing temperatures as it enhances the immune response of birds, particularly during heat stress. The use of CuO-NPs helps reduce degenerative changes induced by heat stress (El-Kassas et al., 2018).

According to Mroczek-Sosnowska et al. (2017), the femurs from the CuO-NPs group exhibited increased weight and volume, and they displayed significantly higher resistance to fracture when compared to the control group. CuO-NPs helped the proliferation of PCNA-positive cells in the long bones of chickens. A higher number of PCNA-positive cells in the bones of chickens treated by CuO-NPs compared to the control group (137 and 122, respectively) suggested a stimulating effect during embryogenesis.

**Silver nanoparticles**

Argentum is necessary for the functioning of the endocrine glands, brain, liver, and other organs (Hassan et al., 2020). It is the most deficient trace element and the best conductor of heat and electricity among metals. Argentum is a heavy metal with a negative effect on the body. With prolonged use, Ag accumulates in the body and causes toxic reactions. Silver nanoparticles (Ag-NPs) have antimicrobial activity and are potential candidates for replacing antibiotics in animal husbandry (Gallocchio et al., 2017).

The Ag-NP is a precipitated form of metal ions constantly generated and removed from surfaces when they bind to biological substrates. High concentrations of ions are locally created at the surface of the particles. They are harmful to microbes, but harmless to the organism. At the same time, it provides a milder, prolonged action of Ag cluster and colloid product (Shaikh et al., 2021).

Ag-NPs have a positive effect on the integrity of the intestine without affecting the immune organs. However, some residue of these particles remains in chicken muscles, necessitating further studies on the concentration and size of NPs, as well as the route of administration and withdrawal time to prevent any potential harm to the chicken muscles (Salem et al., 2021).

The supplementation of Ag-NPs improves the growth performance of broiler chickens, as evidenced by higher body weight, increased muscle mass, and enhanced feed efficiency. Notably, acid-insoluble ash digestibility increases significantly, and there is a tendency for increased Ag digestibility. In addition, various indicators have indicated significant improvements, including triiodothyronine content in blood plasma, muscle Ag and nitrogen content, and mRNA levels in muscle tissue such as insulin-like growth factor-1, glucose transporters, citrate synthase, and glutathione peroxidase (GSH-Px). However, no changes have been observed in mRNA levels of atrogin-1, fatty acid synthase, acetyl-CoA carboxylase, lactate dehydrogenase, and carnitine palmitoyl transferase 1A (Saleh and El-Magd, 2018). In a 22-day *in vivo* investigation involving oral administration of 20 nm spherical Ag-NPs coated with polyvinylpyrrolidone to chickens, Ag was found to accumulate in the liver and yolks, while no significant accumulation was observed in muscles, egg protein, and kidneys of the experimental group (Gallocchio et al., 2017).

The surface of Ag NPs is easily oxidized in biological systems, leading to the release of the toxic $Ag^+$ ion. These ions interact with nucleic acids, lipid molecules, and proteins, inducing oxidative stress and DNA damage. Consequently, the antioxidant systems are depleted as a result of this process (Siddiqi et al., 2018).

A study by Dosoky et al. (2021) indicated no toxicity in broiler embryos after administration of a solution containing 50 mg/kg of Ag-NPs. Moreover, Ag-NPs had no effect on the activity of liver transferases (ALT, AST), alkaline phosphatase, and serum cholesterol, glucose, and triacylglycerol concentrations. Ag-NPs were not detected to cause genotoxicity in liver DNA, as indicated by the concentration of 8-oxo-2'-deoxyguanosine, a biomarker of oxidative stress and associated DNA damage. The results demonstrate that low doses of Ag-NPs are safe for poultry with limited or zero toxicity (Xu et al., 2013). However, further research is needed to identify potential toxic effects and safe doses of nano-Ag supplements for different poultry species.

Another study revealed that Ag-NPs could cause oxidative reactions in the blood, wall of the small intestine, liver, and pectoral muscles of chickens, especially at doses exceeding 9.47 mg/bird (Kulak et al., 2018). A study by Pineda et al. (2012) examined the effects of using water solutions with varying concentrations of Ag-NPs on nitrogen utilization and blood plasma IgG concentration in chickens. The microbial population in the digestive tract, energy metabolism, and growth performance of the chickens remained unaffected by the addition of Ag-NPs to their diet. Moreover, the excretion and nitrogen utilization efficiency were not altered, and there were no changes in the bacterial populations in the intestinal samples. However, there was a significant finding regarding the blood plasma IgG concentration in the experimental broilers. On day 36 of Ag-NPs administration, the concentration of immunoglobulin (IgG) in the blood plasma decreased.

On the other hand, Vadalasetty et al. (2018) have discovered that the administration of Ag-NPs at a concentration of 50 ppm via drinking water reduced the growth of broilers, depressed the immunity, and had no antibacterial effect on different intestinal bacteria. It limits the usage of Ag-NPs against *Campylobacter jejuni* in broiler chickens. Different clinical and biochemical effects were observed after using various Ag-NPs concentrations (1-5 ppm). The most efficient and safe dose of Ag-NPs (up to 2 ppm) was confirmed for the growth traits (body weight, body weight gain, and feed conversion ratio) as well as hematological parameters. On the contrary, higher concentrations (3-5 ppm) caused dose-dependent mild to moderate adverse effects regarding the hematological, biochemical, and oxidative parameters (MDA, T-AOC, and GSH-Px; Fouda et al., 2021).

The serious cardiovascular problems with a decrease in myocardial contractility and an increase in the internal diameter of the left ventricle could occur due to high doses of Ag-NPs (Raieszadeh et al., 2013). Therefore, it is necessary to determine the amount of NPs introduced to the chickens carefully. Experimental studies (Husseiny et al., 2021) have shown that *in ovo* injection of 20 ppm Ag-NP improved muscle development in chick embryos, compared to a 40 ppm administration. This confirms the effectiveness of using Ag-NPs to improve poultry productivity.

$SiO_2$-Ag-NPs have no negative effect on the growth performance and hematological, biochemical, and oxidative parameters of broiler chickens. In addition, immunohistochemistry revealed minimal inflammatory reactions and lymphoid depletion at the 8 mg/kg dose. Therefore, $SiO_2$-Ag-NPs can be considered a promising and safe nano-growth stimulator in broilers during its introduction to the poultry diet at a dose of 4 mg per kg of food (Dosoky et al., 2021).

### Gold nanoparticles

Gold nanoparticles (Au-NPs) have been extensively studied, but there is still a lack of comprehensive information regarding their immunogenic potential, meaning their ability to trigger immune responses in the body. Au-NPs are non-ionic, nanocrystalline, chemically pure particles of 5 nm in size that are synthesized by the photochemical method. When Au-NPs are ingested, they tend to accumulate in the intestinal wall. The extent of this accumulation depends on the dose and duration of exposure. Au-NPs negatively affect the absorption of Ca, iron, and potassium in the jejunum *in vitro* (Sembratowicz et al., 2016). The interaction between Au-NPs and chitosan involves several mechanisms, including the antioxidant effect, the strengthening of the protective effects of antioxidants in the deactivation of free radicals, and the activation of electron transfer in redox reactions. Au-NPs with a size of 15 nm inactivate nitroxyl free radicals by oxidation to carboxyl derivatives. These systems stimulate the immune system and are practically non-toxic (Suchomel et al., 2018).

Chickens treated by Au-NPs for a shorter period, had an increase of the respiratory activity of leukocytes and a decrease of the lysozyme activity of the blood. Higher doses (1.5 and 2.0 mg/kg body weight/day) of Au-NPs with longer duration of taking caused an increase of the interleukin 6 level and ceruloplasmin activity, as well as erythrocyte sedimentation rate. These are signs of a pro-inflammatory effect. In addition, Au NPs increased the content of IgA and IgY in the blood, but the immune response of chickens depended on both the dose and the duration of feeding. Long-term administration of high doses of Au NPs causes an inflammatory response, which could be avoided if the dose does not exceed 1.0 mg/kg body weight/day (Sembratowicz and Ognik, 2018).

Hassanen et al. (2020) confirmed that the addition of 15 ppm Au-NPs to drinking water caused significant blood damage due to oxidative stress, histopathological changes, increased IL-6, Nrf2 gene, and DNA fragmentation in the studied immune organs of broiler chickens. The study results showed a significant decrease in antibodies against Newcastle and avian influenza viruses. On the other hand, the 5 ppm Au-NPs group showed better growth performance with an increase in the final feed conversion ratio without any differences in the preliminary toxicological and immunological parameters, compared to the control groups.

The implementation of Au-NPs has a high potential for application in poultry farming when coated with bacterial outer membrane proteins. This combination enhances their ability to induce an immune response, making them promising candidates for vaccine development against poultry salmonellosis. (Anwar et al., 2021).

### Iron nanoparticles

Iron is one of the most important trace elements. Iron deficiency can lead to various growth retardation and pathological conditions, such as anemia and disorders of the skin and feathers (Rehman et al., 2020). Ferrum (Fe), commonly known as iron, is an essential element for living organisms and plays a crucial role in various biological processes. It is a component of many important proteins and enzymes that are involved in key cellular functions. Some examples of iron-containing proteins and enzymes include cytochrome, peroxidase, oxidase, catalase, hemoglobin, and myoglobin. Iron preparations, in their conventional macroergic form (e.g., iron salts or chelates), are commonly used to supplement iron in cases of iron deficiency or related conditions. However, these preparations have certain limitations, including limited bioavailability and a low toxic threshold. Therefore, iron nanoparticles ($Fe_2O_3$-NPs) can contribute to the solution of this problem.

The physical, biological, and pharmacological properties of $Fe_2O_3$-NPs depend on the particle size. Larger particles are better captured by macrophages, but smaller ones, as a rule, circulate longer in the bloodstream and penetrate the capillary wall. There were no accumulations of $Fe_2O_3$-NPs in the liver, spleen, as well as in chyme of duodenum. Iron levels in the liver and duodenal villi reflect the bioavailability of the released iron due to the transformation of $Fe_2O_3$-NPs in the acidic environment of the stomach. A duodenal gene expression study associated with iron uptake from $Fe_2O_3$-NPs indicates enhancement of the iron-over-iron pathway, supporting a role for mucins. As a result, oral administration of iron oxide nanoparticles is a safe way to deliver drugs in low doses of NPs (Chamorro et al., 2015).

Based on the physicochemical properties, $Fe_2O_3$-NPs have diverse applications, such as detoxifying biological fluids, antimicrobial therapy, and tissue regeneration. However, their properties can lead to significant biodegradation of Fe, resulting in the release of its ions. In a study involving $Fe_2O_3$-NPs supplement plus xylanase, a significant weight gain (54.5%) was observed by the fifth week compared to the control group of chickens. Despite the supplementation, there was no notable rise in iron concentration in the muscles, and no morphological changes were observed in liver cells (chickens were injected with 40 ncat/mg endoxylanase and 15 ppm $Fe_2O_3$-NPs). Nevertheless, these experimental findings show promising potential for utilizing Fe2O3-NPs in poultry farming to produce safe and high-quality meat (Rehman et al., 2020).

The pollution caused by metal nanoparticles is on the rise due to their increased utilization in industrial and domestic applications. These NPs are toxic to established microorganisms, potentially impacting their associated functions and nutrient cycling in agroecosystems. A study conducted by Kamran et al. (2020) showed the toxic effect of Fe2O3 NPs on soil microbes, affecting carbon and nitrogen mineralization in applied manure, consequently influencing the nitrogen cycle within the manure-substrate-plant continuum on the farm.

Ferroptosis is a form of cell death associated with iron-dependent lipid peroxidation. Basaki et al. (2021) used a chick embryo model to investigate whether ferroptosis was involved in the molecular mechanism underlying the potential effects of $Fe_2O_3$-NPs on maternal brain development. Ferroptotic cells appeared in cerebral tissue after exposure to low doses. Oxidative stress and ferroptotic cells were more evident. Low doses caused stronger oxidative stress in cerebral tissue. The TAC and MDA increased, and GSH-Px expression and activity decreased in the $Fe_2O_3$-NPs treated groups. According to the results of a study by Basaki et al. (2021), maternal exposure to $Fe_2O_3$-NPs was associated with ferroptosis in the brain.

**Calcium nanoparticles**

The widest group of NPs is characterized by Ca compounds, as one of the main elements in poultry nutrition. It should be noted that the use of Ca macroergic salts, particularly in the form of carbonates and phosphates, is common in the rations of both layer and broiler poultry. However, recent studies (Ganjigohari et al., 2018; Abo El-Maaty et al., 2021; Gutiérrez-Arenas et al., 2021) have proven the clinical effectiveness of using various Ca salts in nanoform-calcium carbonate ($CaCO_3$-NPs), dicalcium phosphate ($Ca_2PO_4$-NPs), and complex Ca particles with an extract of *Sargassum latifolium algae* (SL-Ca-NPs).

The results of studies addressing the effects of CaCO3-NPs on the performance of laying hens have indicated that $CaCO_3$-NPs can replace $CaCO_3$-NPs in lower amounts while reducing the concentration of Ca in the diet (up to 1.43 %) and decreasing the productivity of laying hens, egg quality, shank thickness, and Ca content in the blood. At the same time, laying hens treated by $CaCO_3$-NPs had a better average feed conversion rate and increased relative eggshell mass and eggshell mass/surface area (Ganjigohari et al., 2018).

Nano dicalcium phosphate ($Ca_2PO_4$-NPs) produced by co-precipitation can be used as a substitute for dicalcium phosphate (DP), a mineral involved in poultry metabolism and development. The digestibility of dietary sources of phosphorus improved in the group receiving 0.35% $Ca_2PO_4$-NPs. The study conducted by Gutiérrez-Arenas et al. (2021) revealed that the dietary source of phosphorus ash and diameters exhibited significant increases when supplemented with additional Ca2PO4-NPs, as compared to the control treatment. Consequently, the utilization of 0.35% Ca2PO4-NPs was identified as the optimal dosage for chickens in terms of enhancing digestibility, absorption rates, and the amount of phosphorus from the diet that gets deposited in the breast tissue.

In a study investigating the effects of feed supplements containing *Sargassum latifolium* combined with Ca-NPs, researchers observed that laying hens fed with these supplements exhibited higher rates of egg weight and shell weight. All groups of laying hens treated with SL-Ca-NP showed the most significant improvements in shell thickness and shell weight per unit surface area. Supplementing SL-Ca-NP into the chicken diet, up to a dosage of 1.5 g/kg of feed, resulted in increased levels of calcium and inorganic phosphorus in the blood serum The ultrastructure of the eggshell in laying hens showed well-developed palisade and mastoid layers when treated with SL-Ca-NP. Additionally, the number of apical cells along the branched tubular gland was greater in SL-Ca-NP-treated hens compared to those not receiving the treatment. Importantly, electron microscopic examination did not indicate any negative effects on the quality of eggshells (Abo El-Maaty et al., 2021).

Therefore, the development of nanostructured Ca compounds can become an effective alternative to commonly used feed additives and increase the profitability of the poultry industry.

**Manganese nanoparticles**

The British United Turkeys recommended introducing Mn supplement into the diet of young turkeys (Jankowski et al., 2019). The reduction of the Mn2O3-NPs dose from 100 to 50 and even 10 mg/kg did not negatively affect the antioxidant defense of young turkeys. In addition, using Mn supplement increases the Mn content in the bloodstream. Moreover, 50% reduction of the recommended level of MnO-NPs increased lipid oxidation processes. It can be assumed that replacing MnO-NPs with Mn2O3-NPs in turkeys' diet will increase cell apoptosis in young turkeys. On the other hand, the reduction of Mn supplement amount in the turkey diet reduces apoptosis, regardless of Mn form (Jankowski et al., 2018).

As stated by other researchers, reducing Mn supplementation at rational rates decreases ileal absorption and Mn accumulation in the liver, pectoral muscles, and skin. The reduction in dietary Mn supplementation in turkeys could potentially lead to an increase in intestinal Zn absorption and a decrease in the accumulation of Zn and Cu in the liver, breast muscle, and skin. Additionally, this reduction in Mn supplementation stimulated B-cells to produce immunoglobulin M and release cytokine IL-6. However, it is worth noting that apoptosis and oxidation in the liver cells and pectoral muscles of turkeys were not intensified by this dietary change (Jankowski et al., 2019).

In a study by Ognik et al. (2019), experimental investigations were conducted using two forms of Mn (MnO-NPs and $Mn_2O_3$-NPs) added to turkeys' diet at three doses of 100, 50, and 10 mg/kg. The results showed that the most effective form was $Mn_2O_3$-NPs at a dose of 10 mg/kg, which significantly induced lipid oxidation. Regardless of the dose of Mn2O3-NPs, this supplement reduced protein nitration more effectively, compared to MnO-NPs. However, reducing the dietary dose of Mn from 100 to 50 mg/kg, and then to 10 mg/kg, led to increased oxidation of proteins and DNA in cells, decreased the activity of antioxidant enzymes, and lowered the level of glutathione. At the same time, it caused an increase in global DNA methylation. Therefore, any reduction in the dietary dose of both forms of Mn was found to be unfavorable in terms of the observed effects on oxidative stress and DNA methylation.

Another study by Orzołek et al. (2021) investigated the improvements in turkey sperm parameters during long-term storage. The researchers evaluated several sperm parameters after 2, 24, and 48 hours of storage, including motility, activity of the cell membrane, mitochondrial membrane potential (MMP), and the percentage of sperm showing NO and SOD activity. The addition of $Mn_2O_3$-NPs to sperm diluent increased the mitochondrial membrane potential and activity of the plasma membrane. As a result, all spermatozoa showed good motility during the storage period.

**Chromium nanoparticles**

Lin et al. (2015) found that the addition of CrPic-NPs and CrPic to the diet of broiler chickens was more effective than the addition of $CrCl_3$. It significantly increased the concentration of chromium in blood serum. However, it was observed that the triglyceride level in the blood serum was lower with the addition of $CrCl_3$, compared to CrPic. It was established that CrPic-NPs supplement improved chromium utilization and reduced serum cholesterol in broiler chickens more effectively than the CrPic supplement. Additionally, NPs supplementation was found to be beneficial in reducing the negative effects of heat stress in broiler chickens. This was attributed to changes in the immune system, including the expression of IFN-γ immune. The addition of Cr and CrPic-NPs improved the weight gain and feed conversion ratio of heat-stressed chicks. The CrPic and CrPic-NPs dietary supplements enhanced antibody titers of heat-stressed broilers as well as antibody titers against avian influenza and infectious bronchitis (supplements of CrPic-NPs at 1000 ppb and CrPic at 1500 ppb from days 21 to 42 of age (Hajializadeh et al., 2017).

Berenjian et al. (2018) compared quails' diet with CrPic-NPs and a diet without CrPic-NPs. At 23 days of age, there was a positive linear relationship between dietary CrPic-NPs levels, feed intake, and average daily gain. The increase of CrPic-NPs in quails' diet led to a general increase in white blood cells and hematocrit levels. In 35-day-old quails, indicators of body weight gain, energy use, and the number of leukocytes in the blood increased due to the addition of CrPic-NPs to the diet. Thus, the optimal concentration of CrPic-NPs in the quails' diet facilitated the negative consequences of physiological stress in quails.

The Cr supplements in both forms (salts and nanoforms) in a dose of 3 mg/kg were found to increase serotonin levels and decrease norepinephrine levels. These supplements also regulate the levels of hormones involved in carbohydrate metabolism, leading to an increase in insulin secretion and a decrease in glucagon secretion. However, it was observed that Cr supplements at this dose had a negative impact on the antioxidant potential of the liver and pectoral muscles of chickens. Therefore, further research is needed to identify new doses of these supplements that would avoid compromising the antioxidant defense system (Stępniowska et al., 2019).

It became obvious that Cr accumulates in the wall of the ileum, liver, pectoral muscles, bones, skin, and feathers of chickens. According to *in vivo* research by Stępniowska et al. (2020), the deposition of Cr in the chickens' ileum did not affect the intestinal absorption of the mineral. However, the introduction of Cr to the chickens' diet reduced the level of phosphorus in the femur. Hamidi et al. (2022) noted the positive effect of CrPic and CrPic-NPs supplementation on the status of heat-stressed broilers, namely on their growth, hormonal changes, immune biomarkers, and IFN-γ gene expression.

Summarizing the obtained results, the authors of the current study note that the use of metal NPs in feed additives for poultry is an urgent scientific task and requires further detailed research, especially in the field of nanotoxicology. The main parameters of the positive effect of metal NPs on the poultry body, a combination of antioxidant, antibiotic, and immunomodulatory impact, are shown in Figure 1.

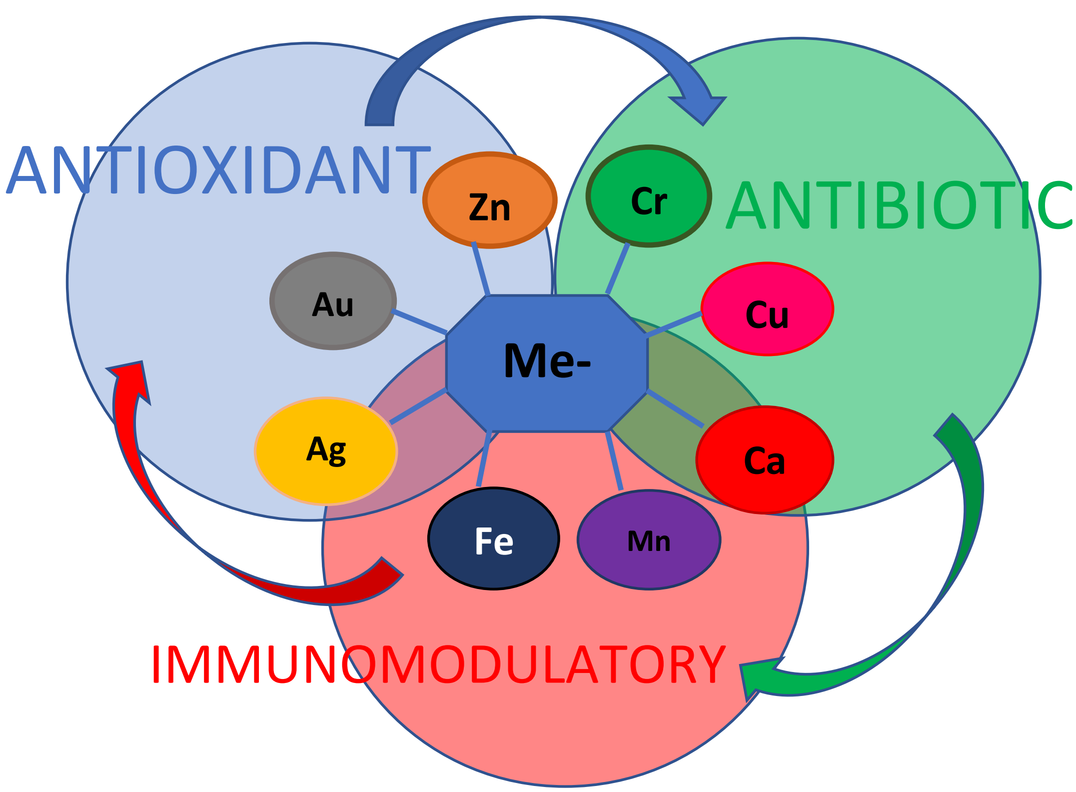


**Figure 1.** Effect of metal nanoparticles (including Ag, Au, Ca, Cu, Cr, Fe, Mn, and Zn) on the poultry body using safe doses

**Table 1.** Dose-dependent effects of metal nanoparticles on increasing feed digestibility, growth performance stimulating, influencing gene expression, and maintaining hormonal balance

| Parameters | Me-NPs | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Ag** | **Au** | **Ca** | **Cu** | **Cr** | **Fe** | **Mn** | **Zn** |
| IFD | ↓↓ | ↑↑ | ↑ | ↑↑↑ | – | ↑ | ↑ | ↑↑↑ |
| GPS | ↓ | ↑ | ↑ | ↑↑↑ | ↑↑ | – | ↑↑ | ↑↑↑ |
| IGE | ↑↑ | – | – | ↑↑ | – | – | – | ↑↑ |
| MHB | ↑ | – | – | – | ↑ | – | – | ↑↑ |

Me-NPs: Metal nanoparticles, including Ag, Au, Ca, Cu, Cr, Fe, Mn, and Zn, IFD: Increasing feed digestibility, GPS: Growth performance stimulating, IGE: Influencing gene expression, MHB: Maintaining hormonal balance, ↑↑↑: Pronounced positive effect in therapeutic doses, ↑↑: Positive effect in medium doses; ↑: A positive effect is observed only when administered in low doses, ↓↓↓: Pronounced negative effect in low doses, ↓↓: Negative effect in medium doses, ↓: Negative reactions are noted for the introduction of high doses of NPs, – : There is no information about the presence of influence on this parameter.

In addition, the obtained data indicate a pronounced dose-dependent effect of the introduction of metal NPs (in particular, nanoparticles of zinc, copper, and chromium have pronounced redox properties; nanoparticles of silver and gold exhibit an antibiotic effect, while nanoparticles of manganese, calcium, and iron have a positive effect on the immunity of poultry); therefore, feed additives based on these nanoparticles can lead to a complex antioxidant, antibacterial, and immunomodulatory effects (Table 1).

## CONCLUSION

Metal NPs have become widely used in the poultry industry and have attracted the interest of numerous researchers. These bioavailable and effective compounds possess unique properties, but their study has been ongoing for more than a decade. A comprehensive analysis of specialized literature revealed a substantial volume of experimental studies exploring the impact of metal NPs on birds' bodies and their products, as well as a comparative analysis with macroergic compounds.

Zn-NPs offer a broad spectrum of effects on various types of poultry, including antioxidant, antimicrobial, and immunomodulatory properties, which contribute to enhanced productivity and increased resistance against internal diseases. Introducing these NPs into diets at a dose of 20-80 mg/kg of body weight allows for a comprehensive beneficial effect on the body. Similarly, Cu-NPs demonstrate comparable properties and, at the same time, exert a pronounced impact on mineral homeostasis, immune protection, and bone tissue, with an effective dose ranging from 5-50 mg/kg of body weight.

Ag-NPs and Au-NPs serve as potential alternatives to antibiotics due to their antioxidant properties. Their comprehensive impact on antioxidant protection helps in preventing cardiovascular and nervous diseases and also improves fertility. Introducing these nanoparticles into diets at appropriate doses (Ag-NPs at 20-40 mg/kg of body weight and Au-NPs up to 1 mg/kg of body weight) can effectively reduce the occurrence of infectious diseases and bolster the immune system.

Fe-NPs can serve as viable substitutes for macroergic agents in the treatment of anemia and diarrhea; however, their use should be limited due to their pro-oxidant properties. On the other hand, Ca-NPs can be safely introduced into diets in the form of various salts at a dose of up to 1.5 g/kg of body weight.

Mn-NPs play a vital role in turkey feed, and their high bioavailability and low toxicity make them excellent replacements for pharmacopeial drugs. Moreover, their properties contribute to preserving rooster sperm during refrigerated storage. Cr-NPs serve as effective anti-stressors, enhancing the bird's resistance to thermal and other physiological stresses. It is noteworthy that they can regulate hormonal balance and impact the antioxidant status.

As a result, the poultry industry should prioritize the implementation of nanotechnological advancements, including metal NPs. However, special attention should be paid to NPs with strong antioxidant properties and a low toxicity threshold. When conducting studies related to nanoform compounds, careful consideration should be given to combining assessments of toxicological parameters with the effects on redox status. This approach is crucial for the safe and beneficial integration of metal NPs in poultry feed additives.

## DECLARATIONS

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**Authors' contributions**

Svitlana Naumenko and Vsevolod Koshevoy conceptualized the research idea, developed the structure of the work, and reviewed and edited the manuscript. Olena Matsenko, Olha Miroshnikova, Iryna Zhukova, and Iryna Bespalova took part in the selection of literature sources and drafting of the manuscript. All authors have read and approved the final version of the manuscript for publication in this journal.

**Competing interests**

The authors have declared that no competing interest exists.

**Ethical consideration**

Ethical issues under current regulations, including plagiarism, consent to publication, misconduct, data fabrication and/or falsification, double posting and/or submission, and redundancy, have been verified by the authors and warranted against the aforementioned violations.

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# Effects of Dietary Supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on Growth and Production Indicators of Broiler Chickens

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## ABSTRACT

In response to the 2006 EU ban on the use of antibiotics as growth promoters, researchers have sought alternatives, leading to a focus on the beneficial effects of probiotics on chickens. The aim of this study was to evaluate the effect of the probiotic mixture containing *Lactobacillus* (*L.*) *farciminis* CNCM-I-3699 and *Lactobacillus rhamnosus* CNCM-I-3698 on the growth, production indicators, and edible organs of broiler chickens. Three trials were conducted, each consisting of 260 newly hatched Ross 308 broiler chicks (males and females) from a commercial hatchery, randomly allocated into control (n = 130) and probiotic-supplemented groups (n = 130). The dietary treatments were basal diet for the control group and basal diet + the mixture of *L. farciminis* CNCM-I-3699 ($2.10^{10}$ GU/g) and *L. rhamnosus* CNCM-I-3698 ($2.10^{10}$ GU/g) at a rate of 4g/10kg of diet for the probiotic supplemented group. Broilers were raised until day 35 of age, and their body weight and feed intake were recorded on days 1, 7, 14, 21, 28, and 35. All broiler chickens were weighed on the first day. The investigated parameters included average weight gain, feed conversion ratio, cumulative feed intake, and the European Broiler Index. Daily mortality was recorded. The average organ's relative weight was calculated for each group on days 1, 7, 14, 21, 28, and 35. Although both groups yielded positive results regarding growth and production indicators, no significant differences were observed between the two groups, suggesting that probiotics may not provide expected outcomes when appropriate conditions and age-related requirements are met. The probiotic-supplemented group exhibited significantly accelerated growth in the heart and liver. However, relative organ weights did not differ significantly between the groups.

**Keywords:** Body weight, Edible organs, Poultry, Probiotic, Productivity

## INTRODUCTION

Poultry industry in the European Union (EU) is one of the fastest-growing agricultural industries. In Latvia, the poultry industry is growing very fast due to an ever-growing need for affordable and good-quality protein. Among meat products, chicken is the most consumed meat. The EU is one of the largest poultry meat producers and a net exporter of poultry products, with an annual production of around 13.4 million tons (European Commission, 2022).

Since 2006, when the EU banned antibiotic use as growth promoters in animal feed to help fatten livestock, meeting consumer demand for quality products has become challenging (Cogliani et al., 2011). Thus, there has been extensive research on the beneficial effects of prebiotics, probiotics, and acidifiers on chickens to improve their health. The aim was to reduce the necessity for antimicrobial medicine and improve commercial productivity (Abudabos et al., 2015; Babazadeh and Asasi, 2021; Marwi et al., 2021; Mirzaei et al., 2022).

A chicken's intestinal tract holds a microbial profile, including *Lactobacillus* (*L.*) spp., which is sensitive to stress, making chickens prone to health issues and productivity (Patterson and Burkholder, 2003). Probiotics help maintain gut microbial flora, improve resistance against harmful pathogens, and enhance the overall development and growth of animals (Wang et al., 2016;

Shah et al., 2021; Agustono et al., 2022). Chen et al. (2018) found that adding *L. rhamnosus* strain to the feed of broiler chickens improved live weight and average daily growth. The ability of *L. rhamnosus* to improve growth has been reported in current studies (Bampidis et al., 2020b; Fesseha et al., 2021). Similar findings have been reported for *L. farciminis* (Bampidis et al., 2020a), which improves stress resilience, leading to improved intestine health and growth (Ait-Belgnaoui et al., 2006). On the contrary, probiotics have no significant effect on live weight in broiler chickens (Qorbanpour et al., 2018; Zhu et al., 2020; Atsuti et al., 2022).

The effect of probiotics on animal feed consumption has not been fully confirmed. Several studies have shown that the addition of different types and doses of probiotics did not affect feed consumption indicators (Awad et al., 2009; Sugiharto et al., 2018; Atsuti et al., 2022) or did not reduce feed consumption and feed conversion rate (FCR) in broiler chickens (Zhu et al., 2020; Agustono et al., 2022). However, some studies have indicated that the dietary inclusion of *Lactobacillus* spp. (*L. farciminis*, and *L. rhamnosus*) significantly reduce daily feed consumption and FCR (Hussein and Selim, 2018; Bampidis et al., 2020b).

Higher body weight (BW) affects inner organ development, but this connection has not always been observed. Shah et al. (2021) have shown that probiotics (*E. faecium* and *P. acidilactici*) could improve live weight gain, but not the relative weight of inner organs (liver, heart, gizzard). The addition of a mixture of *Bacillus* spp. to the diet does not affect the weight of inner organs (heart, liver, gizzard) as well as live weight (Sugiharto et al., 2018). Atsuti et al. (2022) found that adding *L. casei* to the feed did not impact the final weight, but the live weight increased. It could increase the relative weight of the spleen and heart, but reduce the relative weight of the liver.

Although there are studies on probiotics, such as *L. farcimins*, *L. rhamnosus*, *Lactobacillus* species of Kefir, and their impact on chickens' growth, clear information is missing on whether it impacts feed consumption, FCR, and inner organ weight (Awad et al., 2009; Vahdatpour and Babazadeh, 2016).

Therefore, the objective of the present study was to evaluate the effects of the probiotic mixture containing *L. farciminis* CNCM-I-3699 and *L. rhamnosus* CNCM-I-3698 on the growth, production indicators, and edible weight of organs in broiler chickens.

## MATERIALS AND METHODS

### Ethical approval

All issues related to growing the chickens were conducted according to the Republic of Latvia Cabinet Regulation No. 98, 2010, "Welfare Requirements for Keeping and Use of Chicken for Meat Production" (Republic of Latvia, Cabinet Regulation No. 98, 2010). The study was approved by the Research Committee of the Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Latvia (protocol No.2021/1).

### Experimental design and management of broiler chickens

The study was conducted from April to December 2021 and organized into three trials. The study was performed at the Clinical Research Center, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Jelgava, Latvia.

A total of 260 Ross 308 (*Gallus gallus*) newly hatched broiler chicks (female and male) were obtained from a commercial hatchery located in Kekava, Latvia (total number of 780 chicks). The broilers were weighed and completely randomly divided into two groups, namely the control group (Con, n = 130 with an initial weight of 44.64 ± 1.92 g) and the probiotic-supplemented group (ProL, n = 130 with an initial weight of 45.40 ± 2.03 g). Broiler chickens were raised until day 35. Broiler chickens were not vaccinated during the study. Strict biosecurity requirements were followed, including closed rooms, disposable clothing, gloves, shoes, disinfection barriers, and the presence of people only during chicken feeding (twice daily). Mortality was recorded once a day. At the end of the study, the total mortality rate was recorded as 2-5%. The cause and pattern of death were recorded.

The chickens were divided into two separate enclosed pens, commonly referred to as bio-chambers. Each pen was designed with a deep litter system featuring a floor made of clean pine and spruce shavings. The size of each pen was 9 $m^3$. The rooms were equipped with full microclimate control (temperature, humidity, and air supply, control of incoming and outgoing air composition, and light mode), as well as video surveillance. The lighting setting on the first day was 23 hours of light and an hour of darkness (23 hours/1 hour). Afterward, the darkness hours were slowly extended to 6 hours until day 26 (18 hours/6 hours). In the last week of the study, the dark time was gradually reduced until it reached 20 hours of light and 4 hours of darkness (20 hours / 4 hours). The ambient temperature for the initial day was 33-34℃, and as the chickens grew, it slowly decreased to 22℃ until the end of the study. The 24-hour lighting and temperature regime were created based on previous studies and the Ros 308 breeder guidelines (Cassuce et al., 2013; Broiler management manual ROSS, 2018).

### Diet and supplementation of *Lactobacillus* spp.

Fresh drinking water was provided *ad libitum* in nipple drinking lines. The basal diet provided to both the Con and ProL groups of chickens was identical and formulated based on the specific age of the chicks. Starter feed was provided from hatching to day 10, grower feed from day 11 to 24, and finisher feed from day 25 until the end of the study. The main sources of protein in the basal diet were wheat grain, soybean, and rapeseed. The analytical composition of the feed is summarized in Table 1. The ration was prepared based on the Ross 308 breeding guidelines (Broiler Management Manual ROSS, 2018). The starter diet contained wheat grains, soy sprouts, vegetable oil, corn gluten, monocalcium phosphate, fish meal, calcium carbonate, sodium chloride, and sodium sulfate (2975 kcal metabolizable energy [ME]). The grower (3075 kcal ME) and the finisher diets (3195 kcal ME) contained no fish meal but rapeseed and fatty acids.

In the study, the mixture (Lot No.: 92024406) of lactic acid bacteria 4g/10kg was manually added to the ProL feed. The mixture was a bioactive substances complex based on heat-inactivated co-culture of probiotic strains, *L. farciminis* CNCM-I-3699 ($2.10^{10}$ GU/g) and *L. rhamnosus* CNCM-I-3698 ($2.10^{10}$ GU/g) in association with their environment (functional metabolites and bioactive peptides). This complex was obtained from thermally processed fermented milk (heat-treated bacteria retain their cell wall structure) and stabilized on the cereal-based carrier. The product was stored according to the manufacturer’s requirements (in a cool, dry, well-ventilated area), with a shelf life of 18 months from the manufacturing date.

To assess the consumption of the supplemented feed containing lactic acid bacteria by the broilers, and to determine the viability of these bacteria post-consumption, the results obtained from the broiler crops in both groups were compared. Immediately after the completion of each trial, data regarding the quantity of lactic acid was collected. A total of 15 samples were taken from each group, with 15 samples from the ProL group and 15 samples from the Con group. The contents of crops were examined at the Scientific Laboratory of Biotechnologies, Molecular Biology and Microbiology Department, Latvia University of Life Sciences and Technologies.

**Table 1.** Analytical composition of the basal diet balanced for broiler chickens

| Components | Starter diet | Grower diet | Finisher diet |
|---|---|---|---|
| Metabolizable energy (kcal) | 2975 | 3075 | 3195 |
| Protein (%) | 22.5 | 21.50 | 19.50 |
| Fibre (%) | 2.40 | 2.86 | 2.83 |
| Fat (%) | 4.24 | 5.20 | 7.22 |
| Ash (%) | 4.32 | 4.73 | 3.68 |
| Carbohydrates (%) | 46.06 | 46.71 | 45.53 |
| Water (%) | 12.82 | 11.08 | 10.98 |
| Other ingredients | 5.46 | 6.12 | 8.27 |
| Lysine (%) | 1.36 | 1.20 | 1.14 |
| Methionine (%) | 0.84 | 0.60 | 0.85 |
| **Minerals** | | | |
| Ca (%) | 0.96 | 1.00 | 0.78 |
| Na (%) | 0.35 | 0.16 | 0.19 |
| P (%) | 0.50 | 0.50 | 0.50 |
| **Vitamins** | | | |
| A (U/kg) | 16,900 | 14,300 | 13,000 |
| $D_3$ (U/kg) | 6,500 | 5,500 | 5,000 |
| E (mg/kg) | 104,0 | 88.0 | 80.0 |
| **Micronutrients** | | | |
| FeSO4 (mg/kg) | 22.1 | 18.7 | 17.0 |
| $Ca(IO_3)_2$ (mg/kg) | 1.63 | 1.38 | 1.25 |
| CuSO4 (mg/kg) | 20.8 | 17.6 | 16.0 |
| $MnO_2$ (mg/kg) | 156.0 | 132.0 | 120.0 |
| ZnO (mg/kg) | 117.0 | 99.0 | 90.0 |
| $Na_2SeO_3$ (mg/kg) | 0.39 | 0.33 | 0.30 |

Ca: Calcium, Na: Sodium, P: Phosphorus

**Growth performance, production indicators, and edible organs**

All broiler chickens were weighed on the first day and subsequently on days 7, 14, 21, 28, and 35 in both the Con and ProL groups using calibrated scales called Soehnl with an accuracy of ± 1 g. The average BW was calculated for each group. The amount of feed prepared for both study groups (Con and ProL) was the same. It was extended twice a day in the feeders with suitably sized openings, reducing the possibility of the chickens scavenging it in the litter. The amount of food consumed by each group was determined once a week by weighing the amount of uneaten food left in the feeders from both groups.

Obtained results were used to calculate average weekly/daily weight gain, daily feed intake (FI), cumulative feed intake (CFI), and consumed feed quantity on 1 kg live weight in consecutive study periods of 1-7, 8-14, 15-21, 22-28, 29-35 days. Slaughtering was performed every 7 days for inner organ analysis, and pathological sampling was performed. The initial broiler count in each group was 130 on the first day of the experiment. The broiler count gradually decreased over time, with 115 chickens on day 7, 100 on day 14, 85 on day 21, 70 on day 28, and 55 on day 35. The FCR was calculated using the formula described by Chen et al. (2018). The European Broiler Index (EBI) was calculated by following the formula described by Banaszak et al. (2022).

Average grams gained/day of rearing × % survival rate)/FCR × 10

On days 1, 7, 14, 21, 28, and 35 of age, 45 chickens per treatment (15 chickens per treatment in each replicate) were randomly selected and slaughtered by cervical dislocation. The edible organs were removed (liver, heart, gizzard and weighed and weighed using calibrated scales (Kern EW 420 - 3NM [± 0.01g], Germany). Average organ relative weight (percentage of each chicken's live weight) was calculated for each group.

**Statistical analysis**

MS Excel and R-Studio were used for the statistical data analysis to determine production indicators, organ weight, and live weight data. The relative weight of each organ was determined by calculating the percentage of proportion between each organ and the chicken's live weight. For each indicator within the group and age, mean and standard deviation (SD) were calculated. To determine whether the results between the two groups were significantly different, the student's t-test was used. P values less than 0.05 ($p<0.05$) were considered statistically significant.

## RESULTS

**Growth performance and production indicators**

Impacts of probiotics containing two strains of *Lactobacillus* spp. (*L. farciminis* CNCM-I-3699 and *L. rhamnosus* CNCM-I-3698) on the growth factors, feed consumption indicators, and organ development were determined every 7 days (on days 7, 14, 21, 28, and 35 of the study). The mean number of lactobacilli over three trials from the broiler crops was determined to be $11.22 \times 10^5$ for the ProL group and $8.42 \times 10^5$ for the Con group. The higher volume of lactic acid bacteria contents in ProL groups crops presented evidence that these broilers consumed supplemented feed with *L. farciminis* and *L. rhamnosus* and that these bacteria can grow and multiply in the digestive tract.

The initial live weight of broilers showed no significant difference between the two groups, meaning that the raw data was the same and could not affect the results (Table 2). The initial weight plays a crucial role in the further development of broiler chickens. On day 7, the live weight for broiler chickens of both groups increased 3.5 times, with no significant differences between groups. During this period, there was an increase in the ProL broiler chickens' weight by 164.48 ± 12.75 g, while the Con group showed an increase of 166.68 ± 17.11 g. The average daily weight gain for the ProL was 23.50 ± 1.82 g, and for the Con, it was 23.81 ± 2.44 g. In the next two weeks, the broiler chicken weight in both groups almost doubled, and on day 28 of the study, the weight reached 1962.12 ± 100.52 g (average daily weight increased by 111.03 ± 9.54 g) in the ProL group and 1957.71 ± 101.94 g (average daily weight increased by 112.73 ± 8.90 g) in the Con group.

The ProL group chickens at this age reached 2835.70 g ± 161.74 g with an average daily weight gain of 79.72 g ± 4.62 g and an overall weight gain of 2790.30 g ± 161.57 g during the study. In comparison, the Con group indicated lower results, an average weight of 2828.02 g ± 115.64 g, a daily gain of 79.53g ± 3.29 g, and an overall weight gain of 2783.38 g ± 115.17g during the study. The average results of daily weight gain and overall weight gain did not differ significantly between groups ($p > 0.05$). The EBI for ProL group chickens also reached 621.27 ± 49.38 at the end of the study, while it was slightly lower in the Con group and reached 619.73 ± 37.40 ($p > 0.05$).

Although there was no significant difference between the ProL and Con group of chickens regarding feed consumption indicators ($p > 0.05$), there was a visible tendency for better results in the experimental group when calculating the average values throughout the study (1-35 per day), supplementing feed with the probiotics reduces FCR by 0.78%, cumulatively consumed feed by 0.51%, and the feed consumed per 1 kg of live weight gain decreases by 0.70% compared with the Con group (Table 3).

**Table 2.** The effect of dietary supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on the growth performance of broiler chickens during 35 days

| Parameter | Time (day) | Values of growth indicators (mean ± SD) | | P value |
|---|---|---|---|---|
| | | **Probiotic group** | **Control group** | |
| Body weight (g) | First day | 45.40 ± 2.03 | 44.64 ± 1.92 | 0.331 |
| | 7 | 209.89 ± 14.30 | 211.32 ± 18.55 | 0.460 |
| | 14 | 588.86 ± 49.50 | 582.43 ± 43.46 | 0.437 |
| | 21 | 1184.88 ± 114.55 | 1168.62 ± 91.11 | 0.428 |
| | 28 | 1962.12 ± 100.52 | 1957.71 ± 101.94 | 0.480 |
| | 35 | 2835.70 ± 161.74 | 2828.02 ± 115.64 | 0.475 |
| Average weekly weight gain (g/chicken/week) | 1-7 | 164.48 ± 12.75 | 166.68 ± 17.11 | 0.434 |
| | 8-14 | 378.97 ± 35.22 | 371.11 ± 25.45 | 0.385 |
| | 15-21 | 596.02 ± 65.10 | 586.19 ± 48.88 | 0.422 |
| | 22-28 | 777.24 ± 66.77 | 789.09 ± 62.33 | 0.417 |
| | 29-35 | 873.59 ± 70.92 | 870.31 ± 38.63 | 0.474 |
| | 1-35 | 2790.30 ± 161.57 | 2783.38 ± 115.17 | 0.477 |
| Average daily weight gain (g/chicken/day) | 1-7 | 23.50 ± 1.82 | 23.81 ± 2.44 | 0.434 |
| | 8-14 | 54.14 ± 5.03 | 53.02 ± 3.64 | 0.385 |
| | 15-21 | 85.15 ± 9.30 | 83.74 ± 6.98 | 0.422 |
| | 22-28 | 111.03 ± 9.54 | 112.73 ± 8.90 | 0.417 |
| | 29-35 | 124.80 ± 10.13 | 124.33 ± 5.52 | 0.474 |
| | 1-35 | 79.72 ± 4.62 | 79.53 ± 3.29 | 0.477 |
| European broiler index | 1-35 | 621.27 ± 49.38 | 619.73 ± 37.40 | 0.484 |

SD: Standard deviation

**Table 3.** The effect of dietary supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on feed consumption indicators of broiler chickens during 35 days

| Parameter | Time (day) | Feed consumption indicators (mean ± SD) | | P value |
|---|---|---|---|---|
| | | Probiotic group | Control group | |
| Feed intake (g/chicken/day) | 1-7 | 31.18 ± 0.71 | 31.32 ± 0.74 | 0.414 |
| | 8-14 | 59.83 ± 2.39 | 60.15 ± 1.97 | 0.435 |
| | 15-21 | 97.96 ± 2.51 | 97.63 ± 3.54 | 0.451 |
| | 22-28 | 146.01 ± 7.58 | 145.90 ± 5.82 | 0.492 |
| | 29-35 | 179.58 ± 10.10 | 182.20 ± 7.63 | 0.369 |
| | 1-35 | 102.91 ± 2.94 | 103.44 ± 1.89 | 0.404 |
| Feed conversion ratio | 1-7 | 1.04 ± 0.08 | 1.04 ± 0.11 | 0.499 |
| | 8-14 | 1.09 ± 0.07 | 1.10 ± 0.08 | 0.397 |
| | 15-21 | 1.12 ± 0.08 | 1.14 ± 0.07 | 0.417 |
| | 22-28 | 1.20 ± 0.05 | 1.20 ± 0.05 | 0.472 |
| | 29-35 | 1.27 ± 0.04 | 1.28 ± 0.03 | 0.378 |
| | 1-35 | 1.27 ± 0.04 | 1.28 ± 0.03 | 0.378 |
| Cumulative feed intake (g/chicken) | 1-7 | 218.26 ± 4.96 | 219.22 ± 5.20 | 0.414 |
| | 1-14 | 637.09 ± 18.05 | 640.24 ± 15.88 | 0.416 |
| | 1-21 | 1322.79 ± 35.51 | 1323.63 ± 39.49 | 0.490 |
| | 1-28 | 2344.88 ± 32.88 | 2344.93 ± 24.24 | 0.499 |
| | 1-35 | 3601.94 ± 102.98 | 3620.33 ± 66.08 | 0.404 |

SD: Standard deviation

**Table 4.** The effect of dietary supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on organ relative weight of broiler chickens during 35 days

| Trial days | Organ | Probiotic group (Mean percentage ± SD) | Control group (Mean percentage ± SD) | P value |
|---|---|---|---|---|
| 1 | Liver | 4.00 ± 0.48 | 3.93 ± 0.52 | 0.303 |
| | Heart | 0.72 ± 0.09 | 0.67 ± 0.07 | 0.006 |
| | Gizzard | 7.53 ± 0.74 | 7.62 ± 0.57 | 0.316 |
| 7 | Liver | 4.83 ± 0.48 | 4.41 ± 0.64 | 0.050 |
| | Heart | 0.73 ± 0.08 | 0.71 ± 0.11 | 0.276 |
| | Gizzard | 4.78 ± 0.65 | 4.77 ± 0.78 | 0.492 |
| 14 | Liver | 3.58 ± 0.27 | 3.58 ± 0.38 | 0.482 |
| | Heart | 0.70 ± 0.11 | 0.64 ± 0.08 | 0.014 |
| | Gizzard | 3.77 ± 0.37 | 3.67 ± 0.40 | 0.159 |
| 21 | Liver | 2.82 ± 0.26 | 2.74 ± 0.36 | 0.174 |
| | Heart | 0.55 ± 0.07 | 0.54 ± 0.11 | 0.322 |
| | Gizzard | 2.94 ± 0.45 | 2.98 ± 0.47 | 0.375 |
| 28 | Liver | 2.56 ± 0.29 | 2.47 ± 0.23 | 0.107 |
| | Heart | 0.47 ± 0.09 | 0.48 ± 0.05 | 0.180 |
| | Gizzard | 2.39 ± 0.32 | 2.36 ± 0.33 | 0.357 |
| 35 | Liver | 2.11 ± 0.26 | 2.18 ± 0.21 | 0.127 |
| | Heart | 0.46 ± 0.05 | 0.46 ± 0.05 | 0.487 |
| | Gizzard | 1.81 ± 0.46 | 1.85 ± 0.51 | 0.389 |

SD: Standard deviation

### Edible organs

On the first day of the study, the two groups did not differ significantly in terms of liver and gizzard weight ($p > 0.05$). Only the heart weight on the first day was significantly higher in the ProL group than in the Con group ($p < 0.05$). The chickens appeared to be in good health upon visual examination, and when samples of their organs were collected for pathoanatomical analysis, no visible abnormalities or pathologies were detected. On day 7, liver weight in ProL group chickens was significantly higher in the Con group than the ProL and Con groups, with percentages of 4.83% and 4.41%, respectively ($p < 0.05$). On day 14, it was found that the relative weight of the liver and gizzard did not differ significantly between the two groups ($p > 0.05$). The relative heart weight in the ProL group was significantly higher than the Con group ($p < 0.05$).

In the remaining phases of the study (days 21 and 28) and at the end of the study (day 35), no significant differences were observed between the two groups regarding the relative weights of organs ($p > 0.05$, Table 4).

## DISCUSSION

### Growth performance and production indicators

In the present study, the initial weight of chickens did not differ significantly between the two groups. According to Mendes et al. (2011), broiler chickens with an initial weight range of 39.29-41.30 g at 42 days of age weigh approximately 1.98% more compared to chickens with an initial weight range of 34.4-35.22 g. Therefore, the initial weight of chickens has a significant impact (95% confidence) on the live weight of broiler chickens at the age of 42 days, meaning that broilers with a lower initial live weight cannot reach the same weight as chickens with a higher initial weight, even if they are grown under the same housing conditions. Similar results were achieved by Patbandha et al. (2017). They reported that BW increased significantly by 7.14% (10.62 g) and 5.52% (19.65 g) on days 8 and 15, respectively, in chickens with high baseline BW (47.76 ± 0.37), compared to chickens with a lower initial BW (41.24 ± 0.23 g). From day 15 until the end of the study (day 43), the weight did not differ significantly between the groups although the weight remained slightly higher until the end of the study in the group where the outgoing weight of broiler chickens was higher.

Although growth rates did not vary significantly between the two groups of the current study, they were numerically better in the ProL group (0.27% resulted in higher live weight, 0.24% increase in average daily growth, and 0.25% higher in the EBI at the end of the study). In a 42-day study conducted by Hosseini et al. (2013), Cobb broiler chickens supplemented with the commercial probiotic of Protexin (which also included *L. rhamnosus*) did not achieve significantly higher live weight at all periods of the study (days 4, 14, 21, 28, 35, and 42). Meanwhile, in a 24-day study by Chen et al. (2018), white leghorn chickens fed with *L. rhamnosus* CF at the end of the study resulted in significantly higher live weight and average daily gain. Positive results were reported in a study by Fesseha et al. (2021), where the Sasso dual-purpose chicken was fed with *L. paracaseis* and *L. rhamnosus* feed supplement, achieving significantly higher live weight. The addition of probiotics containing *L. farciminis* to the feed also demonstrated a positive effect on live weight gain, as reported in a study by Bampidis et al. (2020a). Other studies using different probiotic compositions also showed greater increases in live weight (Awad et al., 2009; Wang et al., 2016; Shah et al., 2021). However, a significant increase in live weight was not gained through research with other probiotic bacteria (Qorbanpour et al., 2018; Sugiharto et al., 2018; Zhu et al., 2020).

Noticeably, results in both groups were also achieved in the EBI rates. Although no significant differences were found, this rate was 0.25% higher in the ProL group. Awad et al. (2009) conducted a 35-day study where Ross 308 chickens were supplemented with probiotics containing *Lactobacillus* spp., resulting in an EBI rate of 265. In contrast, Palamidi et al. (2016) added inactivated probiotics to the feed in their study, achieving an EBI rate of 305. The significant difference between these studies and the present study can be attributed to the average daily weight gain in the ProL and Con groups, which were 79.72 ± 4.62 g and 79.53 ± 3.29 g, respectively. Additionally, the ProL group exhibited an FCR of 1.27 ± 0.04, while the Con group had an FCR of 1.28 ± 0.03.

Despite not finding significant differences in feed consumption results, it is noteworthy that during 35 days of probiotic feeding, there was a notable improvement in feed conversion, with a 0.78% reduction. Additionally, there was a 0.51% decrease in cumulatively consumed feed and a 0.70% reduction in food consumed per 1kg of live weight gain.

Under production conditions where 40,000 broiler chickens are raised in a single group, these feed efficiency improvements lead to significant economic benefits. Specifically, there would be a reduction of approximately 400 kg in feed consumption per 1 kg of live weight gain and a total reduction of about 800-1000 kg per group. This represents a substantial economic advantage. Similar to the present study, Hosseini et al. (2013) conducted a 42-day study in which they investigated the effects of feeding the commercial probiotic supplement “Protexin,” which included *L. rhamnosus*, on broiler chicken feed consumption. The study did not indicate a significant decrease in feed consumption as a result of probiotic supplementation. Some other studies have reported similar results, where feeding various probiotic bacteria did not lead to significant improvements in FCR and feed consumption rates (Sarangi et al., 2016; Atsuti et al., 2022). Better results were achieved by Chen et al. (2018), where broilers (white leghorns) were fed with *L. rhamnosus*. At the end of their study, a significantly reduced FCR was observed in *L. rhamnosus* group, compared to chickens that were not fed probiotics. Similarly, Fesseha et al. (2021) managed to reduce FCR, when the efficacy of *L. paracaseis* and *L. rhamnosus* was studied. A significant reduction in FCR has also been achieved by adding *Bacillus subtilis* and *L. acidophilus* in a 1:1 ratio to the basic diet by Zhu et al. (2020).

Overall, noticeable production ratings were obtained from both study groups with no significant differences. This could be explained by the fact that when the chickens are not exposed to the risk of disease and stress, as in the case of the current study, the addition of probiotics to the feed may not give the expected results. This shows the significance and importance of microclimate and housing on broiler chicken production and development (Baurhoo et al., 2007; Ebeid et al., 2021).

**Edible organs**

One of the objectives of the current research was to determine how the consumption of probiotics (*L. farciminis* and *L. rhamnosus*) affects the weight of edible organs since there are discrepancies in the literature about the effects of probiotic consumption and the growth of edible organs, such as the liver, heart, and gizzard. Some studies described a significant effect on the weight gain of these organs (Agustono et al., 2022). However, some authors found no significantly higher relative organ weights after feeding chickens probiotics (Awad et al., 2009; Shah et al., 2021). Some studies described that feed supplemented with probiotics could significantly decrease the relative weight of organs (Sugiharto et al., 2018).

In the current study, adding *L. farciminis* and *L. rhamnosus* to the feed increased heart weight in chickens of the ProL group at the age of 1 and 14 days and liver weight at the age of 7 days. However, in the remaining part of the study, no significant differences were observed in the relative weights of the heart, liver, and gizzard between the two groups. Such a significant difference has not been found in the studies of other authors (Awad et al., 2009; Shah et al., 2021). Unlike the current study, the findings of Agustono et al. (2022) on the chicks from ISA brown strain fed by probiotics (containing *L. acidophilus*, *L. plantarum*, and *Bifidobacterium* spp.) indicated a significantly higher relative weight of the liver and heart at the end of the study. Some studies described that feed supplemented with probiotics could significantly decrease the relative weight of organs. Such results were described by Sugiharto et al. (2018), where the relative weight of the heart in the experimental groups of Lohman broiler chicks fed with multistrain probiotics in different concentrations was significantly lower than that of the Con group. Atsuti et al. (2022) found that chickens' liver weight was significantly lower than that of the Con group when increasing the amount of probiotics in the feed.

## CONCLUSION

The broiler chickens that received the appropriate possible age-related housing conditions then feed supplemented with *L. farciminis* (CNCM-I-3699) and *L. rhamnosus* (CNCM-I-3698) mixtures from hatching to 35 days of age did not significantly improve production rates. However, some production indicators, such as body weight gain, EBI, FCR, and cumulative feed intake, were higher in the experimental group than in the control group. Regarding the ProL group, significantly higher weight gain of the liver was observed at the age of 7 days, and weight gain of the heart was observed at the age of 14 days. No significant differences in the weight of edible organs were observed at later age stages. It can be concluded that the difference between the heart and the liver weight could not be due to probiotic supplementation.

In order to emphasize that these results are important and that the effects of the consumption of probiotics cannot be evaluated unambiguously by other authors, research in this direction should be continued using histological, histochemical, and immunohistochemical examinations.

## DECLARATIONS

### Funding

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### Authors' contribution

Sabine Eglite contributed to data collection, database creation, and preparation of the manuscript. Lauma Mancevica participated in data collection, compilation, and preparation of the manuscript. Aija Ilgaza did the statistical analysis, managed the whole process, and made corrections. All authors checked and approved the final version of the manuscript for publishing in the present journal.

### Competing interests

The authors have declared that no competing interest exists.

### Ethical consideration

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

### Availability of data and materials

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

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# Effects of Supplementation of Saviotan Feed (Chestnut Tannin) on Blood Parameters and Yolk Cholesterol Concentration in Japanese Quails (*Coturnix japonica*)

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## ABSTRACT

Tannins are secondary metabolites and active compounds widely present in plants. Tannins have several properties, such as astringent, antiparasitic, anti-diarrheal, anti-bacterial, and antioxidant. Hence, plants containing tannins are a major study subject for a natural alternative to in-feed antibiotics or antioxidants. The functions of tannin extracted from chestnut wood, namely Saviotan Feed (SF) in poultry, especially in quails, have not yet been fully understood. The current study aimed to examine the effect of SF supplementation on some plasma metabolites, including glucose (GLU), triglyceride (TG), total cholesterol (TCHO) concentration, and yolk cholesterol in quails (*Coturnix japonica*). A total of 100 unsexed quails were divided into 4 groups, with 25 quails in each group. These quails were then placed into 20 experimental pens, with 5 quails per pen. Each treatment was replicated 5 times, and the quails were fed a commercial rations diet supplemented with different SF doses of 0% (control), 0.1%, 0.2%, and 0.3%. Quails were provided with SF supplementation from 14 to 56 days of age. A sample of 40 plasma and eggs were randomly collected and analyzed for GLU, TG, and TCHO. The results indicated no significant effects of SF on plasma GLU and TG concentration, but a significant effect was found regarding TCHO. Moreover, supplementation of SF from 0.1 to 0.3% significantly decreased TCHO concentration in the yolk. In conclusion, it has been determined that supplementation of SF may play a significant role in decreasing TCHO in yolk eggs in quails.

**Keywords:** Chestnut tannins, Cholesterol, Glucose, Japanese quail, Plasma metabolites, Triglycerides

## INTRODUCTION

Poultry production as a monogastric animal grows continuously to produce meat and eggs (Mohammed Hassan et al., 2020). Quail farming is one of the livestock sectors that efficiently provides quality animal protein (Handarini et al., 2008). Recently, the population of quail farming has developed in Indonesia. Based on the Indonesian Directorate General Livestock and Animal Health (2020), the population of quails in Indonesia was 14,819,755 heads. Subekti and Hastuti (2013) stated that quails can quickly grow and multiply. In about 42 days, quails have been able to produce eggs and can produce three to four offspring within a year. In addition, quails can produce 250-300 eggs with relatively little feed consumption (about 20 g/head/day). Quail eggs are rich in Vitamin A, riboflavin, and thiamine which benefit vision and immunity. In addition, quail eggs could be an alternative to chicken eggs for patients who cannot consume chicken eggs due to ovomucoid (Khalifa and Noseer, 2019). It was reported by the United States Department of Agriculture (2018) that the concentration of cholesterol in quail eggs was higher than that of chicken eggs (844 and 372 mg/ 100g, respectively). Rahmat and Wiradimadja (2011) stated that cholesterol concentration in the blood may directly affect cholesterol concentration in eggs and meat. Hypercholesterolemia could be affected by consuming high cholesterol concentrations of quail eggs (Khalifa and Noseer, 2019). Hence, the effort to decrease cholesterol content may benefit consumers. Previous studies have reported some methods for decreasing the cholesterol content of egg yolks. For instance, Warren et al. (1988) demonstrated that blended hexane with solvent egg yolk could produce a mixture with 62.2% cholesterol. In addition, Borges et al. (1996) mixed the emulsifying of yolk and acetone with a ratio of 1:12 (weight/weight) to maintain these emulsifying properties. Tannins are found in the plant kingdom and compounds of the polyphenolic group (Huang et al., 2018). Tannins extracted from Chestnut wood (*Castanea sativa* Mill), a common plant species in the Mediterranean area, are an example of hydrolyzable polyphenols characterized by gallic acid moiety (Field et al., 2012). Tannins are valuable because they could potentially replace antibiotics in chicken feeds (Huang et al., 2018). Although the inclusion of tannins in rations of monogastric animals has been discouraged over the years due to their antinutrient contents (Huang et al., 2018), they could have positive effects on monogastric animals if tannins were supplemented at appropriate levels (Huang et al., 2018). Moreover, tannins may reduce the spread of zoonotic pathogens and some risks of diseases in animals. Recently, investigations on using tannins in the bird production sector indicated favorable results (Amirmohammadi et al., 2014; Brus et al., 2018).

Since the EU banned antibiotic growth promoters (AGP) in animal feed in 2006, many strategies have been proposed for replacing AGPs and maximizing growth performance (Schiavone et al., 2008; Mirzaei et al., 2022). Previous reports revealed that supplementation of 3% grape extract tannins in the ration of broiler chicken decreased growth performance, but 1% of grape extract tannins inhibited the growth of pathogenic bacteria (Hughes et al., 2005). In addition, Schiavone et al. (2008) revealed that supplementation of 0.2% chestnut wood tannins accelerated growth and reduced mortality in broilers. In terms of its effect on physiology, it was revealed that supplementation of tannin extract at 75 mg/kg body weight/day significantly reduced total cholesterol (TCHO) and low-density lipoprotein (LDL) levels but did not affect TG and high-density lipoprotein (HDL) levels in hypercholesterolemia in white rats (Umarudin et al., 2012). Budiarto et al. (2016) revealed that tannin compounds can precipitate proteins on the small intestine's surface to reduce fat absorption.

The metabolic performance of the body could be evaluated by plasma metabolite measurements (Weikard et al., 2010). Some factors may contribute to metabolic changes in plasma metabolites, such as natural factors, genetic type, farm conditions, age, physiological state, and nutrients (Erwan et al., 2014; 2018; 2021). Several studies have been carried out on the influence of tannins on several types of livestock, both ruminants and non-ruminants, but a few studies have been carried out on quails. Therefore, this study was conducted to determine the effect of Saviotan Feed (SF) supplementation in the commercial ration on plasma metabolites and egg yolk cholesterol in quails.

## MATERIALS AND METHODS

### Ethical approval

This research was conducted under strict regulations in accordance with the recommendations in the Guide for the Care and Use of Animal, at the Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru, Indonesia.

### Study animals

One-day-old Japanese quails were obtained from a commercial farm located at Garuda Sakti, Indonesia. The study was carried out from May to July 2023 at the Livestock Production Technology, Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Indonesia. The current study investigated 100 laying quails from hatching to 56 days of age. The quails were kept in 20 cages, and each cage was 50×50×20 cm. Before being treated, quails were weighed to obtain a homogeneous initial weight of 100 heads and transferred to cage units of 5 heads each. The addition of SF to the commercial ration started when the quails were 14 days old and continued until they reached 56 days old. The study lasted for 42 days, during which ration and water were provided *ad libitum*.

Each pen unit consisted of 5 quails. A commercial diet (Metabolizable energy of 3050.34 kcal/kg, crude protein of 23.5%) was supplemented with different SF doses (0%, 0.1%, 0.2%, and 0.3% of diet). The SF was provided from Gruppo Mauro Saviola, Mantova, Italy. Table 1 shows the nutritional contents of the commercial feed (Indonesia).

**Table 1.** Nutrient contents of commercial feed of quails

| Nutrients | Content values |
|---|---|
| Crude protein (%) | 23.50 |
| Crude fiber (%) | 1.88 |
| Crude fat (%) | 5.87 |
| Calcium (%) | 0.29 |
| Phosphor (%) | 0.15 |
| Metabolizable Energy (Kcal/kg) | 3050.34 |

## Research procedure

### *Preparation of Saviotan Feed*

The commercial feed was mixed with SF powder every week for each treatment group.

### *Preparation of cage*

The cages and equipment were cleaned and sanctified using disinfectants. Treatment groups were selected on the basis of their body weight to ensure uniformity among the groups. The location of each cage was determined through randomization and each cage was then coded, making it easier to record data.

### *Measured parameters*

The parameters measured in this study were plasma metabolites, including glucose (Glu), triglyceride (TG), and TCHO, using the method by Erwan et al. (2018). The cholesterol concentration of egg yolk was also measured. Blood sampling was carried out at 56 days of age. Two heads of quails from each cage were used as samples, resulting in 40 quails. Blood sampling was carried out after the quail was fasted for 10 hours. This involved slaughtering the quails by cutting the jugular vein and then collecting approximately 3 ml of blood samples using an Eppendorf tube containing ethylenediaminetetraacetic acid (EDTA) to prevent clotting. The blood samples were stored in an ice flask. The quails were decapitated without anesthesia. Then, the samples were taken to the livestock production technology laboratory of the faculty of agriculture and animal science, State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru, Indonesia. The cholesterol concentration of egg yolks was analyzed from 40 eggs using the Liebermann-Burchard method. The analysis was performed using a UV-visible spectrophotometer at Andalas University Biotechnology Laboratory, Padang City, West Sumatera, Indonesia.

### Statistical analysis

One-way ANOVA was used for analyzing all the data and the Tukey-Kramer test was performed as a post-doc test. Significant differences were indicated by $p < 0.05$. Values were presented as means ± Standard Error Mean. Statistical analysis was performed using the commercially available package (SAS, 1998). Thompson rejection test as ($p < 0.05$) to eliminate outliers was applied for all data before being used for data analysis.

## RESULTS AND DISCUSSION

### Plasma glucose concentration

Table 2 shows the effect of dietary supplementation of SF on Glu levels. No Significant effect was observed between treatments regarding Glu ($p > 0.05$). This outcome was predictable since tannin content in chestnut wood was relatively small (0.1-0.3%/kg), so it did not affect blood glucose levels. Feed consumption also may influence glucose levels. According to Purnamasari (2009), the influential factors in blood glucose are metabolic food intake and glucostatic activity of the liver. Although there were no significant effects among treatments, the trend of Glu concentrations decreased in SF-supplemented treatments, compared to control.

Tannins can also have the function of stimulating glucose and fat metabolism. Hence, the deposits of both sources of calories in the blood can be avoided, resulting in cholesterol and blood sugar decrease (Kurnia et al., 2010).

**Table 2.** Effects of supplementation of different Saviotan doses on plasma glucose of quails aged 14 to 56 days

| Treatment | Glucose (mg/dL) |
|---|---|
| Control (0%) | 424.80 ± 57.80 |
| SF (0.1%) | 332.20 ± 39.07 |
| SF (0.2%) | 395.60 ± 33.96 |
| SF (0.3%) | 339.00 ± 38.20 |

Values are mean ± SEM, SF: Saviotan Feed

### Total plasma triglycerides

The TG concentration of plasma in quails treated with commercial rations supplemented with SF is shown in Table 3. As can be seen in Table 3, supplementation of commercial ration of quails with 0.3% of SF up to 56 days of age did not have a significant effect on TG levels ($p > 0.05$). Generally, TG serves as energy reserves. This result is consistent with that of Jannah et al. (2018), who reported that high TG levels can cause a rise in triglycerides leading to disturbances in blood circulation.

The SF supplementation level in the current study was probably not optimal to affect TG level. Schiavone et al. (2008) reported that hydrolyzed tannins (HT) from chestnut wood, such as castalagin, have antimicrobial effects against several types of microbes, like *Escherichia coli*, *Bacillus subtilis*, *Salmonella enteritica*, *Clostridium perfringens*, and *Staphylococcus aureus*.

Based on Table 3, the TG content with the supplementation of 0.1-0.3% SF was 198.9-328.30 mg/dL.

The results of this study were relatively higher than the research by Widowati et al. (2012), indicating that turmeric flour (*Curcuma longa L.*) influences TG levels of quails ranging from 86.8-115.8 mg / dL.

**Table 3.** Effects of supplementation of different Saviotan doses on plasma triglycerides of quails aged 14 to 56 days

| Treatments | Triglycerides (mg/dL) |
|---|---|
| Control (0%) | 242.80 ± 47.32 |
| SF (0.1%) | 328.30 ± 34.69 |
| SF (0.2%) | 198.90 ± 59.55 |
| SF (0.3%) | 247.60 ± 86.92 |

Values are mean ± SEM, SF: Saviotan Feed.

**Plasma total cholesterol**

Table 4 shows the plasma concentration of TCHO levels at the end of treatment. As can be seen, there was a significant difference among the treatments ($p < 0.05$).

**Table 4**. Effects of supplementation of different Saviotan doses on plasma total cholesterol of quails aged 14 to 56 days

| Treatment | TCHO (mg/dL) |
|---|---|
| Control (0%) | 211.00 ± 26.14[a] |
| SF (0.1%) | 230.10 ± 27.07[ab] |
| SF (0.2%) | 319.20 ± 49.84[b] |
| SF 0.3%) | 161.20 ± 18.28[a] |

[ab]Means different superscript letters were significantly different at $p < 0.05$). Values are mean ± SEM, SF: Saviotan Feed, TCHO: Total cholesterol

Some factors, such as feed, influence the concentration of TCHO in the blood. Cholesterol is a hydrophobic molecule that can be transferred in the blood through spherical macromolecules in the plasma lipoproteins, such as chylomicrons, HDL, LDL, and VLDL. The SF supplementation affected quail blood plasma TCHO levels in the current study. Based on the results, the concentration of TCHO was the lowest in the group supplemented with 0.3% SF, compared to other treatments. It was speculated that SF could lower the TCHO content in the direction of the flow of free fatty acids, which can reduce the lipoprotein formation that carries cholesterol. Previous studies have demonstrated that tea tannins inhibit increased serum cholesterol concentration when administered to rats fed a peroxidized corn oil diet (Okuda et al., 1984).

In addition, Gato et al. (2013) reported that plasma total cholesterol levels significantly decreased when humans consumed tannin-rich fiber. The TCHO concentrations in this study ranged from 161.20 to 319.20 mg/dl, which was higher than a study by Blaszczyk et al. (2006), who reported that quail blood cholesterol content ranges from 180 to 220 mg /dl.

**Egg yolk cholesterol of quail**

Changes in egg yolk TCHO levels following supplementation of different doses of SF are shown in Table 5. The tannin content of chestnut wood may decrease the cholesterol concentration of yolk eggs in quails. The concentration of active compounds in the form of tannins decreases cholesterol concentration in egg yolk by inhibiting cholesterol absorption in the digestive tract. Previous research has reported that tannin compounds may inhibit fat absorption by binding fat to intestinal mucosal epithelial cells and increasing cholesterol binding in fiber. As a result, cholesterol can be excreted through feces and not absorbed into the body (Josten et al., 2006). Furthermore, Kurnia et al. (2010) stated that tannins can stimulate glucose and fat metabolism, so the accumulation of both sources of calories in the blood can be avoided, resulting in decreased cholesterol and blood sugar.

Results of the current study indicated a decrease in cholesterol concentration of egg yolk in quails fed SF supplemented ration. Minieri et al. (2016) revealed that a diet with chestnut tannin extract decreased cholesterol by about 17% in Mugellese and 9% of yolk in the Leghorn chicken breed. Mello and Santos (2004) reported that the content of tannin compounds in feed can inhibit the performance of several digestive enzymes, including trypsin, amylase, and lipase, then decrease the availability of amino acids that support the egg formation process, including cholesterol content. Tugiyanti et al. (2016) stated that tannins could inhibit the absorption of food substances, including fat and cholesterol, in the digestive tract by the mucus layer. Winarno (1989) stated that the cholesterol concentration could decrease in the body through two pathways, firstly by being converted into bile acids, and secondly by excreted from the body in the form of neutral sterols in feces. The dietary supplementation of SF in a practical dose may decrease the cholesterol concentration. For human consumption, eggs with a lower cholesterol concentration could be recommended as an alternative for controlling heart disease (Minieri et al., 2016). Further studies are needed to evaluate the relationships between SF and other factors to regulate cholesterol in other poultry species.

**Table 5**. Effects of supplementation of different Saviotan doses on yolk total cholesterol of quails aged 14 to 56 days

| Treatment | Egg yolk cholesterol (mg/dL) |
|---|---|
| Control (0%) | 1,031.14 ±56.95[a] |
| SF (0.1%) | 869.30 ± 53.85[b] |
| SF (0.2%) | 826.26 ± 27.66[b] |
| SF (0.3%) | 869.20 ± 41.32[b] |

SF: Saviotan Feed, [ab]Means different superscript letters were significantly different at $p < 0.05$, Values are Mean ± SEM.

## CONCLUSION

Supplementation of SF in the commercial ration at a 0.1-0.3% level could decrease cholesterol in the egg yolk of quails but did not affect plasma Glu and TG. It is concluded that the optimal level of dietary supplementation of SF for decreasing the cholesterol concentration of yolk eggs in quails is 0.2%.

## DECLARATIONS

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### Availability of data and materials

Data from the current study are available at the editors' request.

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### Authors' contributions

Edi Erwan, Isbul Irfan, and Wawi Ibrah conducted the research, prepared data, performed the statistical analysis, and wrote the first draft. Afriadi and Muhammad Rodiallah edited the manuscript. All authors have checked and approved the final version of the manuscript.

### Competing interests

The authors declare no conflicts of interest.

### Ethical consideration

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

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# Design and Analysis of Ventilation System for Closed Poultry House in Tropical Climate Conditions

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## ABSTRACT

The climate significantly impacts the temperature in different parts of the world. A moderate environment makes it simple to construct a chicken farm. Nevertheless, raising the birds in tropical places where typical temperatures can exceed 40-45°C is difficult because they can only survive at temperatures between 30°C and 35°C. As a result, the current study aimed to design a chicken house with a ventilation and cooling system to prevent excessive heat. The effectiveness of ventilation systems in maintaining liveable and constant conditions at the chicken house was assessed using computational fluid dynamics modeling to mimic internal and external airflows. In this study, a water evaporator-based cooling system and an exhaust fan-based ventilation system were built within a poultry house. ANSYS CFD was utilized to create the design and examine the flow of the model. The findings of each model were generated individually, and these results were compared to those of the other models to determine which model could decrease the temperature within the chicken coop. The proposed model's maximum temperature was around 30-32°C. A poultry house can be constructed using this idea to maintain chickens at a suitable temperature range of 30-32°C.

**Keywords:** Computational fluid dynamic, Evaporator, Exhaust fan, Poultry house, Ventilation

## INTRODUCTION

Poultry is one of the agricultural sectors driving the fastest growth in the Indian economy. Crop output has grown at a rate of 1.5-2% per year, whereas egg and poultry meat production has grown at a rate of 8-10% per year (Mondal and Mishra, 2022). According to FAOSTAT data, India holds the sixth position in the global chicken market. The domestic poultry industry is currently experiencing exceptional growth, boasting a remarkable 18% growth rate. Moreover, investments in the production of chicken meat, which happens to be India's most popular meat, have seen substantial increases in this sector (Reddy and Bhatia, 2022). The annual broiler meat production in India is estimated to be 4.8 million tons. The broiler and egg industries in India generate $12.96 billion in income per year (Saner and Shekhawat, 2022). The number of eggs produced climbed from 30 billion in 2000 to 65 billion in 2014, while annual per capita egg consumption increased from 28 to 65, transforming India into one of the world's largest chicken markets (Das and Samanta, 2021). In India, more than 87% of chicken farms have double-winch curtained side vent apertures for natural ventilation; only circulating fans are used in the summer (Thapa, 2022). They struggle to maintain environmental controls, including appropriateness, stability, and uniformity of the interior climate, due to their inadequate ventilation systems, which causes significant stress on the hens, subpar disease control, and lower productivity (Guo et al., 2022). The broiler houses have been updated, but because of their expanded size to facilitate automation and high production, their interior temperature has not yet risen considerably (Costantino et al., 2022).

In various common types of broiler houses in India, previous studies have examined air temperature, relative humidity, gas concentrations, chicken weight, sudden death syndrome, mortality rates, and other factors (Ahmad et al., 2022). Many researchers performed field investigations in India to learn how ventilation affects the climate within broiler houses. Only a few measurement sites were located within the home, and these trials only

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lasted one season (Choab et al., 2019; Chimankare et al., 2023). Therefore, using their data to evaluate the reliability, stability, and appropriateness of the inner environment would be difficult. They also failed to submit a new ventilation plan. To meet India's three unique seasons, broiler house ventilation systems must be quickly modified. The fundamental problem with most field experiments was that the weather was frequently unexpected and variable, making it hard to affect it (Sanz et al., 2021). Computational fluid dynamics is one of the best and most efficient tools for analyzing agricultural aerodynamics (CFD, Han et al., 2021). It is typically the most straightforward, affordable, and precise method to assist design choices that take into account all of the nuances of an actual fluid flow. It offers trustworthy information and is applicable to early design cycles (Vinuesa and Brunton, 2022). However, the skill, knowledge, and experience of the designer have a big impact on CFD accuracy. More relevant and reliable data are needed to evaluate CFD validation and determine whether the problem's physics has been appropriately defined due to the growing quantity and quality of numerical simulations of flow fields (Gan et al., 2022).

In an effort to enhance and optimize the thermal well-being of broilers in current facilities, this study makes an effort to explain the air temperature and airflow patterns within a broiler house heated by an industrial metal furnace.

## MATERIALS AND METHODS

A thorough explanation of the design's parameters and component elements is presented in this section. Four CFD models were simulated using FLUENT v19.1, a commercial software program. Based on the work by Xiao et al. (2022), the classic k-turbulence model with improved wall functions was used to create CFD models.

### Poultry house design

The chicken coop was created as a component of a closed system. Brick walls, concrete floors, zinc-coated sheet metal, and mechanical ventilation and cooling systems were used to construct modern poultry houses. A total of 7500 birds can fit within the poultry house, which is 45.72 meters long, 9.14 meters wide, 4.57 meters high, 0.60 meters overhung, and has a roof with a 25% slope. On average, there were 17 birds per square meter. In the current study, chickens were kept on a litter floor. Both the foundation and the floor were constructed out of concrete. Figure 1 depicts a schematic of the poultry house.

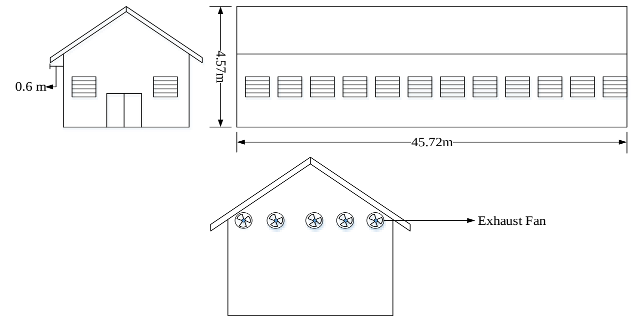


**Figure 1.** Schematics of poultry house

### Window design with shutter

To promote airflow, windows were constructed on either side of the hen house. To guarantee adequate air circulation, 11 windows were constructed on each side of the poultry house by the design of the current idea. Maintaining the temperature within the ideal range of 32°C to 35°C is crucial for the effectiveness of the ventilation systems in providing the best air supply for the chickens inside the building. To achieve this, the design of the current model incorporates a window with a movable shutter. This adjustable shutter is a vital component as it allows for precise control over the amount of air entering the poultry house. As a result, it is crucial to keep the window shutter at a particular angle. The research results on the window ventilation system by Tao et al. (2021) showed that the outlet louvers could significantly influence temperature. The optimal louver angle of $45^{o}$ can increase natural airflow for two types of glazing by 10 to 14%, improving ventilation, compared to a condition without louvers. On the other hand, downward-opening louvers (112.5-1500) would lessen the natural flow by 6% to 9% for every 100-degree increase in louver angle. To measure the impact of typical solar conditions on the efficiency of natural ventilation, empirical models were also built. Figure 2 illustrates the window with ventilation schematics.

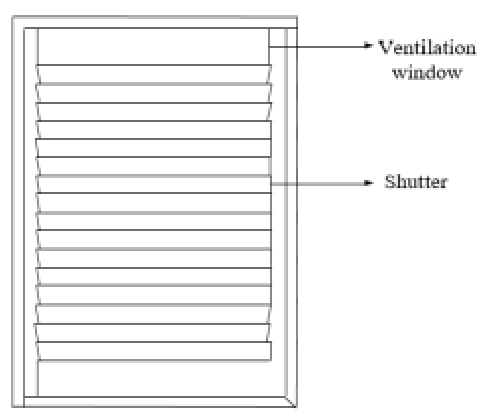


**Figure 2.** Ventilation window with shutter for poultry farm

**Water sprinkler**

The roof of the chicken house was made of zinc-coated sheet metal because the birds would be exposed to the sun's heat. Inside of the house was kept from heating up by direct evaporative cooling systems. A homogenous misting technique was applied at roof height, with a mist evaporation ratio of 0.33, a misting flow rate of 0.04 to 0.06 kilogram per second, and water output of 2.40-3.60 liters per minute. The water sprinkler's schematics are shown in Figure 3.

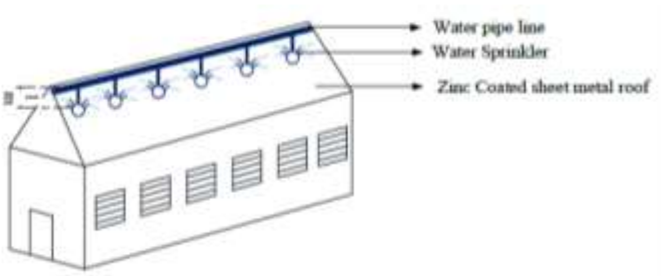


**Figure 3.** Schematics of water sprinkler for poultry farm

**Exhaust fan for ventilation**

The chicken's body temperature and the structure's interior warming up due to the climatic conditions contributed to the heating process. To evacuate the warm air from the poultry house, a ventilation system with an exhaust fan must be used. The ventilation system for the negative pressure tunnel in the current design consisted of five exhaust fans. The chicken house was equipped with an exhaust fan system to circulate the inside air outdoors. The fan was fixed inside the home on a side wall. Each fan had a 100 cfm (cubic feet per minute) output capacity. When the fan was running, the shutters were maintained open, and gravity drew them shut when it was not. The exhaust fan schematics for the ventilation system are presented in Figure 4.

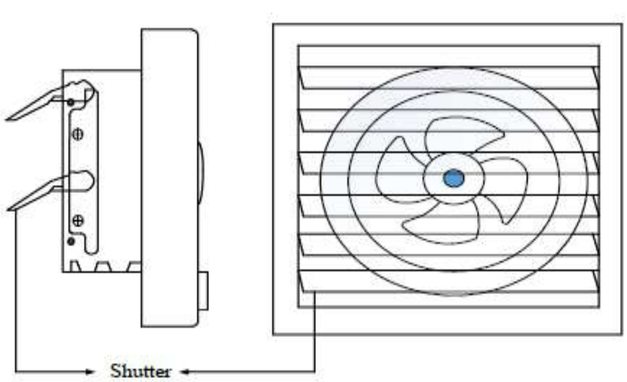


**Figure 4.** Schematics for exhaust fan in the poultry house

**Computation fluid dynamics design evaluation**

In this part, the thermal behavior of a chicken coop was explored using computational methods and fluid dynamics (CFD) data structures. Complex issues needed fast supercomputers, and software was created to increase accuracy and speed in difficult modeling situations. Initial validation typically entailed experimental methods, such as wind tunnels, and comparisons with prior research were made. The ANSYS CFD tool was used to simulate the flow.

**Geometry**

After starting ANSYS CFD, the model's geometry was created. To put the design into practice, there were two methods. To design using building parameters in ANSYS CFD, the model was first created in design software (SolidWorks, CATIA), imported into ANSYS, and then exported. ANSYS CFD was used to create the model. The design geometry schematic is shown in Figure 5.

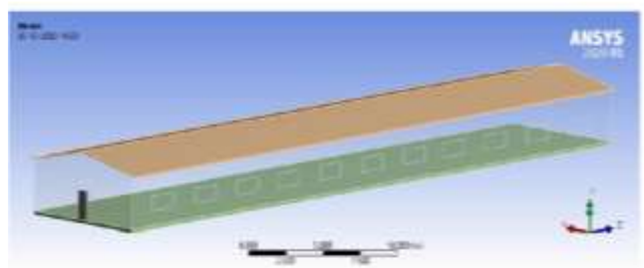


**Figure 5.** The geometry of poultry house in CFD

**Meshing**

Meshing the design was part of the second analytical step after the geometry generation stage. Similar to finite element simulations, a fluid body and its boundary were applied to a numerical grid in CFD meshing. For the current model, 691017 elements and 174741 nodes were mesh. A schematic illustration of the meshing is illustrated in Figure 6.

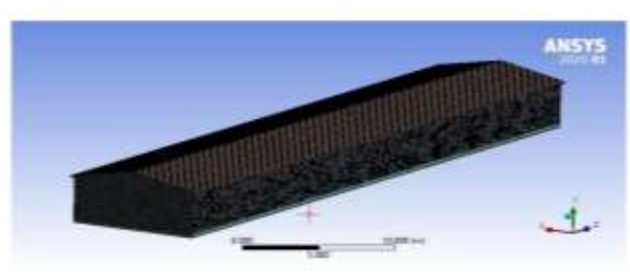


**Figure 6.** Schematics of the meshed design for poultry house

**Setup**

It is the third stage of the model's flow analysis. The model was built up using the appropriate domain (solid or fluid) choices. The inlet, outlet, roof, floor, and exhaust fan material qualities and processing parameters were set for each domain. The type of consequence has been demonstrated in this section. For the current model, two configurations that contain the Navier strokes equation and the continuity equation were activated.

***Continuity equation***

According to the continuity equation in fluid dynamics, the rate at which mass enters and leaves a system is the same as any steady-state process (Alabdalah et al., 2020). The continuity equation's differential form follows Formula 1.

$$\frac{\partial \rho}{\partial t} + \nabla\ (\rho u) = 0 \tag{1}$$

Where, t is time, ρ denotes fluid density, and u signifies flow velocity vector field.

***Navier strokes equation***

The Navier strokes equations, partial differential equations, were used in fluid mechanics to explain the movement of viscous fluids. These equations are advanced versions of those created in the eighteenth century by Leonhard Euler to explain the flow of incompressible and frictionless fluids. Mathematically speaking, the Cauchy momentum equation may be separated from the Navier-Stokes momentum equation. The Formula 2 shows primary convective pattern.

$$\frac{Du}{Dt} = \frac{1}{\rho} \nabla \cdot \sigma + g \tag{2}$$

The Cauchy momentum equation is obtained by multiplying a pressure value by the volumetric stress (Formula 3), which is the deviator stress term in the Cauchy stress tensor.

$$\rho \frac{Du}{Dt} = - \nabla p + \nabla \tau + \rho g \tag{3}$$

Where, ρ determines density, u refers to flow velocity, ▽ is divergence, p refers to pressure, t denotes time, and τ accounts for the deviator stress tensor.

**Solution**

In the fourth stage of the analysis process, the iteration was set to yield the most accurate result based on the setup details. The current design model had a 200-iteration cap, and the final calculation run was performed. Figure 7 explains the setup and solution's design.

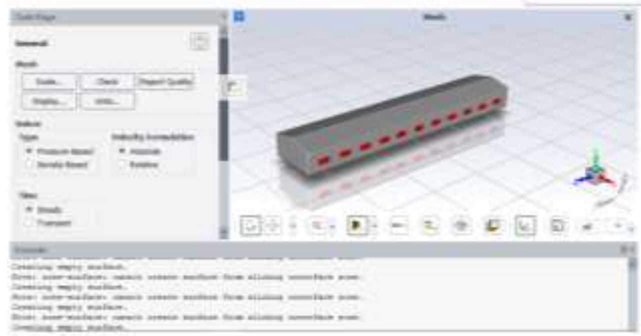

**Figure 7.** Setup and solution for poultry house

## RESULTS AND DISCUSSION

The outcome was produced using four stages. There are four types of chicken houses, namely poultry houses without an exhaust fan or vaporizer, poultry houses with an exhaust fan outlet but no vaporizer, and poultry houses with both an exhaust fan and a vaporizer. The CFD was used to calculate how the microclimate in a mechanically ventilated broiler house varied in space throughout the summer and winter in research by Küçüktopcu et al. (2022). To contrast the predicted results, actual data on temperature, relative humidity, and airspeed were gathered within the chicken farm. The study simulated alternative remedies for two problems, including winter stagnation zones and high summer temperatures, which may cause poultry heat stress. An evaporative cooling pad system might lower the temperature by around 3°C when the mean air temperature in the residence exceeded 25°C in the summer. To address the issue of hot and humid air buildup in stagnant areas of the poultry house during the winter, the installation of four 500-mm circulation fans spaced 20 meters apart could be a practical solution. These fans would help in improving airflow and eliminating the temperature and humidity variations within the chicken house. This study demonstrated how CFD may be effective in creating the best heating, cooling, and ventilation systems in chicken barns.

To create practical designs for functional indoor hen settings, Chen et al. (2021) assessed cage-free home ventilation systems. The interior conditions, particularly at the hen level, could be dependably managed within the survival temperature range, according to quantitative assessments of airflow, temperature, and pressure distribution contours. The hen house's ventilation rates inside four ventilation systems were at the higher end of the recommended ventilation range, another sign that the farm could be relied upon to maintain appropriate air quality during cold weather. This study highlighted the value of employing computational fluid dynamics modeling as a research tool to address issues with animal welfare in animal housing designs.

A study by Ferraz et al. (2022) set out to assess the climate inside a heated commercial broiler house. The proposed CFD model rendered accurate simulations of the experimental data. This investigation discovered that numerous areas of the broiler house experienced heating system issues during the trial periods, which may have negatively impacted the comfort of the birds and, eventually, their productivity and monetary losses. The information gathered might be used as a guide for choosing the ideal setting to support the development of chicks.

In a cage-free hen house, Chen et al. (2021) investigated how successfully ventilation systems maintained constant and suitable living conditions for hens. Four full-scale, floor-raised hen coop CFD models in three dimensions were created. In addition to the conventional top-wall inlet sidewall exhaust (TISE) ventilation architecture, these models each represented one of three alternative ventilation systems. For each ventilation scheme, 2,365 individual birds were simulated using simple forms. The average airflow at the bird level of the standard TISE model was 0.35 m/s (69 ft/minutes), according to the simulation findings of the mid-wall inlet ridge exhaust and mid-wall inlet attic exhaust. Neither model considerably differed from this value.

The effectiveness of three passive cooling systems in meeting thermal and indoor air quality criteria in a semi-arid chicken barn was examined by Al Assaad et al. (2021). Direct evaporative cooling was 6.8% more expensive than using a dew-point evaporative cooler. Costs were decreased by 4.7% when customized ventilation was used instead of standard ventilation using dew point devices, simultaneously maintaining the same air quality and temperature.

According to Obando Vega et al. (2022), compost-bedded pack barns (CBP) needed sufficient ventilation and maintain stable temperatures to create the ideal environment for a good composting process. As a consequence, this study was carried out utilizing a compost barn construction model and dimensional analysis methods for naturally ventilated buildings. Using CFD, three-dimensional models of compost barns with different ridge patterns and wind directions were created, along with a visual depiction of how these factors influenced the airflow through the structure. The findings showed that the CFD model and simulations for barn ventilation matched the actual data and provided precise airflow forecasts via the design of the CBP barns for various roof ridge types. The findings also indicated that the open ridge with a chimney was the ideal roof design for a west-to-east wind direction in the winter.

***Poultry house without exhaust fan and vaporizer***

In this model, the intake and outlet at the exhausted home were just the windows, and the outcome was calculated. Figures 8 and 9 display the temperature and velocity results from ANSYS CFD.

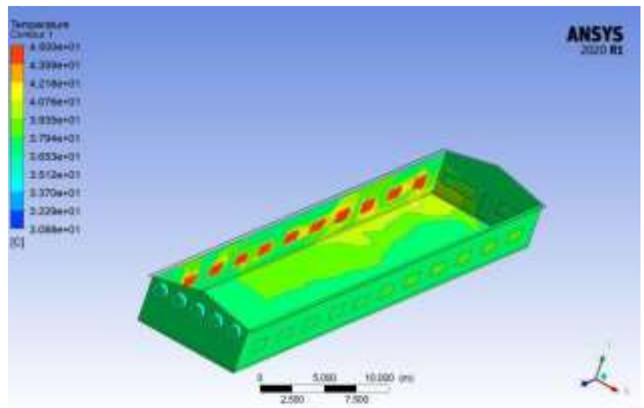


**Figure 8.** The temperature of the poultry house without using an exhaust fan and vaporizer

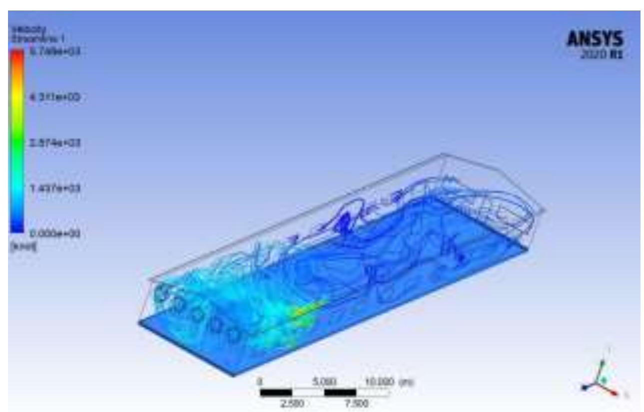


**Figure 9.** Velocity streamline of poultry house without using an exhaust fan and vaporizer

***Poultry house with vaporizer and without exhaust fan***

In this analysis, the input and outflow were provided as windows, and the springer was regarded as the evaporator for the cooling water. Figures 10 and 11 display the temperature and velocity results from ANSYS CFD.

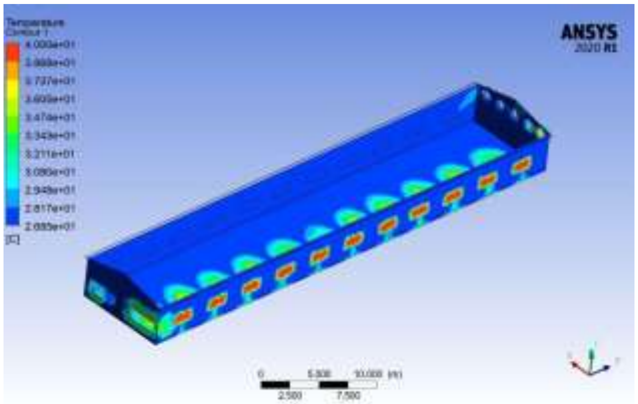


**Figure 10.** The temperature of poultry house with vaporizer and without exhaust fan

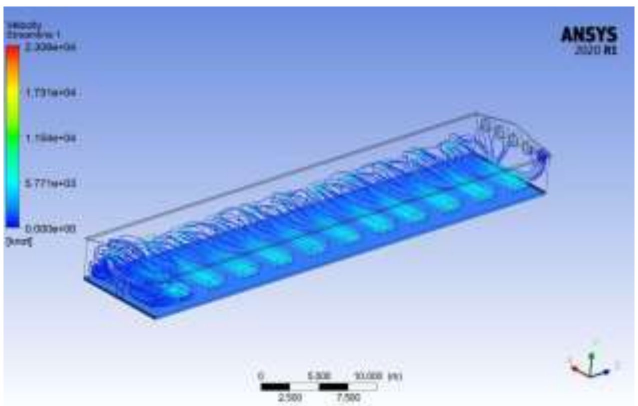


**Figure 11.** Velocity streamline of poultry house with vaporizer and without exhaust fan

***Poultry house with exhaust fan outlet and without vaporizer***

In this study, the intake was represented by windows, while a wall-mounted exhaust fan represented the output. Figures 12 and 13 show the ANSYS CFD result with temperature and velocity.

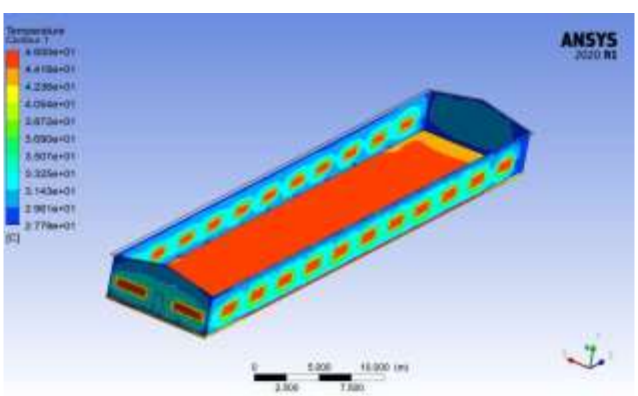


**Figure 12.** Temperature for poultry house with exhaust fan outlet

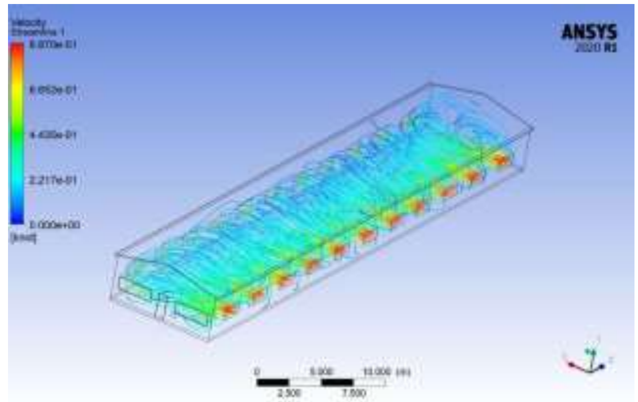


**Figure 13.** Velocity for poultry house with exhaust fan outlet

***Poultry house with vaporizer and exhaust fan***

In this part, the evaporator is employed as the cooling system, the windows are used as the input, and the exhaust fan is used as the outlet. Figures 14 and 15 show the temperature and velocity results from ANSYS CFD.

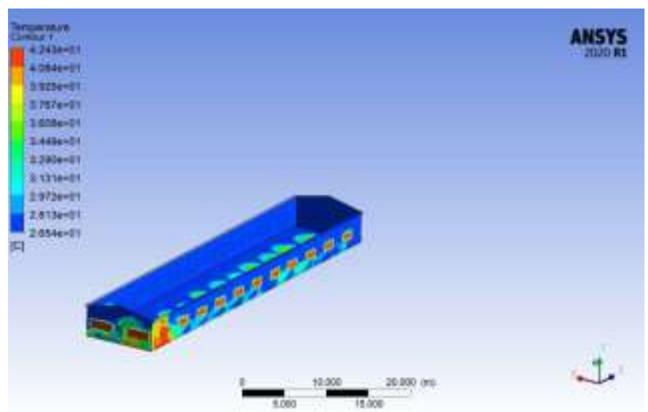


**Figure 14.** Temperature for poultry house with evaporator and exhaust fan

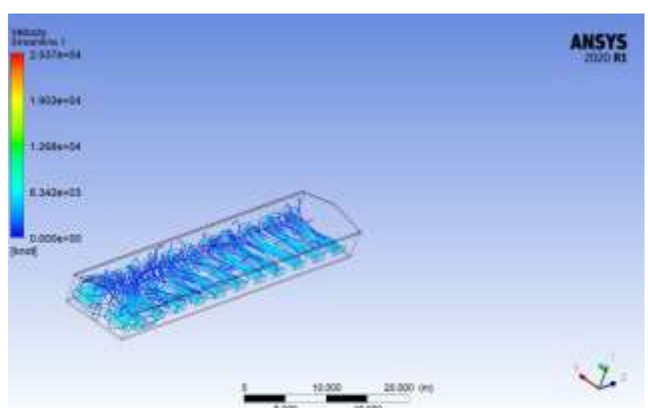


**Figure 15.** Velocity streamline for poultry house with evaporator and exhaust fan

**Comparative analysis**

The results were produced by the CFD tool under four distinct setup settings. The ambient temperature and mass flow rate for all models were 12 m/s and 40°C, respectively. Figure 8 depicts the temperature distribution on the poultry house for the first modal (poultry house without exhaust fan and vaporizer). Since it was determined from the findings that the heating is high on the floor area, the highest temperature ever recorded in the house using this model is 46°C.

The second model, a chicken coop with a vaporizer but no exhaust fan, was shown to have a maximum inside temperature of 37°C based on the temperature reduction on the floor and walls. The third model, a chicken coop without a vaporizer but with an exhaust fan outlet, showed that the outlet zone had a high temperature. The model's overall temperature is depicted in Figure 12, with the greatest temperature recorded as 36°C. The chicken house in its final form has an exhaust fan and a vaporizer as part of its ventilation and cooling systems. Figure 14 shows the results of CFD.

The x-axis in Figure 16 shows the names of models 1, 2, 3, and 4, while its y-axis displays each model's temperature. The temperature inside a chicken house was dramatically lowered by the installation of an exhaust fan and a vaporizer. Therefore, a temperature differential of 10°C may be created by the water evaporator (water springer) and exhaust fan as the air circulation system with this model. The four models' comparison graph is shown in Figure 16.

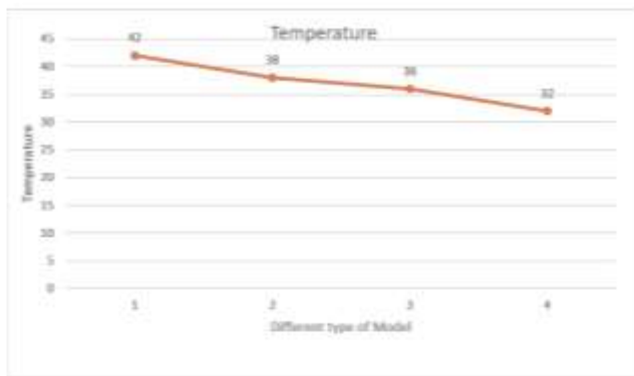


**Figure 16.** Result comparison chart for four analysis models.

***Comparative analysis of theoretical and computation fluid dynamics analysis***

This study made use of numerical analysis and computational fluid dynamics. The flow analysis was carried out using ANSYS CFD, and the numerical analysis was finished and published by Saner and Shekhawat (2022). Table 1 compares the results of theoretical and numerical analysis.

**Table 1.** Parameter comparison between numerical and computational fluid dynamics analysis

| Input parameters | Numerical analysis | CFD analysis |
|---|---|---|
| Mass flow rate (kg/s) | 12 | 12 |
| Temperature ambient (°C) | 40 | 40 |
| Exhaust fan (rpm) | 100 | 100 |
| Ventilation window (Shutter angle in °C) | 45 | 45 |
| Approach | Energy balance equation (calculation) | Continuity equation and Navier strokes equation (flow analysis) |

CFD: Computational fluid dynamics

***Output comparisons***

The internal temperature of the design was calculated numerically to be 28°C for a mass flow rate of 12 m/s and an ambient temperature of 40°C. According to the results of the CFD research, the second model suggested in the current study had an ambient temperature of 40°C and an interior temperature of 30°C with a mass flow velocity of 12 m/s. Therefore, 28°C and 30°C were the final results of both studies. As a result, the average error could be calculated as 2°C.

## CONCLUSION

The ANSYS CFD was used in this project to design and assess the poultry house. Ventilation and cooling were accomplished by the evaporator and the exhaust fan. The design of the model incorporated four different analytical techniques. A poultry house without an evaporator or exhaust fan came in first, a poultry house with an evaporator but no exhaust fan was in the second phase, a poultry house with both an evaporator and an exhaust fan were in third, and a poultry house with both was presented in fourth stage. According to the CFD results, the evaporator and exhaust fan may be used to lower the temperature in the chicken house to the lowest possible level. This model could reach temperatures as high as 32°C. By applying this theory, a chicken coop could be built, keeping the birds between 30 to 32°C. Future studies on sophisticated automation and monitoring technology can enhance the inside atmosphere of chicken coops. Depending on the situation in real-time, sensors, data analytics, and machine learning algorithms may change the temperature, humidity, and ventilation. It allows dynamic adjustments to lower interior heat and provides a comfortable avian environment.

## DECLARATIONS

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### Authors' contributions

Kapil A. Saner wrote the original draft, methodology, study conception and design, analysis and interpretation of results, and reviewing and editing. Sanjay P. Shekhawat has conceptualized, collected data, reviewed, and edited. The authors confirmed their contribution to the paper as follows, and all authors reviewed the results and approved the final version of the manuscript.

### Availability of data and materials

Not Applicable

### Ethical consideration

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

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### Competing interests

The corresponding author states that the authors have no competing interests on behalf of all authors.

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# Effects of Partnership Patterns on Broiler Chickens' Performance in the Agribusiness System of Indonesia

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## ABSTRACT

The partnership system is standard broiler cooperation in Indonesia. This system influenced agribusiness performance. Hence, the current study aimed to analyze the broiler agribusiness system in Kediri Regency, Indonesia, addressing three main areas, including partnership patterns, production performance, and financial performance. In this study, data was gathered from participants using a cross-sectional survey approach, capturing information from individuals at a specific moment in time. The research was performed from July to September 2022, utilizing both primary and secondary data. Primary data was obtained through direct observations and interviews with relevant stakeholders, while secondary data was sourced from various databases, such as the Indonesian Statistical Bureau and the Agriculture Ministry of Indonesia. Both types of data were subjected to quantitative descriptive analysis. The results indicated that the broiler partnership pattern consisted of three subsystems, including the chicken production facility providers (day-old chicks, feed, and medicines), the farming unit responsible for production process management (housing, feeding, drinking, and biosecurity), and the marketing subsystem focusing on chicken prices). The farmers in the farming unit showed effective production performance with a feed conversion ratio of 1.69, an index performance of 307, and an average body weight of 2.03 kg/head. Moreover, the farmers demonstrated a profitable financial performance with the revenue-cost ratio exceeding 1, reaching 1.07.

**Keywords:** Agribusiness**,** Broiler, Income, Partnership, Profitable, Revenue

## INTRODUCTION

The broiler producer industry holds significant promise as a vital animal commodity due to its efficient chicken meat production, which serves as a major source of animal protein consumption in society (Azizah et al., 2013). According to Fitrah (2013), broilers are a poultry product with better performance and productivity than other products, such as beef, goat, and pig. Additionally, the Indonesian chicken commodity demonstrates substantial growth potential, driven by an increased market demand resulting from the country's rising population. According to the Indonesian Central Bureau of Statistics (2023), there was an increase of 6% in chicken production in Indonesia between 2019 and 2021, aligning with the massive Indonesian population growth of 273.52 million.

Moreover, the broiler industry plays a crucial role in meeting the ever-growing demand for meat among consumers. The chicken population is enormous, resulting in widespread farm production across all corners of the country (Ratnasari et al., 2015). Many factors contribute to the development of chicken commodities, such as the availability of breed, feed, medicine, and industry equipment. Similarly, some factors strengthen the upstream industry, such as breeding, animal feed, veterinary medicine, and farm equipment companies, and the downstream industry in the form of chicken meat processing (Amam, 2022). Hence, the broiler is a necessary commodity with substantial business potential for growth.

The partnership pattern is a standard broiler cooperation system chosen by farmers (Ulfa et al., 2021). A partnership is a business strategy by two or more business parties within a certain period to achieve profits with the principle of mutual benefit between the partnering parties (Kalangi et al., 2021). The partnership pattern in the chicken sector is one way of cooperation between small farmers called plasma farmers and private companies as the core. The reasons for choosing this system by farmers include limited resources on all sides, the shift in the position of the main actors from the government and the private sector to the community, and other complex problems, such as lack of funding, market competition, feed access, and business risk (Ulfa et al., 2021). The core of the broiler partnership approach must provide facilities for livestock production, technical and management advice, and accommodating and marketing production outcomes. Plasma farmers provide cages and carry out cultivation activities. The proceeds from the sale of chickens are handed over to the core party at a price adjusted to the contents of the cooperation agreement contract. According to Ridwan (2022), breeders want to join the partnership system to get the intensity of assistance with production facilities and earn income. In this case, the farmer's satisfaction depends on the profits with various receipts because they work on different business scales.

The Livestock Service Office of East Java Province stated that in 2022 the broiler population in East Java would reach 252,918,032 chickens, with meat production reaching 442,478.71 tons per year (Livestock Service of East Java Province, 2022). In this case, the human population of East Java reached 39.74 million people in 2022 (Ministry of Communication and Technology, 2022). Compared to the total population of East Java people, chicken production is still low since the total chicken meat consumption increased by 8.62% per year in 2022 (Indonesian Ministry of Agriculture, 2022). Given these circumstances, East Java can be an important province for broiler development. Kediri Regency is also a critical area for this commodity development since it has a large area of 1,588.79 $km^2$ with a population of 1,140,809 people. Within this region, broiler farms have been established, ranging from small to medium and large-scale operations. Many factors affect the scale of the business, including investment, capital, cages, land, and equipment. The investment and capital in this scenario may influence the number of broilers and the production costs. Hence, the present study aimed to examine the broiler farming agribusiness system in Kediri Regency, Indonesia, with a particular focus on the issues mentioned above.

## MATERIALS AND METHODS

### Ethical approval

All subjects participated voluntarily and provided written informed consent to participate in this study. The study was approved by the ethics committee of the Animal Science Faculty, the University of Brawijaya (register number KEP.29/07/2022)

### Study area

The current research was performed in Kediri Regency, Indonesia, due to its status as the largest production center of broilers in the region. Furthermore, Kediri Regency stands out for having the highest number of farmers engaged in partnerships with private companies using a partnership pattern in East Java Province (Mahendra, 2023). The research was carried out in July-September 2022 using a cross-sectional survey method, collecting data from respondents at a single point in time.

### Respondent criteria

The respondents were 80 plasma farmers and 6 broiler partnership core companies. Respondents were determined using purposive sampling with the plasma farmers as the samples with a chicken population between 2,000-12,000 chickens. The farmers had a three-year minimum partnership period and carried out broiler farming activities for at least three production cycles within 2022. Moreover, the partnership core companies were the plasma farmers' business partners, with more than 5 years of experience.

### Data collection

The data collection method employed in this research involved field observation and interviews. Field reflection was carried out through direct observations and interviews with respondents on-site to gather primary data. Ethical considerations were given utmost importance, and consent was obtained from all participants in adherence to the standard ethical guidelines and regulations set forth by the University of Brawijaya.

The primary data were predominantly acquired through interviews and questionnaire distribution to both farmers and partnership companies. Questionnaires were administered directly to the respondents, encompassing various aspects, such as farmer identity, production costs (including the quantity and price of inputs), and revenue (entailing the quantity and price of output). Similarly, partnership companies' data included respondent identity, broiler input development, and output prices.

The secondary data included information on the broiler population and the development of broiler input and output prices from relevant agencies. These agencies encompassed the Livestock Service Office of the government, Animal Husbandry Companies, Trade Office, and the Indonesian Central Bureau of Statistics.

### Production performance analysis

#### *Feed conversion ratio*

Feed Conversion Ratio (FCR) is defined as the amount of feed required to produce one kilogram of body weight. Ideally, 1 kilogram of feed should result in 1 kilogram or even more (FCR $\leq$ 1) of body weight (Ulfa et al., 2021). Unfortunately, this condition is not always achieved. In broilers, the target FCR of 1 can usually be achieved before the chickens reach 2 weeks of age. After that, the FCR increases as the chickens grow older. According to Karar et al. (2023), the formula for FCR is as follows, FCR= Total Feed (g)/ Total Weight (g).

#### *Index performance*

The index performance (IP) is one of the main parameters used in measuring the success of a farm. The IP value is obtained through calculations based on the ratio of feed consumption in a given period, the total weight achievement of the livestock at the time of harvest, the average age of the livestock at the time of harvest, and the percentage of mortality rate (Ulfa et al., 2021). The formula for IP is as follows:

IP = ([100-D] × ABW X 100) / FCR × average age

Where, IP defines Performance Index, D denotes the depletion percentage of the chicken population (%), ABW is the average body weight at harvest (kg), FCR refers to feed conversion ratio, and the average age represents the average duration required for raising broiler, typically measured in days.

The IP values in broiler chicken farming are classified into five categories. The IP below 300 is classified as poor, IP in the range of 301-325 is considered fair, IP in the range of 326-350 is good, IP in the range of 351-400 is classified as very good, and IP above 400 is excellent (Santoso and Sudaryani, 2011).

#### *Other parameters of production performance*

Average body weight (ABW) is another parameter for measuring production performance in the broiler industry. According to Santoso and Sudaryani (2011), the ABW in the chicken industry refers to the average weight of a chicken at a specific stage or age. It is commonly used as a measurement to assess the growth and development of chickens. The unit of measurement for ABW is typically kilograms.

### Financial performance analysis

#### *Production costs*

Production costs in the chicken industry refer to the expenses incurred in raising and producing chickens for commercial purposes, including feed costs, housing and equipment cost, utilities, and medicine expenditures (Azizah et al., 2013).

#### *Revenue and income*

In the broiler industry, revenue refers to the total amount of money generated from selling chickens and chicken-related products (Sehabudin et al., 2022). It represents the income generated from the primary business activities of selling live chickens and other chicken-related products. Income, on the other hand, refers to the profit or financial gain obtained from the chicken industry after deducting the production costs and expenses from the revenue (Azizah et al., 2013). It shows the net earnings after deducting all expenditures, such as feed, labor, housing, veterinary charges, utilities, and other operating costs.

#### *Revenue costs ratio*

The revenue cost ratio (R/C), also known as the cost-to-revenue ratio, is a financial metric used in the chicken industry to assess the relationship between revenue and costs (Afandi et al., 2020). It is calculated by dividing the total costs incurred in chicken production by the total revenue generated from the sale of chickens and chicken-related products. The R/C is measured using the formula below.

R/C ratio = Revenue (IDR) / Costs (IDR).

Where, R/C refers to the revenue cost ratio and IDR represents Indonesian Rupiah, the Indonesian currency.

### Statistical analysis

The study examined the resulting data using descriptive analysis (SPSS, version 20, USA) to describe the situation within the samples and the partnership pattern system so that the study would achieve a factual and accurate description of the facts, characteristics, and relationships between the phenomena being investigated.

## RESULTS AND DISCUSSIONS

### The partnership patterns

#### *The subsystem of the production facility provider*

The production facility provider is crucial in day-old chick (DOC), feed, vaccines, medicines, and equipment. Its primary obligation is to provide livestock production facilities for the plasma farm. Some criteria considered in this subsystem are accuracy of time, place, price, type, quantity, and quality. Farm agribusiness operations can run more efficiently when this production provider is available and adequate (Salman, 2020). In Kediri Regency, Indonesia, the farming subsystem of the production facility provider includes providing DOC, feed, and Medicines.

***Day old chick***

The core company provides DOC to plasma farmers using it for chicken rearing. The DOC strains maintained by the majority of farmers are Cobb, Lohman, and Ross, depending on who the core company supplier is and what the manufacturer is. The price of DOC varies in each period but is the same for each plasma stratum because the DOC supply comes from the same core company. The price of DOC is also dependent on the contract list between plasma farmers and core companies. In several cases, the difference in the price happens due to the additional costs, such as the additional costs of the vaccine in the hatchery. Figure 1 shows the average DOC cost to raise chickens for six periods with 34,029,007 Indonesian Rupiah (IDR).

Based on Figure 1, the DOC price fluctuated in each period. Supply and demand influence the DOC price; in this case, the core companies also have a significant role in determining the price. According to Rahmadani (2009), certain seasons also affect the DOC price. For example, a notable increase in activity can be observed during period 4 (P4), which corresponds to the months of July and August. This period coincides with the start of the new academic year, and it also includes holidays when many individuals plan and take vacations. Consequently, this combination of factors contributes to the highest surge in activity during this time.


**Figure 1.** The cost of day-old chicks during six periods of rearing broiler chickens

**Table 1.** Cost of feed during six periods of rearing broiler chicken in Indonesia in 2022

| Period | Feed consumption (kg) | Feed costs (IDR) | Feed costs per bird (IDR/bird) | Increase percentage (%) |
|---|---|---|---|---|
| Period 1 | 12,998.48 | 110,544,612 | 23,978.78 | 0 |
| Period 2 | 14,115.53 | 121,533,879 | 26,362.52 | 10 |
| Period 3 | 14,125.63 | 124,359,330 | 26,975.40 | 2 |
| Period 4 | 14,793.94 | 129,577,390 | 28,107.28 | 4 |
| Period 5 | 14,104.67 | 120,505,348 | 26,139.42 | -7 |
| Period 6 | 13,519.57 | 115,569,573 | 25,068.77 | -4 |
| Average | 13,942.97 | 120,348,355 | 26,105.36 | |

IDR: Indonesian Rupiah.The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

***Feed***

The core companies also provide feed to plasma farmers. These companies include Charoen Pokphand Indonesia Ltd. (Limited liability company), Malindo Feed Mill Ltd., Japfa Comfeed Indonesia Ltd., Cheil Jedang Indonesia Ltd., and New Hope Indonesia Ltd. There are two phases for feeding depending on the chicken's development period. Those phases are the starter phase in the form of granules (crumble) and the finisher phase in the form of pellets. Feed is a crucial element in the financial system as it substantially contributes to business costs with 60-80% of total production costs (Syamsudin, 2000). Table 1 illustrates the different feed consumption of broilers due to harvest time and mortality in each period. The highest consumption occurred in period 4 because of the low mortality rate; thus, more chickens were produced during this period.

Feed costs can affect farmers' income. When feed prices rise, production costs also increase, affecting the selling price of broilers later. Feed costs reach 80% of production costs for plasma-core farmers with supplies from feed manufacturers. This is because the core company supplies the majority of feed without farmers being independent and skilled to make their feed ratio. Similarly, feed and breed costs are the main costs in broiler farming (Suwarta et al., 2012). According to Amri et al. (2017), the core company has set the feed price stated in the partnership contract.

***Medicines***

The core company plays a vital role in supplying medicines to plasma farmers. The administration of these medicines is crucial for preventing diseases that may arise during the rearing of chickens, as well as for preventive measures against certain diseases. Each core company collaborates with livestock medicine companies to support the production process of their plasma farmers. Table 2 shows the average cost of medicines needed to raise chickens for six periods with 1,815,082 IDR.

According to the data presented in Table 2, period 5 stands out with the highest medical costs compared to the other periods. This indicates that during this specific period, more funds were allocated to purchasing medicines for the chickens. The primary reason behind this higher expenditure is that period 5 occurs in September-October, which marks the transition from the dry season to the rainy season. Such shifts in weather conditions can significantly impact the health of livestock, including chickens.

The research findings by Widianingrum et al. (2023) support this observation, as they reported that environmental temperature changes, like those experienced during seasonal transitions, can have notable effects on the prevalence and severity of poultry diseases.

**Table 2.** The cost of medicines used during the six periods of raising broiler chicken in Indonesia in 2022

| Items | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 6 | Average |
|---|---|---|---|---|---|---|---|
| Medicines costs (IDR) | 1,727,983 | 1,761,146 | 1,844,954 | 1,843,068 | 2,004,290 | 1,709,050 | 1,815,082 |

IDR: Indonesian Rupiah.The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

**Table 3.** The average selling price of broiler chicken per kilogram for six periods in Indonesia in 2022

| Items | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 6 | Average |
|---|---|---|---|---|---|---|---|
| Broiler price (IDR/kg) | 19,588 | 20,040 | 20,236 | 20,261 | 19,763 | 19,787 | 19,946 |

IDR: Indonesian Rupiah.The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

**Farming business subsystem**

The farming subsystem includes the production process, such as housing, feeding and drinking management, and sanitation.

***Housing***

The majority housing system is stilt cages with a common area of 50 m × 8 m × 1.5 m (from the ground to the base of the cage) × 2 m (from the base of the cage to the roof) for a capacity of 2,500 chickens (Figure 2). The main advantage of stilt cages is that it is easier for air circulation to enter, considering that the Kediri Regency, Indonesia, is a hot area. Less air circulation can cause respiratory problems and disease in chickens (Brouček and Čermák, 2015). The coop direction is from west to east or vice versa, so there is no direct exposure from sunlight to the chicken. The cage roof is made of asbestos and tiles with a monitor roof. Asbestos and tile materials do not absorb heat like zinc roofs resulting in lower temperatures for the chicken. The use of a monitor roof is to provide air circulation in housing. Fadilah et al. (2007) state that the recommended leg height is 1.5-1.8 m with a width not exceeding 8 m and a length of 30-50 m. Stage cage system has better air circulation than postal cages because the air can enter through the bottom and side directions. In addition, farmers also utilize bamboo for housing systems (Wantasen et al., 2014).

**Figure 2.** Open house system of rearing broiler chicken for plasma farmers (tilt cage) in Kediri Regency, Indonesia, 2022

This bamboo housing system can allow air to enter between the floor gaps and provide air circulation. Similarly, the direction of the chicken coop is arranged from east to west (Rasyaf, 2008). This arrangement is based on the assumption that positioning the coop in this direction helps prevent the chickens from being directly exposed to sunlight. By avoiding direct sunshine, it helps to prevent the accumulation of excessive sunlight inside the coop.

The average chicken density for DOC, 1-week-old, 2-week-old, 3-week-old, 4-week-old, and 5-week-old was 350, 85, 44, 15.5, 10, and 7 head/$m^2$, respectively. Figure 3 shows the density of the cages on one of the plasma breeders in Kediri Regency, Indonesia. Fadilah et al. (2007) had a different opinion regarding broiler density in stage pens. They suggested a density of 6-7 individuals per square meter. The density of chickens in the coop varies between the rainy season and summer. During the rainy season, when the temperature is lower, a higher density is needed to provide sufficient warmth and prevent the chickens from experiencing cold stress. In contrast, during the summer, when the temperature is higher, a lower density is recommended to avoid overheating and ensure the comfort of the chickens.

**Figure 3.** Feed and drinking management of broiler chicken in plasma farmers in Kediri Regency, Indonesia, 2022

***Feed and drinking management***

The feeding system is *ad libitum* feeding from the DOC to the finisher phase in Kediri Regency, Indonesia. The amount of feed and time varies from day to day. This difference is due to the distinct daily feed requirements. Feeding at 1-14 days is in the crumbled form, and the feed from 15 days until harvest is in the form of pellets. According to Fadilah et al. (2007), a balanced diet with the proper proportions of protein, carbohydrates, fats, vitamins, and minerals is necessary for broiler chickens. The feed should be thoughtfully prepared and modified to meet the unique nutritional requirements of the broilers at various growth stages. This comprises starter feed for the initial growth stage, followed by grower feed, and finisher feed. According to Rasyaf (2008), the consumption of broiler rations is a reflection of various nutritious components entering the chicken's body. This amount of feed is what chicken needs for production and body maintenance.

The drinking system is also *ad libitum,* in Kediri Regency, Indonesia. Drinking water must always be available because the water content in the chicken's body is more than 70% (Figure 4). Chicken needs to consume a significant amount of water to prevent stress and dehydration, to keep body temperature, and to maintain body water content steady. Fadilah et al. (2007) stated that the temperature inside the cage impacts the water consumption of chickens. Specifically, as the temperature increases, chickens tend to drink more water.

**Figure 4.** The layout of the feed and drink system in the broiler open house cage in Kediri Regency, Indonesia, 2022

***Beosecurity management***

Sanitation plays a crucial role in biosecurity efforts for livestock farming by maintaining cleanliness and preventing the spread of diseases. Dewulf and van Immerseel (2019) emphasize that sanitation aims to prevent diseases by ensuring the cleanliness of the cages, environment, and equipment. Fadilah et al. (2007) highlight that biosecurity and sanitation measures are essential in stopping the transmission of infectious and zoonotic diseases. Cleaning cages and conducting fumigation after harvesting is an example of a sanitation practice in the farm, Kediri Regency, Indonesia. This helps eliminate potential disease-causing agents, reduce the cycle of diseases, control flies and odors, and safeguard the environment. The farmers also protect the chickens by vaccination, which is vital for maintaining the chicken's immune system against various diseases. According to Ravikumar et al. (2022), administering vaccines requires careful consideration of several factors. For instance, the vaccinated chickens should be in good health, the correct dose of the vaccine should be used, the appropriate vaccine type should be selected based on the chicken's age, and sterile equipment and proper packaging should be utilized. Vaccines can be administered at different ages and through various methods. For instance, the avian influenza vaccine can be given via subcutaneous injection at 3 days of age (Alkie et al., 2018), the Gumboro vaccine can be administered through drinking water at 12 to 14 days of age (Khan et al., 2017), and the New Castle Disease Clone vaccine can be administered via spray at 20-22 days of age (Dimitrov et al., 2017). It is important to note that vaccine administration may vary among farmers due to their experience and the specific history of diseases on their farms (Müller et al., 2012).

### The marketing subsystem

#### *Prices*

Partnership patterns have different prices in each period, but the price set is not far from the market price. The average price of harvested broilers fluctuated in each period. Market prices fluctuate (not fixed) and tend to rise because the number of broiler market demands is increasing daily, and the supply of broilers is decreasing. For instance, during holy shrine holidays, there is a lot of market demand, but chicken availability does not increase, causing broiler prices to rise. In the partnership system, there is a contractual agreement about the price between the core companies and plasma. Hence, price similarities exist even though the plasma farms differ in stratum and scale. The main reason is that the core companies determine the final price for the plasma farm. Table 3 shows the average selling price earned by farmers for six consecutive periods. The highest price is in period 4, with a value of 20,261 IDR per kg of chicken.

**Table 4.** The average selling price of broiler chicken per kilogram for six periods in Indonesia in 2022

| Items | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 6 | Average |
|---|---|---|---|---|---|---|---|
| Broiler price (IDR/kg) | 19,588 | 20,040 | 20,236 | 20,261 | 19,763 | 19,787 | 19,946 |

IDR: Indonesian Rupiah.The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

**Table 5.** Average Body Weight values generated for six periods of raising broiler chicken in Indonesia in 2022

| Items | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 6 | Average |
|---|---|---|---|---|---|---|---|
| Average body weight (kg) | 1.91 | 2.09 | 2.02 | 2.15 | 2.07 | 1.96 | 2.03 |

IDR: Indonesian Rupiah.The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

### The production performance

#### *Average body weight*

Average Body Weight (ABW) results from production in the form of average harvest weight obtained from broiler rearing activities. The ABW unit is kg/head. Table 4 shows the ABW values generated in each period. In this case, each period has a different value. The results showed that the average value was 2.03 kg/head, which all farmers have the probability of producing in each period.

#### *Feed conversion ratio*

Production performance is an essential factor in determining a farm's effectiveness. Farmers can see how effective their farms are by using variables such as the FCR value and IP. According to Rodde et al. (2020), the FCR value helps calculate how much feed is consumed to produce the desired livestock products such as eggs, meat, and milk. The lower the FCR means, the lower the feed costs required. In addition, farmers apply the IP value to assess the efficiency of broiler farming based on the percentage of live chickens, body weight, harvesting age, and FCR (Maharatih et al., 2017). Table 5 shows the amount of feed given and the total weight of the chickens produced each period. The second period has the best feed effectiveness compared to the other periods, as evidenced by a low FCR value. Overall, the effectiveness of feeding in broilers can be seen from the FCR value with an average of 1.69, and it is categorized as a good performance. The FCR value is used to calculate the efficiency of the feed consumed by broilers. The effectiveness of animal feed is inversely proportional to the FCR value; in other words, as the FCR value increases, the effectiveness of the feed decreases, and vice versa.

#### *Index performance*

Furthermore, Table 5 shows the average IP value of 307.14; this means that the farmer's performance is excellent and stable. According to Fadilah et al. (2007), a higher IP value indicates better production performance in chickens. They categorized IP values ranging from 300-350 as indicative of stable and effective production performance. In other words, achieving an IP value within this range is considered desirable for optimal performance in chicken production.

**Table 6.** Feed conversion ratio obtained during six periods of rearing broiler chicken in Indonesia in 2022

| Items | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 6 | Average |
|---|---|---|---|---|---|---|---|
| Total feed (kg) | 12,998.48 | 14,115.53 | 14,125.63 | 14,793.94 | 14,104.67 | 13,519.57 | 13,942.97 |
| Total body weight (kg) | 7,722.85 | 8,453.20 | 8,178.79 | 8,720.60 | 8,393.49 | 7,949.85 | 8,236.46 |
| FCR | 1.68 | 1.66 | 1.72 | 1.69 | 1.68 | 1.69 | 1.69 |
| IP | 310.60 | 313.39 | 299.84 | 304.67 | 307.85 | 306.52 | 307.14 |

FCR: Feed Conversion Ratio, IP: Index Performance.The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

**Table 7.** Total cost for six periods of broiler chicken rearing in Indonesia in 2022

| Items | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 6 | Average |
|---|---|---|---|---|---|---|---|
| Total costs (IDR) | 144,135,629 | 157,614,025 | 160,718,110 | 167,661,744 | 157,057,871 | 150,033,261 | 156,203,440 |
| Total costs per bird (IDR) | 31,950 | 33,858 | 34,887 | 36,825 | 34,316 | 32,985 | 34,137 |
| Total costs per kg (IDR) | 18,578 | 18,520 | 19,416 | 19,250 | 18,670 | 18,777 | 18,869 |

IDR: Indonesian Rupiah. The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

**Table 8.** Farmers' revenue and income during six periods of raising broiler chicken in Indonesia in 2022

| Items | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 6 | Average |
|---|---|---|---|---|---|---|---|
| Total revenue (IDR) | 155,609,988 | 172,538,160 | 168,911,332 | 179,367,644 | 168,447,617 | 162,365,641 | 167,873,397 |
| Total costs (IDR) | 144,135,629 | 157,614,025 | 160,718,110 | 167,661,744 | 157,057,871 | 150,033,261 | 156,203,440 |
| Income (IDR) | 11,474,358 | 14,924,135 | 8,193,222 | 11,705,900 | 11,389,746 | 12,332,380 | 11,669,957 |
| Income/c (IDR) | 2,887 | 3,754 | 2,061 | 2,945 | 2,865 | 3,102 | 2,936 |

IDR: Indonesian Rupiah.The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

**Table 9.** Revenue/Cost **(**R/C) ratio for six periods of rearing broiler chickens in Indonesia in 2022

| Items | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 6 | Average |
|---|---|---|---|---|---|---|---|
| Total Revenue/bird (IDR) | 39,147 | 43,406 | 42,493 | 45,124 | 42,377 | 40,847 | 42,232 |
| Total Costs/bird (IDR) | 36,261 | 39,651 | 40,432 | 42,179 | 39,511 | 37,744 | 39,296 |
| R/C Ratio | 1.08 | 1.09 | 1.05 | 1.07 | 1.07 | 1.08 | 1.07 |
| Percentage of increase (%) | - | 1 | -4 | 2 | 0 | 1 | |

IDR: Indonesian Rupiah.The six periods of broiler chicken rearing refers to the latest six durations during which broiler chickens are raised from day-old chicks to the point of reaching market weight from 2021 to 2022

## The financial performance

### *Production costs*

Production costs are costs incurred by farmers in the process of broiler production. This cost includes various forms such as depreciation, DOC, feed, medicines, vitamins, employee wages, electricity, and so on. Table 6 shows the costs required to maintain broiler chickens for six periods. The table reveals the cost difference in each period, with the highest costs in the fourth period, with 167,661,744 IDR or 36,825 IDR per head.

### *Revenue and income*

Revenue and income are essential factors in measuring financial performance in the livestock business. Farmers receive income from their production activities through sales of livestock products, bonuses from core companies, and sales of manure waste (Azizah et al., 2013). In addition, farmers calculate their income or profit by total revenue minus total production costs (Edwards and Duffy, 2014). Table 7 represents the revenue and income received by farmers from period 1 to period 6.

Farmers obtained the highest revenue and income in period 2, with 172,538,160 IDR and 14,924,135 IDR, respectively. The results indicated in the figures are higher than the average per period, with a revenue of 167,873,397 IDR and an income of 11,669,957 IDR. Table 7 shows that the average farmer's income per head is IDR 2,936.

### *Revenue cost ratio*

Calculating the revenue cost ratio (R/C) is one of the production variables to measure the efficiency of a business farm. According to Gumus (2008), this ratio analysis aims to evaluate the efficiency of input and output activities by comparing total revenue and production costs. Table 8 indicates the analysis of the R/C ratio. The analysis shows that broiler farming is profitable because the R/C ratio is more than 1 per rearing period with an average of 1.07 (Table 9).

## CONCLUSION

In conclusion, the livestock partnership pattern in Kediri Regency, Indonesia consists of three subsystems, namely production facility provider, farming business, and marketing. Through this partnership, various calculations related to production performance, such as the feed conversion ratio and performance index, were carried out. The results indicated that the broiler farms demonstrated profitable performance in this study based on the return on cost (R/C) ratio, the broiler farmers exhibited positive financial performance, which proved to be beneficial for their businesses in the current study.

## DECLARATIONS


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### Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author, Hartono (budihartono_ub@ub.ac.id). The data are not publicly available due to containing information that could compromise the privacy of research participants.


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### Authors' contribution

Budi Hartono was involved in planning and supervising the work. Nanang Febrianto and Muhammad Helmi processed the experimental data, performed the analysis, drafted the manuscript, designed the figures, and performed the calculations in this study. Puji Akhiroh aided in interpreting the results and worked on the manuscript. All authors read and approved the final manuscript.

### Competing interests

The authors declare that there is no existence of competing interests.

### Ethical consideration

The authors have examined ethical issues, including plagiarism checks, publication permission, misconduct, and duplicate publishing.

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# Effects of Supplementation of *Eurca* Seeds as Nutraceutical Feed Additive on Productivity, Antioxidant Activity, and Yolk Cholesterol Level of Laying Hens

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## ABSTRACT

*Eruca sativa* (*ES*) seeds are annual herbs belonging to the *Brassicaceae* family, widely grown in Mediterranean countries, such as Egypt, Italy, and Greece. The *ES* is rich in macronutrient components and phytochemical content, exhibiting potent antioxidant properties and functional properties for vital processes such as digestion and absorption of nutrients. Therefore, this research was conducted to evaluate the effects of dietary *ES* supplementation on laying performance, some blood parameters, and egg yolk cholesterol. A total of 300 Silver Sabahia strain hens, aged 26 weeks, were randomly distributed among four groups of five replicates, each replicate consisting of 15 hens. Chickens in group 1 served as a control and were fed the basal diet. Those in groups 2, 3, and 4 were fed basal diet supplemented with 1, 2, and 3% *ES*s, respectively. Productive performance traits, egg quality traits, hematological parameters, blood parameters, and yolk cholesterol profiles were performed throughout the study. The study lasted for 13 weeks (until week 39 of chickens' age). Results indicated that 3% *ES* supplementation had higher results on egg mass (35.68%), egg production (21.13%), and improved feed conversion ratio by 30.37%, compared to all groups. Furthermore, *ES*s supplementation positively affects the shell thickness and yolk color score compared to the control. Compared to the control, the highest significant blood hemoglobin and lymphocytes were recorded in the groups supplemented with 2% and 3% of *ES*s. The *ES* inclusion at a higher level (3%) in the diet of laying hens led to significantly enhanced serum high-density lipoprotein and total antioxidant capacity, while reducing cholesterol, low-density lipoprotein, and malondialdehyde levels, compared to the control diet. Serum calcium, tri-iodothyronine, and alkaline phosphatase levels increased significantly in response to 3% *ES* treatment, while liver enzymes decreased significantly compared to the control diet. Notably, the addition of 2% and 3% *ES*s to the hens' ration resulted in reduced egg cholesterol content, which is desirable for consumers seeking healthier dietary choices. Finally, adding 3% *ES*s to hens' diet improves productive performance, egg quality traits, hematological parameters, blood parameters, and yolk cholesterol profile.

**Keywords:** Blood parameters, Egg production, *Eruca* seed, Nutraceutical additive, Yolk cholesterol

## INTRODUCTION

Domestic Egyptian chickens appear to have great genetic diversity and can survive harsh environmental conditions. One of the major problems in the Egyptian local breeders is the high conversion rate and low egg production as their egg production curve ends rapidly (Khalil, 2020; El-Saadany et al., 2022a; Farag et al., 2022). Moreover, table eggs from local Egyptian chickens are very popular among consumers, in Egypt but egg yolks are high in cholesterol, which may cause health problems (Deif Allah et al., 2020). Several attempts have been made to improve egg production, feed conversion rate, egg quality, and yolk cholesterol, and one of these ways was to manipulate the diet by using natural products (El-Saadany et al. 2022b,c). Nutraceuticals are biologically active substances found in natural products. They can be added to poultry diets for nutrition and health benefits (El-Sabrout et al., 2023).

*Eruca sativa* (*ES*) seeds are one of the annual herbs of the *Brassicaceae* family. It is easily grown in Mediterranean countries like Egypt, Italy, and Greece. *Eurica sativa* seeds are of great importance for human and animal health. They contain various nutritional and therapeutic properties, such as antibacterial, anti-carcinogenic, antifungal, and antioxidant properties (Kim et al., 2004). These seeds are a good source of essential oils, proteins, and phytochemicals, such as flavonoids and glycosinolates (Barillari et al., 2005; Bell and Wagstaff, 2014), which is incorporated in poultry biological functions for promoting health and productivity. Additionally, it is rich in minerals like Ca, Zn, Cu, Fe, I, K, and other elements (ELSadek, 2014). It also contains carotenoids, vitamins C, E, and K, most types of vitamin B groups, and volatile oils. For all these benefits, Egyptian farmers tend to grow *ES*s to provide a cheap source of phytochemicals and antioxidant products to consumers (El-Gengaihi et al., 2004; Barillari et al., 2005).

Previous studies showed that feeding layers with *ES* increased egg quality by improving eggshell thickness and the density of the yolk color (Al haj et al., 2019). Also, the addition of *ES*s in broiler feed improved immunity and serum oxidation systems, which led to increases in the broilers' productivity, and modulations of intestine histomorphology characteristics under the various types of stresses (Shani, 2019; Al-Shammari and Batkowska, 2021). Shani (2019) stated that dietary broilers with ESs at 2.5 g/Kg had a protective effect against any oxidative stress induced. Moreover, Abou El-Maaty et al. (2021) demonstrated that applying *ES*s to broilers' diets increased serum total antioxidant, follicle-stimulating hormone, and thyroid hormones while decreasing the concerning activities of liver enzymes. Similarly, Abdul-Majeed and Taha (2019) found that adding *Eruca* seeds to the quail diet as a rich omega 3 and 6 sources improved the lipid profile and egg production. Although some aimed to decrease egg yolk cholesterol by adding natural substances, such as sumac and ginger (Gurbuz et al., 2017), green tea extract (Huang et al., 2019), and grape seeds (Sun et al., 2018) to layer diets, there is no information about the impact of *ES*s supplementation on egg yolk cholesterol. Therefore, the current research was carried out to study the impact of *ES*s inclusion into Egyptian layer diets on their physiological state to improve productive performance and reduce egg yolk cholesterol.

## MATERIALS AND METHODS

### Ethical approval

The current study was performed according to the guidelines of the Departmental Committee of Animal and Poultry Production, and the pronouncement of the Ministry of Agriculture in Egypt on animal ethics and welfare (Decree No. 27 (1967) that generally enforces the humane treatment of animals.

### Experimental design

A total of 300 layer hens at 26 weeks old from the Silver Sabahia strain (Egyptian local strain), with average body weight (1751 ± 95 g), were used in this trial. The experiment was conducted during the spring period of 2022, lasting for 13 weeks. Chickens for the study were obtained from two sources, namely El-Sabahia Poultry Research Station in Alexandria, and the Animal Production Research Institute within the Agricultural Research Centre in Alexandria, Egypt. Chickens were vaccinated according to the following program. The chicks received vaccinations against Newcastle disease, Gumboro, and Infectious Bronchitis during the first day. In week 3, they were vaccinated against Gumboro. At week 6, they received vaccinations against Newcastle and Infectious Bronchitis. Week 7 included vaccinations against foul typhoid. In week 14, the chickens were once again vaccinated against Newcastle and Infectious Bronchitis. Subsequently, vaccinations against Newcastle were administered every 4 weeks. All hens, which were from the same hatching batch and had similar body weights, were randomly divided into four groups, each consisting of 15 birds, resulting in a total of 5 replicates per group. These hens were individually housed in cages measuring 30 * 50 cm. The lighting was controlled artificially, with 16 hours of light per day, and the average temperature was maintained at 25℃, with an average humidity rate of 74%. Throughout the experimental period, which spanned from weeks 26 to 39 of age, feed and water were made available to the hens *ad libitum*.

Regarding the dietary treatments, the control group was provided with the basal diet, while the second group had the basal diet supplemented with 1% *ES*s/kg of diet. In the third group, the basal diet was supplemented with 2% *ES*s/kg of diet, and the fourth group had the basal diet supplemented with 3% *ES*s/kg of diet.

The *ES*s (with no preparation) were purchased from the General Company for Agricultural Agencies in Damanhor, Egypt. The ingredients and chemical composition of the basal diet were prepared according to (NRC, 1994), and the ingredients are presented in Table 1. The methods used for calculated chemical analysis of the basal diet were according to the Association of Official Analytical Chemists (AOAC, 2000).

**Table 1.** Ingredient and chemical composition (g/kg) of the experimental diet for laying hens through 26-39 weeks of age

| **Ingredients** | **(%)** |
|---|---|
| Corn | 66.33 |
| Soybean meal (48% CP) | 24.2 |
| Limestone | 7.5 |
| Dicalcium phosphate | 1.32 |
| Vit+Min Premix[1] | 0.25 |
| NaCl | 0.25 |
| DL-methionine | 0.15 |
| Total | 100 |
| **Chemical composition** | |
| Metabolizable energy ( kcal/kg) | 2700 |
| Dry matter (%) | 90.73 |
| Crude protein (%) | 16.97 |
| Crude fat (%) | 2.45 |
| Crude fiber (%) | 3.96 |
| Ash (%) | 6.37 |
| Nitorgen free extract (%) | 60.98 |

[1]Vit+Min mixture provides per kilogram of diet: vitamin A, 12000 IU; vitamin E, 10 IU; menadione, 3 mg; Vit. $D_3$, 2200 ICU; riboflavin, 10 mg; Ca pantothenate, 10 mg; nicotinic acid, 20 mg; choline chloride, 500 mg; vitamin $B_{12}$, 10 µg; vitamin $B_6$, 1.5 mg; vitamin $B_1$, 2.2 mg; folic acid, 1 mg; biotin, 50 µg. Trace mineral (milligrams per kilogram of diet): Mn, 55; Zn, 50; Fe, 30; Cu, 10; Se, 0.10; Antioxidant, 3 mg. CP: Crude protein

### Production and egg quality traits

The body weight was determined at the age of 26 weeks, and the hens were weighed again at the end of the experiment at the age of 39 weeks. Egg weight was recorded as eggs were weighed individually to the nearest 0.01 g for each replicate, and the average was calculated. Egg mass was calculated by multiplying egg numbers by the average egg weight. The egg production was recorded according to the following equation [(number of eggs / Period) * 100] from 26 to 39 weeks of age. Feed consumption by gram was evaluated for each chicken per day. The feed conversion ratio was calculated as the amount of consumed feed required for producing a unit of egg mass. Fifteen eggs from each group once every four weeks (at 30, 34, and 38 weeks) were randomly taken from the same days of production. Eggs were collected to determine egg quality traits. Shell, albumen, and yolk percent were determined by dividing each previous item by the egg weight and multiplying by 100. The shell thickness was measured by a micrometer to the nearest 0.01 mm. The yolk color intensity was calculated based on the standard color of the yolk using a Roche yolk color fan with a score range of 1-15 from light yellow to dark yellow (Vuilleumier, 1969).

### Hematological and biochemical parameters

At 39 weeks of age, 40 blood samples from the experiment (ten blood samples from each group) were randomly taken from the branchial wing vein in a tube with an anticoagulant (EDTA) and without anticoagulant for biochemical parameters. 40 Blood (10 from each group) samples were used to determine blood morphology, including hemoglobin (Hb), red blood cells (RBC'S), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscle hemoglobin (MCH), mean corpuscle hemoglobin concentration (MCHC), white blood cells (WBC'S), and their fractions (percentage of lymphocytes and heterophils). The RBCs were counted on an acridine orange (AO) bright-line hemocytometer using a light microscope at 400x magnification, while the WBCs were counted on an AO bright-line hemocytometer using a light microscope at 100x magnification after diluting blood samples 20 times with a diluting fluid (1% acetic acid solution with a little of Leishman's stain), and their fractions (lymphocytes and heterophils) were determined according to Altan et al. (2003). The Hb was determined by the cyanomethemoglobin method as cited by Coles (1986), while wintrobe hematocrit tubes were used for determining the PCV as a percentage. Serum samples were obtained by centrifuging the blood at 3000 rpm for 20 min, and it was stored at −20 °C for biochemical analysis. Serum total protein, glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium, phosphorus, alkaline phosphatase (ALP), total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured using commercial kits (Diamond Diagnostics Chemical Company, Egypt) following the manufacturer's instructions by a spectrophotometer (SELECTA®UV-2005 SPAIN). The value of serum total tri-iodothyronine (T3) was tested using the radioimmunoassay technique according to (Hollander and Shenkman, 1974) by chemical commercial kits (Diamond Diagnostics Chemical Company, Egypt).

### Yolk's cholesterol profile

Yolks were carefully separated without albumen for determination of yolk cholesterol (Allain et al., 1974), high-density lipoprotein (HDL, Lopez-Virella, 1977), and low-density lipoprotein (LDL, Wieland and Seidel, 1983) using commercial Kits (Diamond Diagnostics Chemical Company, Egypt).

### Statistical analysis

This study used an entirely statistical randomization design. All results were subjected to standard statistical one-way analysis of variance (ANOVA) in the Statistical Package for the Social Sciences, SPSS, 2008, version 17. Duncan's multiple range test was implemented to evaluate whether the means of the variables differed significantly or not (Duncan, 1955). Means were considered statistically significant at $p \leq 0.05$.

## RESULTS

### Productive and egg-quality traits

Table 2 shows the influence of *ES*s supplementation on the productivity of laying hens. As can be seen, there was an improvement in egg production (21.13%), egg mass (35.68%), and feed conversion ratio (30.37%) by adding 3% *ES*, compared to other experimental groups ($p \leq 0.05$). Table 3 illustrates the influence of *ES*s on the quality of eggs. Results indicated that Eggshell, albumin, and yolk percentage were not affected by *ES*s supplementation, while the administration of *ES*s in laying diets significantly improved shell thickness and yolk color score ($p \leq 0.05$).

**Table 2.** Productive performance of laying hens as affected by the supplementation of *Eruca* seeds through 26-39 weeks of age

| Traits \ ***Eruca*** **seeds levels** | Control | 1% | 2% | 3% | SEM | P value |
|---|---|---|---|---|---|---|
| Initial bW (g) | 1741.00 | 1753.11 | 1762.33 | 1750.44 | 95.55 | 0.008 |
| Final bW (g) | 1905.33 | 1777.66 | 1866.78 | 1810.33 | 99.11 | 0.313 |
| Egg Weight (g) | $52.45^{d}$ | $55.64^{a}$ | $54.29^{c}$ | $54.84^{b}$ | 0 | 0.000 |
| Egg Mass (g/h/d) | $29.01^{b}$ | $29.02^{b}$ | $29.60^{b}$ | $39.36^{a}$ | 1.34 | 0.000 |
| Egg Production (%) | $57.13^{b}$ | $53.74^{b}$ | $53.86^{b}$ | $69.20^{a}$ | 1.58 | 0.000 |
| Feed Intake (g/h/d) | $120.54^{a}$ | $118.68^{a}$ | $108.33^{b}$ | $113.71^{ab}$ | 2.96 | 0.026 |
| Feed Conversion ratio (g feed/g egg) | $4.28^{a}$ | $4.27^{a}$ | $3.71^{a}$ | $2.98^{b}$ | 0.22 | 0.000 |

$^{abc}$ Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the means, BW: Body weight

**Table 3.** Egg quality traits of laying hens as affected by the supplementation of *Eruca* seeds through 26-39 weeks of age

| Traits \ ***Eruca*** **seeds levels** | Control | 1% | 2% | 3% | SEM | P value |
|---|---|---|---|---|---|---|
| Shell weight (%) | 10.11 | 9.63 | 10.70 | 10.59 | 0.29 | 0.138 |
| Albumin weight (%) | 57.60 | 58.71 | 56.78 | 56.19 | 0.69 | 0.063 |
| Yolk weight (%) | 32.29 | 31.67 | 32.52 | 33.22 | 0.66 | 0.457 |
| Shell thickness (mm) | $0.32^{c}$ | $0.34^{b}$ | $0.35^{b}$ | $0.38^{a}$ | 0.01 | 0.000 |
| Yolk color score | $5.83^{c}$ | $6.79^{b}$ | $7.31^{b}$ | $8.19^{a}$ | 0.27 | 0.000 |

$^{abc}$ Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the mean

### Hematological parameters

Table 4 presents the effects of *ES*s on the hematological traits of laying hens' diets. The results revealed that the supplementation of *ES*s could significantly improve the Hb content at different levels of *ES*, compared to the control group ($p \leq 0.05$). Similar trend was observed on MCHC and Lymphocytes as they significantly improved by *ES*s addition at different levels compared to the control ($p \leq 0.05$).

### Biochemical parameters

Table 5 summarises the impact of *ES*s on laying diets' biochemical parameters. Results showed that cholesterol profile, liver enzymes, antioxidant parameters and Ca significantly improved in the treated groups with *ES*s, compared to the control group ($p \leq 0.05$). However, total protein, glucose, and phosphorous were not affected by the tested material ($p \geq 0.05$).

### Yolk cholesterol profile

Table 6 indicates the impact of *ES*s on the cholesterol profile of yolk. It was found that administration of *ES*s at different levels significantly decreased yolk cholesterol and LDL ($p \leq 0.05$) and significantly increased the HDL, compared to the control group ($p \leq 0.05$).

**Table 4.** Hematological parameters of laying hens as affected by the supplementation of *Eruca* seeds through 26-39 weeks of age

| Traits / ***Eruca* seeds levels** | **Control** | **1%** | **2%** | **3%** | **SEM** | **P value** |
|---|---|---|---|---|---|---|
| Hb (g/dL) | 10.17$^{b}$ | 10.32$^{ab}$ | 10.60$^{a}$ | 10.67$^{a}$ | 0.12 | 0.028 |
| RBC ($10^6$/mm$^3$) | 2.28 | 2.37 | 2.54 | 2.45 | 0.08 | 0.262 |
| PCV (%) | 31.51 | 31.95 | 32.80 | 33.00 | 0.36 | 0.028 |
| MCV (fI) | 138.08 | 135.02 | 130.28 | 137.12 | 4.06 | 0.709 |
| MCH (pg) | 44.56 | 43.60 | 42.10 | 44.32 | 1.31 | 0.719 |
| MCHC (g/dL) | 32.28$^{b}$ | 32.29$^{ab}$ | 32.32$^{a}$ | 32.32$^{a}$ | 0.11 | 0.021 |
| WBC ($10^3$/mm$^3$) | 13.47 | 13.63 | 13.67 | 13.93 | 0.15 | 0.218 |
| Heterophils (%) | 23.00 | 23.33 | 23.67 | 24.00 | 0.70 | 0.801 |
| Lymphocytes (%) | 41.67$^{c}$ | 43.33$^{bc}$ | 45.00$^{ab}$ | 47.33$^{a}$ | 0.80 | 0.001 |
| H/L ratio | 0.55 | 0.54 | 0.52 | 0.51 | 0.02 | 0.392 |

$^{abc}$ Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the mean, Hb: Hemoglobin concentration, RBC: Red blood cell, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscle hemoglobin, MCHC: Mean corpuscle hemoglobin concentration, WBC: White blood cell

**Table 5.** Blood parameters of laying hens as affected by the supplementation of *Eruca* seeds through 26-39 weeks of age

| Traits / ***Eruca* seeds levels** | **Control** | **1%** | **2%** | **3%** | **SEM** | **P value** |
|---|---|---|---|---|---|---|
| Total Protein (g/dl) | 6.07 | 6.03 | 6.33 | 5.33 | 0.28 | 0.184 |
| Glucose (mg/dl) | 175.00 | 176.67 | 168.33 | 157.00 | 10.84 | 0.315 |
| Cholesterol (mg/dl) | 178.00$^{a}$ | 168.00$^{b}$ | 156.33$^{c}$ | 154.67$^{c}$ | 1.85 | 0.000 |
| HDL (mg/dl) | 29.23d | 30.53$^{c}$ | 35.57$^{b}$ | 39.00$^{a}$ | 0.41 | 0.000 |
| LDL (mg/dl) | 92.67$^{a}$ | 89.00$^{b}$ | 84.67$^{c}$ | 78.67$^{d}$ | 1.20 | 0.000 |
| AST (U/L) | 220.50$^{a}$ | 133.50$^{b}$ | 213.50$^{a}$ | 146.00$^{b}$ | 8.44 | 0.000 |
| ALT (U/L) | 43.07$^{a}$ | 42.60$^{ab}$ | 42.03$^{b}$ | 41.73$^{b}$ | 0.30 | 0.037 |
| Calcium (mg/dl) | 17.90$^{b}$ | 18.30$^{b}$ | 18.78$^{ab}$ | 19.77$^{a}$ | 0.30 | 0.017 |
| Phosphorus (mg/dl) | 5.86 | 6.18 | 6.04 | 6.25 | 0.09 | 0.075 |
| T3 (ng/dl) | 2.03$^{bc}$ | 2.18$^{ab}$ | 1.99$^{c}$ | 2.19$^{a}$ | 0.05 | 0.022 |
| T4 (ng/dl) | 4.72 | 5.26 | 4.95 | 5.16 | 0.11 | 0.061 |
| Alkaline Phosphatase (ng/dl) | 453.57$^{a}$ | 450.50$^{a}$ | 422.67$^{b}$ | 408.50$^{b}$ | 4.07 | 0.000 |
| TAC (mmol/ml) | 1.79$^{b}$ | 1.96$^{c}$ | 2.26$^{b}$ | 2.43$^{a}$ | 0.04 | 0.000 |
| MDA (Mmol/ml) | 2.62$^{a}$ | 2.10$^{b}$ | 1.92$^{c}$ | 1.72$^{d}$ | 0.06 | 0.000 |

$^{abc}$ Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the mean, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, T3: Triiodothyronine, T4: Thyroxin, TAC: Total antioxidant capacity, MDA: Malondialdehyde

**Table 6.** Yolk Cholesterol profile in eggs as affected by the supplementation of *Eruca* seeds

| Traits / ***Eruca* seeds levels** | **Control** | **1%** | **2%** | **3%** | **SEM** | **P- value** |
|---|---|---|---|---|---|---|
| Cholesterol Egg (g/g yolk) | 14.99$^{b}$ | 15.68$^{a}$ | 12.61$^{c}$ | 12.57$^{c}$ | 0.18 | 0.000 |
| HDL (mg/g yolk) | 4.28$^{c}$ | 4.85$^{b}$ | 5.01$^{b}$ | 5.53$^{a}$ | 0.15 | 0.000 |
| LDL (mg/g yolk) | 9.91$^{a}$ | 9.57$^{a}$ | 6.79$^{b}$ | 6.56$^{b}$ | 0.11 | 0.000 |

$^{abc}$ Means in the same row having different superscripts are significantly different ($p \leq 0.05$). HDL: High-density lipoprotein. LDL: Low-density lipoprotein

## DISCUSSION

There has been some recent inquiry into the potential use of novel natural materials as nutritional supplements in chicken diets. These components must provide nutritious and high-quality meals (El-Sabrout et al., 2023). Chickens may benefit from nutraceuticals by increasing their well-being and the quality of their products (Khalifah et al., 2021; Elazab et al., 2022). Plant seeds, such as pumpkin, garden cress, and grapes are examples of these nutraceuticals, and they have long been utilized in industrial chicken farms to maintain chickens' health and boost productivity (Taaifi et al., 2023).

In this study, authors investigated the effect of *ES*s on the production traits and physiological parameters of laying hens as the *ES*s are a good source of essential oils, proteins, and phytochemicals such as flavonoids and glycosinolates (Bell and Wagstaff, 2014).

The results showed that the addition of 3% *ES*s significantly improved the productive performance of laying hens. This result was confirmed by Jabbar et al. (2015), who found that the inclusion of *ES*s in the feed of quails improved egg weight, egg mass, and egg production. The positive effects of *ES*s on layer productive performance could be correlated to the availability of a large number of nutrients in *ES*s, such as proteins, essential oils, vitamins (A, B, and C), minerals, and glycosides, which can serve as antioxidant properties that keep chickens healthy and improve their productive performance. The *ES*s oil is rich in essential oils which improve sexual hormones by increasing mRNA levels (in sexual hormone-related genes (GnRHR, FSHR, LHR, and StAR mRNA levels, Alagawany et al., 2019). Moreover, Janeczka (2021) found that *ES* acts as a phytoestrogen because their content of 17 B-estradiol (247 picogram/gram dry weight [pg/g D.W]), which is found in flavonoids such as kaempferol, and quercetin (Shani, 2019)**.** This phytoestrogen may be involved in promoting steroid formation, resulting in an improvement in the rate of egg production in Silver Sabahia hens. The current study results indicated that adding 3% *ES*s to the diet significantly reduced feed conversion ratio values. In agreement, Abozid et al. (2014) mentioned that *ES*s contain about 35% oil, and unsaturated fatty acids contain 82.1% of total fatty acids. These essential oils affect the digestive system of chickens by enhancing digestive enzyme secretion and intestinal mucosa (Jamroz et al., 2006; Jang et al., 2007) and reducing the number of pathogens in the alimentary tract (Zeng et al., 2015). Abozid and Ayimba (2014) confirmed that *ES* oil contains high amounts of omega 3 and 6 fatty acids, reducing the feed conversion ratio for layer hens.

The addition of *ES*s improved eggshell thickness (Table 5), which is beneficial for breeders to enhance table egg safety and quality. This is in agreement with Rozan and Boriy (2022), who proved that *ES* contained a high amount of calcium (1223.5 mg /100 g). Moreover, El-Saadany et al. (2022a) confirmed that *ES*s contain quercetin, leading to an increase in eggshell thickness. Englmaierova et al. (2013) reported that the consumer preferred the yolk color to be deep, and the significant increase in yolk color in the present study was probably due to the presence of quercetin, carotenoids and essential oils in *ES*s. According to El-Saadany et al. (2022 a), quercetin administration increases the color of the yolk by producing a yellowish pigment. In addition, Al-haj et al. (2019) attributed the increase in yolk color score to the feeding hens *ES* as a source of essential oils, which elevate yolk lipids content that contains pigments (EL-Saadany et al., 2022 b).

Table 3 indicated that the improvement of hematological parameters such as hemoglobin and lymphocytes for hens treated with *ES*s could be related to the ability of secondary plant components (phytochemicals) to enhance the digestion and absorption of nutrients and subsequently improve immunity and health (Al-Shammari and Batkowska, 2021). Perhaps the significant increase in blood hemoglobin for hens treated with 2 and 3% of *ES*s is related to the presence of iron and copper in *ES*s, where iron is one of the important factors that enter into the process of producing RBC, while copper increases the absorption of iron from the digestive system to make RBC (Rowely, 1998). Moreover, *ES*s contain vitamins (B12, Niacin, B6, B2, B1, and B1), which have a role in Hb biosynthesis in the body **(**Martinez-Sánchez et al., 2008; Gulfraz et al., 2011). The results of Shani (2019) supported these previous assumptions. The increase in the number of serum lymphocytes in the *ES*s groups (2 and 3% of diet) compared to the control group may have a positive role in raising the immune responses and healthy status of chickens. These findings are consistent with those of El-Saadany et al. (2022c), who discovered that supplementing the diet with photogenic extract of pumpkin and garden cress increased lymphocyte values in local laying hens. In addition, Khalil et al. (2015) found that *ES*s contain vitamins, flavonoids, and glucosinolates, which act as antioxidants for improving growth and immune functions. Moreover, *ES* oil is rich in omega-3 and omega-6 fatty acids that improve lymphocytes in layer chickens, as mentioned by El-Saadany et al. (2022 b).

It was found that *ES* supplementation in laying hens decreased serum cholesterol and LDL, whereas HDL levels were enhanced. The results may be due to the fact that *ES*s have strong antioxidant properties. El-Fadaly et al. (2017) and Jin et al. (2009) demonstrated that glucosinolates in *ES* can inhibit lipid peroxidation. Shani (2019) reported that *ES* is rich in flavonoids like quercetin, which can be associated with the inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA), the first step enzyme in cholesterol formation (El-Saadany et al. (2022b). Additionally, El-Gengaihi et al. (2004) indicated that *ES*s have β-Sitosterol, which reduces cholesterol absorption from the small intestine. Abozid et al. (2014) reported that *ES*s contained a large amount of omega-3 and omega-6 fatty acids that inhibit lipogenesis. Abou El-Maaty et al. (2021) reported that unsaturated fatty acids in *ES*s, such as linoleic and linolenic acids, can elevate HDL and reduce cholesterol and LDL. Abdul-Majeed and Taha (2019) found that vitamin C and carotenoids in *ES*s could increase thyroid activity, which is one of the important glands for controlling cholesterol and lipid metabolism. In the current study, the obtained serum thyroxin hormone levels (increased in the group fed 3 % *ES*s) supported this assumption.

The AST and ALT activity levels are the most useful indicators for liver function. According to Table 5, adding 3% of *ES*s in the hens ration decreased the activity of AST and ALT enzymes. This could be explained by the presence of more than one active ingredient, such as quercetin, carotenoids, and essential oils in the *ES*s, and the effects are cumulative. The *ES*s are rich in carotenoids, which maintain the body's cell membranes and prevent the release of AST and ALT enzymes into the blood (El-Saadany et al., 2022b). Also, *ES*s are rich in antioxidants, such as Kaempferol, quercetin, and glucosinolates **(**Jin et al., 2009; El-Fadaly et al., 2017**),** which can activate the regeneration of the liver and reduce the activity of AST and ALT. According to Alam et al. (2007), *ES*s have a high content of Sulphur, which activates the liver function and immune system. Abou El-Maaty et al. (2021) reported that *ES*s (as a source of antioxidants) enhanced the health status of broilers by decreasing the serum lipid profile and AST and ALT.

The *ES* dietary supplementation in hens at 2 and 3% decreased serum ALP activity, whereas the highest Ca level was recorded for hens fed a diet with the addition of 3% *ES*s. Al-Daraji and Razuki (2012) demonstrated that *ES*s are rich in vitamin C, which decreases the activity of ALP and calcium in the blood. El-Saadany et al. (2022a) demonstrated that the application of quercetin to laying hens increases calcium levels in their blood. This increase in calcium level is due to increasing calcium absorption from the intestinal epithelium and stimulating the activity of vitamin D receptors, as mentioned by Inoue et al. (2010). Finally, the high blood calcium level recorded in the current study may be due to the high calcium concentration of *ES*s (1223.5 mg /100 g, Rozan and Boriy, 2022).

According to Table 5, adding 3% of *ES*s to laying chickens ration increased serum T3, which translated into increased metabolism and thus increased egg production. Thyroid hormones are involved in the regulation of anabolic and catabolic pathways of protein, lipid, and carbohydrate metabolism (Lachowicz et al., 2008; 2009). Abd El-Hady et al. (2020) indicated that treating broilers with phytogenic extracts of herbs Such as garden cress increased the T3 hormone and metabolic cycle. Abou El-Maaty et al. (2021) found that broilers fed the *ES*s diet significantly increased T3 and T4. Also, results were confirmed by the finding of Yadav et al. (2016), who found that *ES* meal probably causes improvement in the transport of sodium-iodide and increased absorption of iodide, resulting in increased production of T3 and T4.

The present study showed that treated chickens with a diet supplemented with *ES*s induced the most substantial effect on the antioxidant enzymes compared with the control. The *ES*s are rich in carotenoids, phenolics, glucosinolates, vitamin C, and flavonoids, which have a powerful antioxidant ability (Barillari et al., 2005; Bennett et al., 2006; Keyata et al., 2021). These active components can remove free radicals by potentially improving the TAC and MDA enzymes. Shani (2019) found that adding *ES* to the broiler diet significantly decreased MDA. Moreover, *ES*s contain essential oils that enhance catalase activity, detoxifying hydrogen peroxide and converting lipid hydroperoxides to non-toxic substances (Fki et al., 2005). Furthermore, quercetin in *ES* has antioxidant properties due to the presence of a C-ring, many hydroxyl groups, and conjugated orbitals (Rice-Evans et al., 1997). El-Saadany et al. (2022a) reported that quercetin can reduce MDA production and inhibit cell membrane lipoperoxidation.

Table 6 indicated that groups fed 2 and 3% *ES*s had lower significant concentrations of egg yolk cholesterol by 15.88 and 16.14%, respectively, and lower LDL levels by 31.48 and 33.80%, respectively, compared with the control group. The *ES* groups significantly increased yolk HDL levels by 17.06 and 29.21 % in the 2 and 3% groups, respectively, compared to the control. This is an indicator of increasing the nutritive value of eggs. Recommendations state that dietary cholesterol should be constrained to less than 300 mg/day **(**Weggemans et al., 2001). As a result of the high cholesterol levels in eggs, many consumers reduce their consumption of eggs (especially among elderly or diabetic individuals) to avoid heart disease. In recent years, a variety of photogenic plants (natural antioxidants) have been widely used as alternative nutritional strategies to reduce cholesterol in eggs as grape, pumpkin, and garden cress seeds (Peipei et al., 2018; El-Saadany et al., 2022c). However, there is no information about the effects of *ES*s administration on yolk cholesterol. The decreased cholesterols content in the egg yolk seen in the present study might be due to the high content of polyunsaturated fatty acids and phytochemicals in ESs. These ingredients may cause multiple effects on laying hens, including reduced absorption or synthesis of cholesterol in the gastrointestinal tract, increased cholesterol excretion in the feces, and inhibition of hepatic cholesterol synthesis (Huang et al., 2019).

## CONCLUSION

Adding *Eurca* seeds to the laying hens' diets at a high level of 3% improves production performance, egg quality traits, some hematological parameters, and blood chemical analysis. Also, *Eurca* seeds administration at 2 and 3% levels succeeded in reducing yolk cholesterol levels according to the current demands of consumers. Results in this way allow the producers to use natural feed additives to improve the quality of the final products of eggs. Future studies should be focused on more natural alternative feed additives to improve the health of poultry, products, and consumers.

## DECLARATIONS

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### Authors' contribution

El-Sahn, El-Barbary, Farag and Khalifah deveoped the idea and designed the study. Iraqi, Farag, and EL-Prollosy collected data. El-Sahn, Iraqi and El-Barbary wrote the paper and performed the statistical analysis. Khalifah drafted the manuscript and approved the final manuscript. All authors checked and confirmed the final analysis data and the last revised manuscript before publication in the journal.

### Competing of interests

The authors declared that they have no competing interests.

### Availability of data and materials

The data presented in this study are available on request from the corresponding author.

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### Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors before the submission. The final results of the statistical analysis have also been checked and confirmed by all authors.

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# Genetic Diversity and Structure of Local Chicken Populations Raised in Five Agroecological Zones of Togo

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**ABSTRACT**

Local chickens are the most commonly raised poultry breed in rural areas of Togo, where they help in alleviating poverty and food insecurity in households. The current study aimed to ensure the sustainable management of this genetic resource by evaluating the genetic diversity, phylogenetic relationships, and population structure of local chicken populations from five agroecological zones (Dry Savannah, Atakora, Forest, Wet Savannah, and Littoral) in Togo. Genotyping was carried out using 15 microsatellite markers on 30 unrelated individuals per agroecological zone. Genetic diversity was assessed by estimating the number of alleles per locus, observed heterozygosity, unbiased expected heterozygosity, and the polymorphic information content (PIC). The genetic structure of the populations was analyzed using a Bayesian-based approach. The results revealed a high genetic diversity but weak population structuring among local chickens. Moreover, 98 alleles were detected in all population groups, varying from 3 to 12 per locus, with an average of 6.53 ± 2.67 alleles per locus. The PIC values varied from 0.436 to 0.690, with an average of 0.550 ± 0.087. The mean number of alleles per population across all markers ranged from 4.4 ± 1.4 (Dry Savannah) to 5.4 ± 2.0 (Forest). The unbiased expected heterozygosity was high and varied from 0.58 ± 0.07 (Atakora) to 0.65 ± 0.11 (Forest), while that observed varied between 0.46 ± 0.09 (Dry Savannah) and 0.57 ± 0.14 (Forest). All populations deviated significantly from the Hardy-Weinberg equilibrium. Across populations, $F_{IT}$, $F_{IS}$, and $F_{ST}$ fixation indices were 0.150, 0.132, and 0.021, respectively. The genetic distances were low and varied from 0.022 (between Atakora and Dry Savannah) to 0.045 (between Atakora and Forest). These results could be used in potential genetic improvement programs or the preservation of local chickens in Togo.

**Keywords:** Genetic diversity, Local chickens, Microsatellite markers, Heterozygosity, Togo

## INTRODUCTION

Poultry plays a key role in developing countries by providing protein through meat and eggs (Moula et al., 2013). Domesticated chickens (*Gallus gallus domesticus*) are the main poultry genetic resources worldwide. In Sub-Saharan Africa, over 80% of the total chicken populations are local chickens (Ngeno et al., 2015). These local chickens reduce malnutrition and poverty (Osei-Amponsah et al., 2015). In Togo, local chicken is the most common poultry breed raised, particularly in rural areas, so it plays an important role in fighting against food insecurity in households and improves the livelihood of populations. The local chicken is found in all agroecological zones of Togo, where it is known to be well-adapted (Dao et al., 2015). To date, little is known about the genetic diversity of the breed.

One of the fundamental biological characteristics of local chickens is their rusticity since they are disease-resistant and better adapted to survive under harsh environmental conditions and poor rearing practices (Ben Larbi et al., 2018). According to Bakare et al. (2021), local chickens are good scavengers, efficient mothers, independent, resilient, and need little care to grow. Additionally, these authors reported that consumers prefer their products because of their taste, leanness, and suitability for special dishes.

However, the generalization of the use of commercial hybrids (resulting from terminal crosses) and uncontrolled crossbreeding to improve the productivity of the local chickens constitute a real threat of loss of their genetic originality in relation to their products' quality and their rusticity (Ben Larbi et al., 2018). Weigend et al. (2004) foresaw that this menace may lead to an unrecognized replacement of local genotypes with commercial hybrids, which have a higher production potential based on high nutrient requirements but are not selected for survival in such a harsh environment. According to Leroy et al. (2012), this situation is due to poor conservation strategies and a lack of incentives for a continued and sustainable use of local chicken populations. For these scientists, a perfect characterization of genetic structure and an assessment of the genetic diversity of local chicken populations are requisite for the development of conservation strategies.

The assessment of genetic diversity is a key step towards identifying and preserving valuable genetic resources to deal with changes in environmental conditions, changes in consumer preferences, and adaptation to different production practices (Suh et al., 2014). Genetic marker polymorphisms are a way of assessing diversities in chickens, and different genetic markers have been used. Microsatellites are markers that have been widely used in genetic diversity studies because of their codominance, availability throughout the genome, and high polymorphic nature (FAO, 2011; Suh et al., 2014). Microsatellite-based studies from Côte d'Ivoire (Loukou et al., 2009), Benin (Youssao et al., 2010), Ghana (Osei-Amponsah et al., 2010), and Burkina Faso (Yacouba et al., 2022) indicated high genetic variation within local chicken populations in these countries. However, studies on the characterization of local chickens in Togo are only phenotypic, including adult body phaneroptic and measurements (Dao et al., 2015). It is, therefore, important to assess the genetic diversity of these agroecologically adapted chicken populations using molecular biomarkers to offer insights into their improvements.

This study aimed to investigate the genetic diversity, phylogenetic relationships, and population structure of local chickens raised in the five agroecological zones of Togo using 15 microsatellite loci.

## MATERIALS AND METHODS

### Ethical approval

The authors confirm that the sampling procedures and the collection of blood samples for this study were performed in accordance with the guide for the care and use of agricultural animals in research (008/2021/BC-BPA/FDS-UL) edited by the Faculty of Sciences of the University of Lomé (Togo).

### Study areas

The study covered the national territory of Togo, divided into five agroecological zones (Dao et al., 2015), including Dry Savannah, Atakora, Wet Savannah, Forest and Littoral (Figure 1).

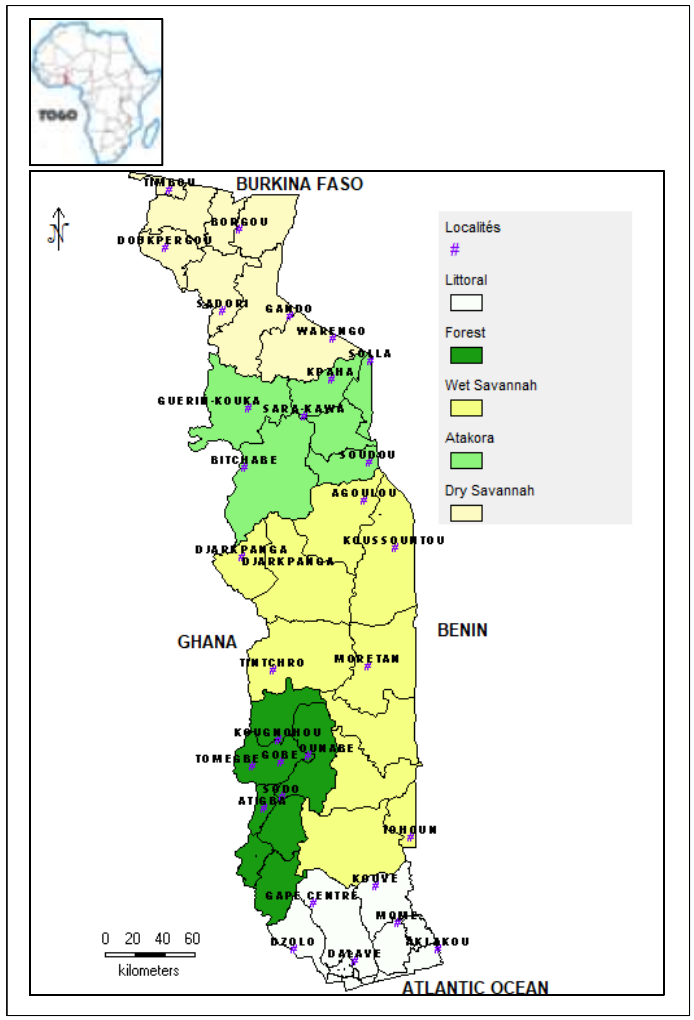



**Figure 1**. Map of Littoral, Forest, Wet Savannah, Atakora, and Dry Savannah agroecological zones of Togo with localities of sampled local chickens

The Dry Savannah zone is a lowland area in the extreme north of Togo, whose flora is dominated by the Sudanian Savannah. The climate is Sudanese, with annual rainfall between 1000 and 1100 mm and the annual average temperature of 28.5°C.

The Atakora zone is the northern mountainous area with a studio-Guinean climate. The vegetation is made of a mosaic Savannah, forest with *Isoberlinia doka*, and compact dry forests. The annual rainfall is around 1300 mm, with a maximum in August-September when it rains every other day.

The Wet Savannah is a vast plain in the central and southeast of Togo whose flora consists of Guinean Savannah characterized by numerous fragments of compact forests. The area is characterized by a humid tropical climate with unimodal rainfall. The annual rainfall varies between 1200 and 1500 mm for a number of rainy days, reaching 120 days in the rainy season. The annual temperature variation is between 20 and 32 °C.

The Forest zone corresponds to the southwestern part of the mountains of Togo. The vegetation of the area is made of authentic semi-evergreen forests with a subequatorial climate characterized by bimodal rainfall. The annual average rainfall oscillates between 1500 and 1800 mm. Average annual temperatures are between 22 and 27 °C. The Forest zone is an area of excellence for producing coffee, cocoa, oil palm, plantain, avocado, and citrus fruits.

The Littoral zone corresponds to the coastal plain covered by a mosaic of semi-deciduous forests, Savannah, and grasslands. It is subject to a four-season subequatorial climate (two rainy seasons and two dry seasons) with total annual rainfall varying between 800 and 1200 mm from the south to the north of the area. For temperatures, the absolute maximum is between 32 and 35°C in February and the minimum of 21°C is recorded in the rainy season.

**Blood sampling and DNA extraction**

Blood samples were collected from 120 hens and 30 cocks of 8 to 10 months of age belonging to five agroecological zones of Togo. In each agroecological zone, 30 individuals of local chickens were sampled from six villages, keeping predominantly local chickens and located at least 20 km apart. Five chickens per village and only one chicken per household were randomly sampled to avoid including genetically related individuals. Approximatively, 2 ml of blood was collected per chicken by a puncture at the wing vein using 19G VENOJECT® needles into Vacutainer EDTA-containing tubes. Prior to DNA extraction, blood samples were stored at -20°C.

DNA was extracted from blood samples using the QIAGEN® kit (QIAGEN, Valencia, CA, USA) at the CIRDES genotyping platform in Bobo-Dioulasso (Burkina Faso). A NanoDrop Spectrophotometer (ThermoFisher Scientific™ Nanodrop 2000, Wilmington, USA) was used to quantify the total DNA extracted, which was stored at +4°C until DNA amplification by polymerase chain reaction (PCR) and genotyping.

**Microsatellite genotyping**

The DNA polymorphism was assessed using a set of 15 microsatellite loci which were included in previous studies (Loukou et al., 2009; Osei-Amponsah et al., 2010; Youssao et al., 2010; Yacouba et al., 2022) and part of the 30 ISAG-FAO recommended microsatellite markers (FAO, 2011) for chicken genetic diversity assessment. The names of the 15 microsatellite loci, their chromosomal location, and PCR conditions are presented in Table 1.

The PCR was performed in 15 µl reaction volume containing 8.1 µl of sterilized water, 1.6 µl of 10X PCR buffer, 1.6 µl of dNTPs (2.5mM), 0.8 µl of $MgCl_2$ (25mM), 0.2 µl FM13 (Forward) primer (Hillel et al., 2003), 0.3 µl of reverse primer (10 µM), 0.1 µl of Qiagen Taq DNA polymerase (5U/µl); 0.3 µl of fluorochrome dye (Dye 700), and finally 2 µl of DNA samples. The amplifications were performed using an automated thermal cycler (Applied Biosystems Veriti 96 Well, Thermal Cycler) and programmed for 1 cycle of an initial denaturation of DNA and enzyme activation step at 94°C (3 min), followed by 35 cycles consisting of denaturation at 94°C (30 seconds), primer annealing at temperature varying from 58 to 64°C (30 seconds), and extension at 72°C (45 seconds), then a final cycle of extension at 72°C (8 minutes).

The amplified products were then migrated using vertical high voltage electrophoresis (1,500 volts for 1 hour and 30 minutes) in an acrylamide gel on a Li-Cor® automated sequencer (DNA Analyzer Model 4300; LI-COR Biosciences-GmbH, Germany) following the manufacturer's procedures. The electrophoretic profiles were analyzed using SAGA$^{GT}$ Generation 2.0 software to assess DNA polymorphism.

**Table 1.** Information on the 15 microsatellite loci analyzed in five local chicken populations of Togo

| Microsatellite locus | Chromosomal position | Primer sequence (5' > 3') Forward Reverse | Annealing temperature | Alleles sizes (bp) |
|---|---|---|---|---|
| ADL0268 | 1 | CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT | 60°C | 102-116 |
| ADL0278 | 8 | CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG | 60°C | 114-126 |
| MCW0034 | 2 | TGCACGCACTTACATACTTAGAGA TGTCCTTCCAATTACATTCATGGG | 60°C | 212-246 |
| CW0037 | 3 | ACCGGTGCCATCAATTACCTATTA GAAAGCTCACATGACACTGCGAAA | 64°C | 154-160 |
| MCW0067 | 10 | GCACTACTGTGTGCTGCAGTTT GAGATGTAGTTGCCACATTCCGAC | 60°C | 176-186 |
| MCW0069 | *E60C04W23* | GCACTCGAGAAAACTTCCTGCG ATTGCTTCAGCAAGCATGGGAGGA | 60°C | 158-176 |
| MCW0078 | 5 | CCACACGGAGAGGAGAAGGTCT TAGCATATGAGTGTACTGAGCTTC | 60°C | 135-147 |
| MCW0081 | 5 | GTT GCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC | 60°C | 112-135 |
| MCW0111 | 1 | GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG | 60°C | 96-120 |
| MCW0183 | 7 | ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC | 58°C | 296-326 |
| MCW0206 | 2 | ACATCTAGAATTGACTGTTCAC CTTGACAGTGATGCATTAAATG | 60°C | 221-249 |
| MCW0216 | 13 | GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG | 60°C | 139-149 |
| MCW0222 | 3 | GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC | 60°C | 220-226 |
| MCW0295 | 4 | ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC | 60°C | 88-106 |
| MCW0330 | 17 | TGGACCTCATCAGTCTGACAG AATGTTCTCATAGAGTTCCTGC | 60°C | 256-300 |

All primers of this table are used in the previous studies of Loukou et al. (2009), Osei-Amponsah et al. (2010), and Yacouba et al. (2022).

### Genetic diversity estimates

Data generated were analysed using *CERVUS* version 3.0.7 computer program (Kalinowski et al., 2007) and *FSTAT* version 2.9.4 software (Goudet, 2003) to estimate the number of alleles detected, polymorphic information content (PIC, a measure of how a microsatellite locus is informative in relation to expected heterozygosity (Botstein et al., 1980) observed heterozygosity ($H_O$), unbiased expected heterozygosity ($H_E$). Moreover, *POPGENE* version 1.32 software (Yeh et al., 1999) was used to estimate F-statistics of Wright's (1978), number of successful migrants per generation (Nm) and significance of deviations from Hardy-Weinberg equilibrium for each of locus across the 5 local chicken populations. Deviations from Hardy-Weinberg equilibrium were assessed using a chi-square goodness-of-fit test. The test compares observed genotype frequencies with expected genotype frequencies calculated from alleles frequencies assuming Hardy-Weinberg equilibrium. Within-population genetic diversity, represented by mean number of alleles, private alleles, observed ($H_O$), and expected ($H_E$) heterozygosities and inbreeding coefficient ($F_{IS}$) for each population across loci was estimated using the *GENETIX* version 4.05 software (Belkhir et al., 2004). The Markov chain Monte Carlo (MCMC) algorithm (100 batches and 5,000 iterations per batch, dememorization step of 10,000) as implemented in *GENEPOP* version 4.7.5 software (Rousset, 2008) was used to test the deviations from Hardy-Weinberg equilibrium for each population across loci.

### Genetic relationships and structure

For the estimation of the genetic relationships among the five chicken populations, Nei's $D_A$ distances (Nei et al., 1983) between all pairs of populations were computed based on allele frequencies using the *POPTREE2* computer program package (Takezaki et al., 2010, Kagawa University, Japan). A phylogenetic tree based on $D_A$ distances was constructed using the neighbour-joining method implemented in *MEGA* version 11 software

(Tamura et al., 2021). Furthermore, a bootstrap test (Felsenstein, 1985) with 1,000 resampling of loci was used to evaluate the phylogenetic tree robustness. Moreover, the *GENETIX* version 4.05 software (Belkhir et al., 2004) was used to perform Factorial correspondence analysis (FCA) in order to investigate the differentiation of the individuals within each population. A Bayesian approach, implemented in *STRUCTURE* version 2.3.4 software (Hubisz et al., 2009) was used to reveal probable clustering substructures. The analysis involved 20 independent runs for each number of clusters K (ranging from 2 to 10) with a burn-in period of 50,000 Markov Chain Monte Carlo iterations followed by 120,000 repeat numbers. The most likely number of clusters (K) was determined using the distribution of the ΔK statistic as described by Evanno et al. (2005) and implemented in *STRUCTURE HARVESTER* program (Earl and Vonholdt, 2012).

### Statistical analysis

The significant level of deviations from Hardy-Weinberg equilibrium using a chi-square goodness-of-fit test was set as $p < 0.05$. The statistical significance of deviations from Hardy-Weinberg equilibrium based on the Markov chain Monte Carlo (MCMC) algorithm was set at a p value of 5%.

## RESULTS

### Microsatellite loci polymorphisms

A total of 98 alleles were identified in the 150 chickens assessed at 15 microsatellite loci. The number of alleles per locus across chicken populations varied from 3 (MCW0037) to 12 (MCW0069), with the mean number of alleles 6.53 ± 2.67 in all loci (Table 2). Out of the total alleles identified, 18 were considered private alleles, so they were observed in only one population.

The observed heterozygosity ($H_O$) mean value of 0.522 was lower than the expected heterozygosity ($H_E$) means value (0.616). The values of $H_O$ ranged from 0.393 (ADL0278 and MCW0216) to 0.687 (MCW0034), while that of $H_E$ varied from 0.516 (MCW0067 and MCW0078) to 0.733 (MCW0034).

The polymorphic information content (PIC) per locus ranged from 0.436 (MCW0078) to 0.690 (MCW0034), with an average of 0.550. A total of 67% of microsatellite loci had a PIC value above 0.5, indicating that they were highly informative.

The heterozygote deficiency (as determined by $F_{IS}$ index) at the microsatellite locus level, extended between -0.036 (MCW0081) and 0.351 (ADL0278) with a mean of 0.132 for all loci. The global heterozygosity deficit of individuals within the overall populations ($F_{IT}$) per locus ranged from -0.019 (MCW0081) to 0.388 (ADL0278), and averaged at 0.150. The genetic differentiation among populations (evaluated by $F_{ST}$) estimates was 0.021 on average and varied from 0.006 (MCW0037 and MCW0330) to 0.056 (ADL0278). The average gene flow between populations, estimated by the number of migrants per generation (Nm) in the overall population and across the fifteen microsatellite loci, was 11.909. The chi-square goodness-of-fit test revealed that about two-thirds of microsatellite loci deviated significantly from Hardy-Weinberg equilibrium ($p < 0.05$).

### Genetic diversity within populations

Within the populations, genetic diversity estimates are summarized in Table 3. The mean number of alleles for the overall chicken populations was 4.9 and varied from 4.4 in the Dry Savannah local chicken population to 5.4 in the Forest zone.

The mean $H_O$ ranged from 0.464 (Dry Savannah) to 0.569 (Forest), while $H_E$ ranged from 0.585 (Atakora) to 0.647 (Forest). The inbreeding coefficient ($F_{IS}$) values varied from 0.111 (Littoral) to 0.212 (Dry Savannah) and were different from 0 ($p < 0.05$), indicating a significant deficit of heterozygotes within the population.

### Genetic relationship between populations

The genetic relationships between populations were estimated using the Neighbour-Joining method based on Nei's $D_A$ genetic distances and FACA.

Based on Table 4, the matrix of pairwise genetic distances between populations showed a low genetic distance (0.022) between Atakora and Dry Savannah populations, and between Forest and Wet Savannah (0.025). The highest genetic distance was observed between the Forest and Dry Savannah populations (0.045). The phylogenetic relationship by the Neighbour-Joining tree based on $D_A$ genetic distances showed three main branches (Figure 2). Unlike the other populations, the Littoral chicken population stands alone and constitutes the first main branch. The Forest and Wet Savannah chicken populations were found in the second major branch, while Atakora and Dry Savannah local chicken populations were grouped in the third main branch of the tree.

The FCA was performed using allele frequencies of the 15 microsatellite loci, as an alternative approach to understanding the genetic relationship among chicken populations. Figure 3 shows a weak differentiation between the five local chicken populations. The three axes of the FCA explained 87.17% of the variability and distinguished three groups. Axis 1 separated two groups, including Group 1 (Atakora and Dry Savannah local chicken populations) and Group 2 of Wet Savannah, Forest and Littoral (costal) local chicken populations. Axis 2 isolated the Littoral local chicken population from the Wet Savannah and Forest local chicken populations.

**Genetic structure**

The most consistent gain in information was obtained with K: 3 (Figure 4). The STRUCTURE clustering was graphically illustrated in Figure 5, which displays the individual of each population as a vertical line partitioned into three colored segments that represent the individual's estimated membership coefficients in the three assumed clusters. The proportion of membership of each population in each of the three inferred clusters showed that apart from the Forest local chicken population, which individuals clustered fairly in the three clusters, the four other local chicken populations had more than 40% of their individuals clustered in the Cluster 3. The *STRUCTURE* analysis results revealed a low structuring in the local chicken populations studied.

**Table 2**. Number of alleles, polymorphic information content, observed and unbiased expected heterozygosities, Wright's F-statistics, gene flow and significance of deviation from Hardy-Weinberg equilibrium for each of the 15 microsatellite loci in 5 local chicken populations of Togo

| Locus | Na | PIC | $H_O$ | $H_E$ | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | Nm | HW |
|---|---|---|---|---|---|---|---|---|---|
| ADL268 | 5 | 0.597 | 0.500 | 0.657 | 0.224 | 0.237 | 0.016 | 15.07 | *** |
| ADL278 | 5 | 0.584 | 0.393 | 0.645 | 0.351 | 0.388 | 0.056 | 4.18 | *** |
| MCW034 | 11 | 0.690 | 0.687 | 0.733 | 0.015 | 0.060 | 0.046 | 5.24 | ** |
| MCW037 | 3 | 0.466 | 0.487 | 0.552 | 0.109 | 0.115 | 0.006 | 39.14 | *** |
| MCW067 | 5 | 0.443 | 0.453 | 0.516 | 0.099 | 0.118 | 0.021 | 11.43 | ns |
| MCW069 | 12 | 0.562 | 0.580 | 0.629 | 0.064 | 0.075 | 0.011 | 22.13 | ns |
| MCW078 | 5 | 0.436 | 0.487 | 0.516 | 0.042 | 0.054 | 0.012 | 19.92 | ns |
| MCW081 | 6 | 0.512 | 0.593 | 0.584 | -0.036 | -0.019 | 0.016 | 15.72 | ns |
| MCW111 | 8 | 0.640 | 0.513 | 0.691 | 0.241 | 0.255 | 0.019 | 12.95 | *** |
| MCW183 | 10 | 0.680 | 0.620 | 0.727 | 0.135 | 0.145 | 0.011 | 22.09 | *** |
| MCW206 | 7 | 0.659 | 0.640 | 0.711 | 0.087 | 0.097 | 0.011 | 21.79 | *** |
| MCW216 | 4 | 0.470 | 0.393 | 0.551 | 0.272 | 0.283 | 0.015 | 16.00 | *** |
| MCW222 | 4 | 0.515 | 0.480 | 0.590 | 0.171 | 0.184 | 0.017 | 14.93 | *** |
| MCW295 | 6 | 0.516 | 0.433 | 0.574 | 0.214 | 0.243 | 0.037 | 6.49 | *** |
| MCW330 | 7 | 0.477 | 0.567 | 0.559 | -0.023 | -0.016 | 0.006 | 39.20 | ns |
| **Mean** | **6.5** | **0.550** | **0.522** | **0.616** | **0.132** | **0.150** | **0.021** | **11.91** | |

Na: Number of alleles, PIC: Polymorphic information content, $H_O$: Observed heterozygosity, $H_E$: Unbiased expected heterozygosity, $F_{IS}$: Inbreeding coefficient within populations, $F_{IT}$: Inbreeding coefficient overall populations, $F_{ST}$: Inbreeding coefficient of differentiation among populations, Nm: Number of migrants, HW: Significance of deviation from Hardy-Weinberg equilibrium ($p < 0.05$), ns: Not significant, ** $p< 0.01$; *** $p< 0.001$

**Table 3**. Total, mean, and private number of alleles, observed and expected heterozygosity, and inbreeding coefficient of 5 local chicken populations in Togo

| Population | Na | MNa | NPA | $H_O$ | $H_E$ | $F_{IS}$ | HW |
|---|---|---|---|---|---|---|---|
| Atakora | 73 | 4.87±1.92 | 1 | 0.482±0.150 | 0.585±0.073 | 0.178 | *** |
| Forest | 81 | 5.40±2.03 | 6 | 0.569±0.139 | 0.647±0.105 | 0.123 | ** |
| Littoral | 76 | 5.00±1.73 | 3 | 0.547±0.115 | 0.614±0.109 | 0.111 | *** |
| Wet Savannah | 77 | 5.13±1.81 | 5 | 0.547±0.143 | 0.623±0.089 | 0.124 | ** |
| Dry Savannah | 66 | 4.40±1.40 | 3 | 0.464±0.093 | 0.587±0.083 | 0.212 | *** |
| All | 98 | 6.53±2.67 | 18 | 0.522±0.089 | 0.616±0.075 | 0.132 | |

Na: Number of alleles; MNa: Mean number of alleles; NPA: Number of private alleles; $H_O$: Observed heterozygosity; $H_E$: Expected heterozygosity; $F_{IS}$: Inbreeding coefficient within populations; HW: significance of deviation from Hardy-Weinberg equilibrium ($p < 0.05$); ** $p< 0.01$; *** $p< 0.001$

**Table 4**. Genetic distance between the five local chicken populations in Togo

| | Atakora | Forest | Littoral | Wet Savannah | Dry Savannah |
|---|---|---|---|---|---|
| **Atakora** | - | | | | |
| **Forest** | 0.038 | - | | | |
| **Littoral** | 0.042 | 0.039 | - | | |
| **Wet Savannah** | 0.032 | 0.025 | 0.032 | - | |
| **Dry Savannah** | 0.022 | 0.045 | 0.042 | 0.032 | - |

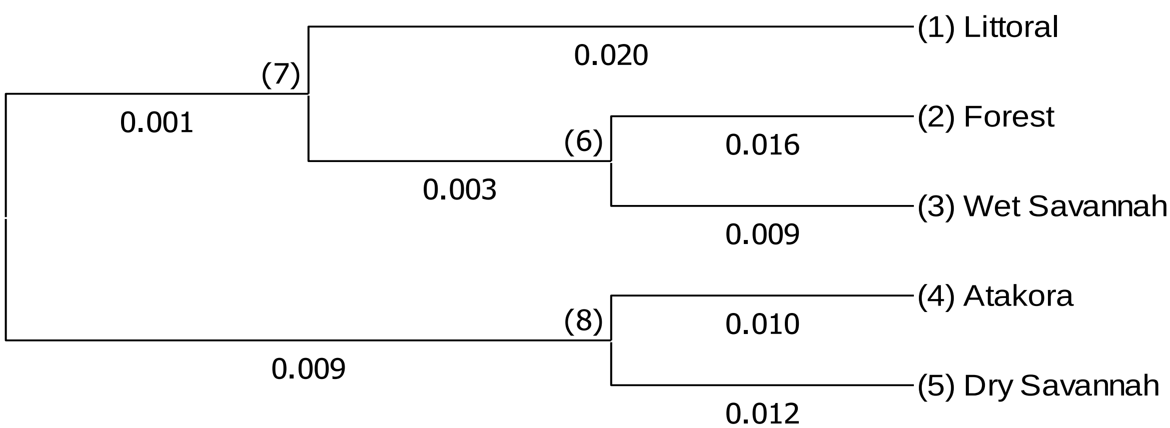



**Figure 2**. Neighbor-joining tree showing genetic relationships among local chicken populations in Togo based on $D_A$ genetic distance

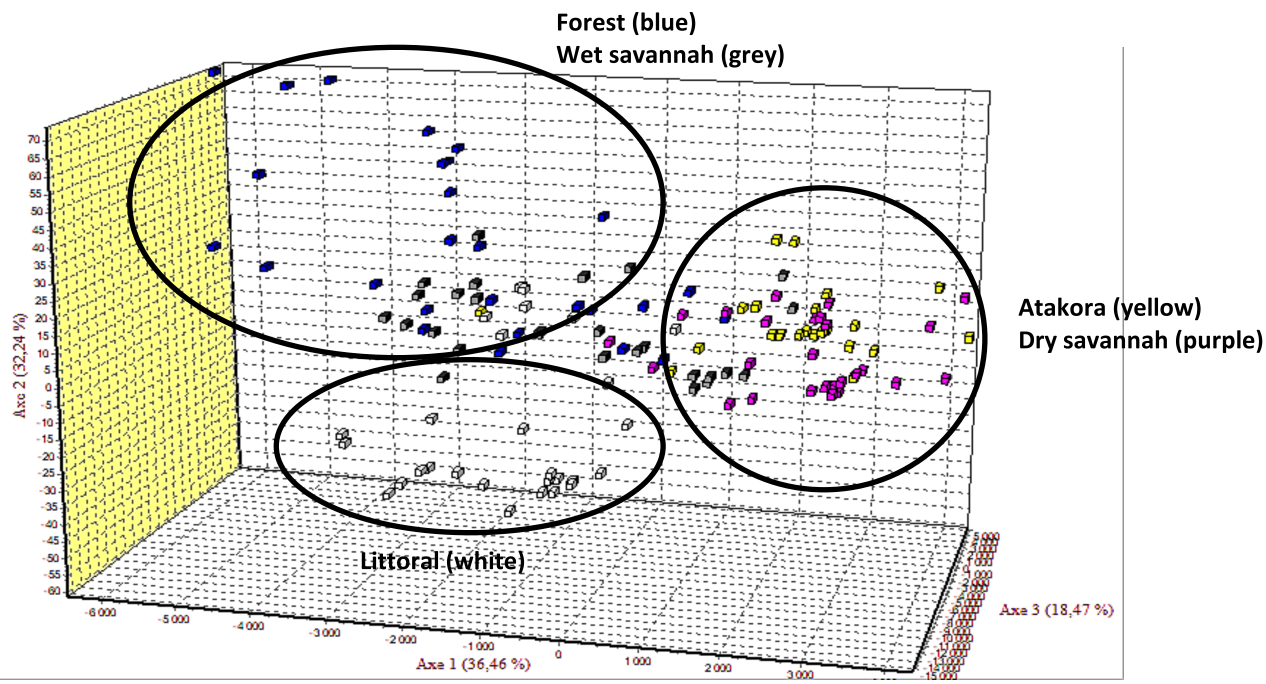



**Figure 3**. Clustering patterns of all individuals analysed using 15 microsatellite loci as revealed by factorial correspondence analysis (FCA) implemented in GENETIX 4.05. Yellow (Atakora), blue (Forest), white (Littoral), grey (Wet Savannah) and purple (Dry Savannah)

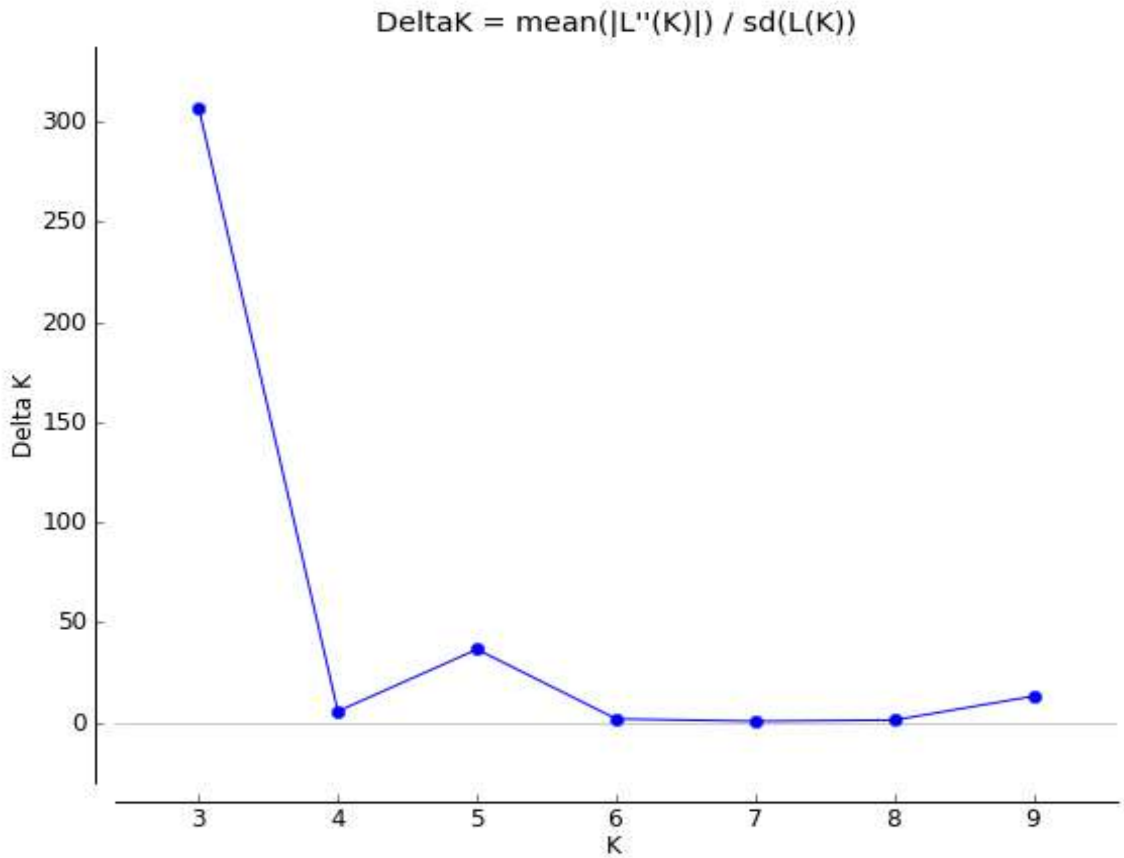
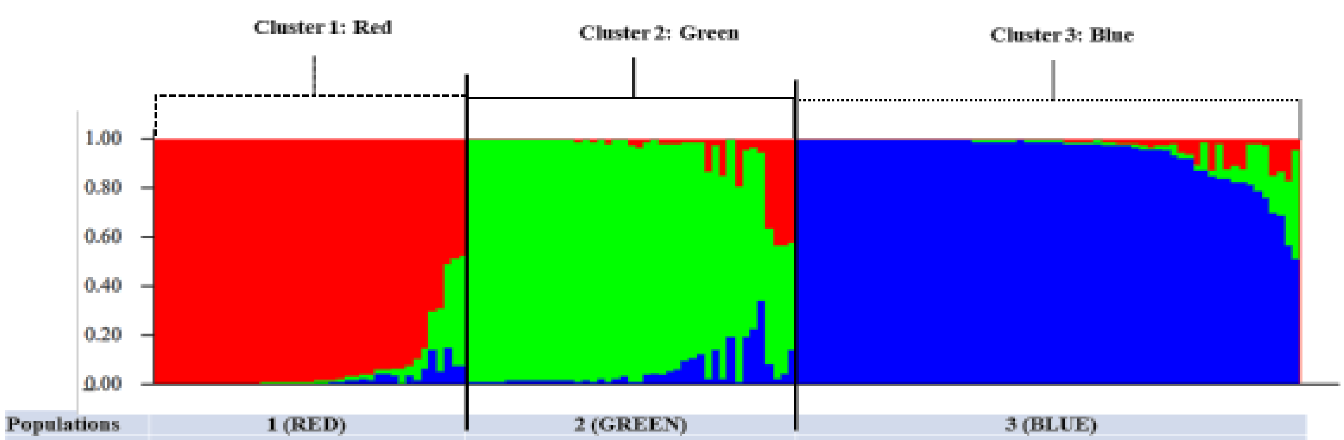



**Figure 4** . Delta K values generated by *STRUCTURE HAVESTER* program estimating the most likely number of clusters of the five local chicken populations in Togo




| Populations | 1 (RED) | 2 (GREEN) | 3 (BLUE) |
|---|---|---|---|
| Atakora | 27% | 28% | 45% |
| Forest | 31% | 32% | 36% |
| Littoral | 27% | 27% | 46% |
| Wet Savannah | 25% | 31% | 44% |
| Dry Savannah | 30% | 24% | 47% |

**Figure 5**. Clustering diagram based on structure analysis of the five local chicken populations in Togo. Each of the 150 chickens is represented by a thin vertical line, which is divided into three colored segments that represent the individual's membership coefficients in the three assumed clusters. The percentages represent the proportion of individuals of each population in each cluster.

## DISCUSSION

The number of alleles per microsatellite locus across all populations ranged from 3 (MCW037) to 12 (MCW069), with an average of 6.53. This result showed that the 15 loci were polymorphic in the local chicken populations of Togo. The present findings were similar to those found across indigenous chickens from agroecological zones of Côte d'Ivoire (Loukou et al., 2009), Forest and Savannah chicken populations of Ghana (Osei-Amponsah et al., 2010), Cameroon indigenous chickens (Fotsa et al., 2011) and in local chicken ecotypes in Burkina Faso (Yacouba et al., 2022). The allele numbers at loci ADL278 (05 alleles) and MCW183 (10 alleles) observed in Cameroon indigenous chicken ecotypes (Keambou et al., 2014) were similar to those obtained in the current study for the two loci.

The mean number of alleles per locus (MNA) obtained in the current study (6.53) was lower than the previous values reported by Mahammi et al. (2016) in Algeria (10.26), Soltan et al. (2018) in Egypt (9.10) and Habimana et al. (2020) in Rwanda (10.89). However, lower mean numbers of alleles per locus were observed in Benin (5.73-5.91) by Youssao et al. (2010), in Egypt (4.92) by Eltanany et al. (2011), in Sudan (5.3) by Berima et al. (2013) and in Tanzania (5.7) by Lyimo et al. (2013). Ramadan et al. (2012) and Yacouba et al. (2022) reported 6.9 in Egypt and 6.35 in Burkina Faso alleles per locus, respectively, which were closer to the values obtained in the present study. Such differences in the mean number of alleles per marker reported across studies could be attributed to the number of ecotypes/populations of studied chickens, the variation in the sample size, and the number and the loci used for the genotyping.

The polymorphic information content (PIC) is considered by Hubisz et al. (2009) as the best index for estimating the polymorphism of a locus, and based on PIC value. Botstein et al. (1980) classified loci as highly informative ( PIC > 0.5), moderately informative (0.25 < PIC < 0.5), and slightly informative (PIC < 0.25). The mean PIC of the microsatellite loci used in the present study was 0.55, indicating their high informativeness and suitability for assessing local chicken populations' genetic diversity in Togo. The mean PIC obtained in the current study was higher as compared to the value reported by Osei-Amponsah et al. (2010) in Forest and Savannah chicken populations of Ghana (PIC = 0.460) but closer to the values reported by Keambou et al. (2014) in five Cameroon chicken populations (PIC = 0.57) and Yacouba et al. (2022) in four Burkina Faso local chicken ecotypes (PIC = 0.541). However, higher mean PIC values were reported by Olowofeso et al. (2016) in three Nigerian chicken populations (0.70) and Rashid et al. (2020) in Bangladeshi native chicken populations (0.7489).

The mean $F_{IS}$ value was 0.132, which was lower than previously reported in Ghana and Egypt (Osei-Amponsah et al., 2010; Ramadan et al., 2012). This result indicated a deficit of heterozygotes. Ten loci out of the 15 used deviated significantly from the Hardy-Weinberg equilibrium. Clementino et al. (2010) and Dorji et al. (2012) reported lower percentages of Hardy-Weinberg equilibrium deviation (44%) and (40%), respectively, in Brazilian chicken ecotypes and native chickens from Bhutan. These differences can be attributed to the farming systems of the local chicken populations in different countries. In the current study zone, mating is random, the proportion of males is lower than that of females, and the roosters remain long in the production systems.

Heterozygosity is one of the basic measures of genetic diversity. The current study indicated that the mean observed heterozygosity ($H_O$) per chicken population varied from 0.464 to 0.569 with an overall mean value of 0.522, while the unbiased expected heterozygosity ($H_E$) ranged from 0.585 to 0.647 with an overall average of 0.616, indicating that there is high genetic diversity in the studied local chickens. These results were closer to those reported in local chickens from Cameroon ($H_E$ = 0.65) by Keambou et al. (2014) but lower than the values ($H_O$: 0.71-0.88 and $H_E$: 0.47-0.85) reported in Korean native chicken lines by Seo et al. (2013). In contrast, heterozygosity values were higher than those reported by Okumu et al. (2017) in Kenya ($H_E$ = 0.40) with 18 highly polymorphic microsatellite loci and by Yacouba et al. (2022) in Burkina Faso ($H_O$ = 0.391, $H_E$= 0.539) using 20 polymorphic microsatellite loci. The differences in observed and expected heterozygosity values between studies can be linked to the population structure, characteristics, and the number of microsatellite loci used in the studies. Furthermore, the expected heterozygosity values were higher than those observed in the five local chicken populations, leading to positive $F_{IS}$ values. The positive value of the $F_{IS}$, indicating heterozygote deficiency in the five local chicken populations, could be due to the population substructure created by clusters of households during the stratified sampling, which could bring the Wahlund effect (Samaraweera et al., 2021). Generally, there are no controls on mating, and very few roosters in village poultry flocks. This can lead to inbreeding and heterozygote deficiency.

The genetic distances (from 0.022 to 0.045) estimated between studied chicken populations were low, indicating that these populations are not genetically isolated from each other. These estimated genetic distances were much lower as compared to the values obtained in five chicken populations of Bangladesh (0.29 to 0.58) using 16 polymorphic microsatellite loci (Abdur Rashid et al., 2020) and in five Korean native chicken lines (0.08 to 0.17) using 15 microsatellite loci (Seo et al., 2013). Also, the global population differentiation ($F_{ST}$) was very low (0.021), indicating that only 2.1% of total genetic variation was due to population variation versus 97.9% due to the genetic variation within- populations. These results indicate that within-population variation is the main source of genetic diversity in the local chicken populations of Togo. The results also highlight a high level of gene flow among chicken populations between the different agroecological zones of Togo.

Based on DA genetic distances, the phylogenetic relationship observed on the Neighbour-Joining tree showed three main branches. The clustering patterns of all individuals, as revealed by FCA, confirmed the Neighbour-Joining results within one group, the Atakora and Dry Savannah chicken populations, in the second group, the Wet Savannah and Forest chicken populations, while the Littoral (coastal zone) chicken population clustered in a third group. These groups were so close and revealed little genetic differentiation among local chicken populations studied which can be explained by the large number of common alleles brought out by different individuals due to the important gene flow between them. The little genetic differentiation revealed by FCA was supported by the structure analysis. these results suggest a very mild population sub-structuring among the five populations of chickens examined in this study. This indicates that the chickens from these populations share a highly mixed and admixed genetic background. The low genetic differentiation observed in the present study is certainly due to the uncontrolled migration of chickens from one agroecological zone to another through the live animals or eggs sharing, thus favoring a permanent gene flow. This result is in accordance with Mtileni et al. (2011), who reported that large effective population sizes as well as continuous gene flow may be one of the forces responsible for the lack of population differentiation among local South –African chicken genotypes in their studies. In addition, the traditional rearing system used in the studied zones allows gene flow between the chicken populations. A similar observation was reported in Burkina Faso by Yacouba et al. (2022). These authors did not observe a sub-structuring in four Burkina Faso local chicken ecotypes using 20 microsatellite loci. Berima et al. (2013) also reported an absence of sub-structuring in five Sudanese native chicken breeds using 29 microsatellite loci. This absence or low sub-structuring in the local chicken ecotypes/breeds reported in most African countries could be due to a lack of genetic improvement programs concerning this poultry species. The advantage here is the high genetic variability observed in the local chicken populations, but the disadvantage is that, without a proper breeding program, uncontrolled crossbreeding will continue with a risk of losing some adaptive traits.

## CONCLUSION

Fifteen microsatellite loci were used for genotyping local chicken from Dry Savannah, Atakora, Wet Savannah, Forest, and Littoral agroecological zones of Togo. The results indicated high genetic diversity in the studied local chickens. The differences between agroecological chicken populations account for a small fraction (2.1%) of the total genetic variation. The analysis of the local chicken populations' genetic structure showed a low genetic structure among the agroecological chicken populations. Present results suggest that there is no specific and isolated genetic group in the local chicken populations raised in the study area. This study offers crucial details on the genetic makeup of indigenous chickens in Togo that could be applied to preservation or improvement efforts for the species. Prior to preservation or improvement, the growth performances and the reproductive potentialities must be evaluated in a controlled environment with the aim to identify any valuable chickens among local ecotypes.

## DECLARATION

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### Authors' contribution

Kossoga Kakom Assota contributed to the conceptualization of the protocol, data collection, data analysis, and manuscript drafting. Dayo Guiguigbaza-Kossigan contributed to the conceptualization of the protocol, data analysis, and revising the manuscript.

Bilalissi Abidi N'nanle Ombortime and Oke O. Emmanuel contributed to revising the manuscript. Tete-Benissan Amivi Kafui contributed to the supervision of the data collection and analysis and to the revising of the manuscript. All authors read and approved the final manuscript.

### Ethical consideration

The authors have made sure that the manuscript complies with the journal's ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) for submission and publication.

### Funding

The present study received no funding.

### Availability of data and materials

The corresponding author can provide all the related data of this study upon a reasonable request.

### Competing of interests

The authors reported no conflict of interest regarding the publication of this manuscript.

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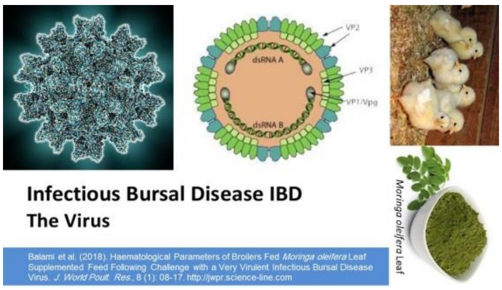


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