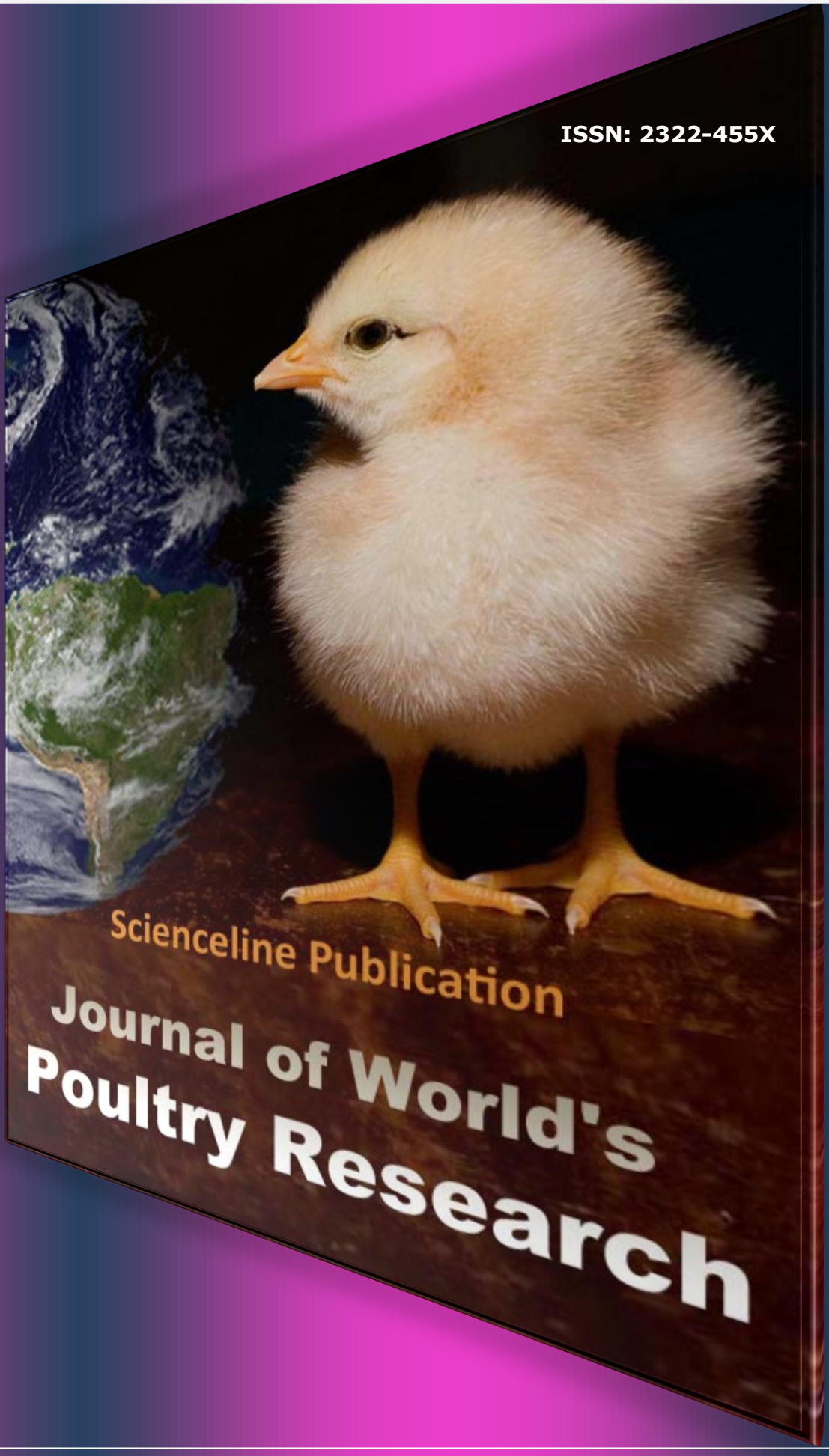
Journal of World's Poultry Research

BOOKLET

ISSN: 2322-455X



Volume 13, Issue 1, March 2023

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13 (1): 01-167, March 25, 2023

Editors-in-Chief

Managing Editor

Associate Editors

Language Editor

Statistical Editor

Technical Editor

Editorial Team

Advisory Board

TABLE OF CONTENT

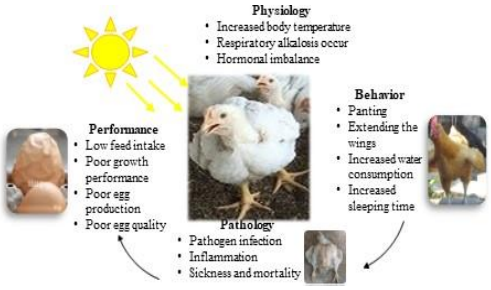
Volume 13 (1); March 25, 2023

Poultry Management Strategies to Alleviate Heat Stress in Hot Climates: A Review

Bhawa Sh, Morêki JC, and Machete JB

J. World Poult. Res. 13(1): 1-19, 2023; pii: S2322455X2300001-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.1



ABSTRACT: Heat stress remains a major challenge affecting poultry production in sub-tropical and tropical environments; hence it continues to receive attention. The present study aimed to discuss heat stress and its effects on poultry production and suggests mitigation strategies to combat the effects of increased environmental temperature on poultry performance. Poultry raised in hot climates suffers from heat stress, which reduces meat and egg production, reproductive performance, feed intake, and feed conversion efficiency leading to poor growth rates. Reduced feed intake results in a reduction in meat quality, growth, egg yield, and quality. A decrease in feed utilization efficiency is the major cause of poor growth performance in hot environments. To counteract the negative impacts of high ambient temperatures on the performance of poultry, a wide range of management practices are widely used, including nutrient manipulations (particularly protein and energy), electrolyte and vitamin supplementation, feed form (especially particle size and moisture content), choice feeding, controlled feeding, time of feeding, wet feeding, water management, and use of new breeds that thrive well in hot environments. These management practices help lower heat load and facilitate evaporative cooling, all of which may positively impact poultry performance and health.
Keywords: Choice feeding, Feed conversion efficiency, Heat stress, Poultry production

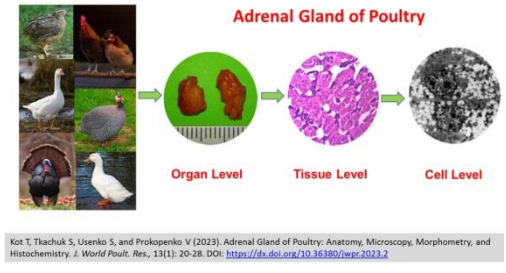
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Adrenal Gland of Poultry: Anatomy, Microscopy, Morphometry, and Histochemistry

Kot T, Tkachuk S, Usenko S, and Prokopenko V.

J. World Poult. Res. 13(1): 20-28, 2023; pii: S2322455X2300002-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.2



ABSTRACT: The adrenal gland plays a crucial role in poultry's body. Its hormones affect growth, tissue differentiation, and metabolism regulation, as well as the bird body's resistance to infections, intoxication, stress, and low temperature. For poultry farming, veterinary medicine, and ornithology, it is of scientific interest to study the morphological features of the adrenal gland of birds. This review aimed to assess poultry adrenal anatomy, microscopy, morphometry, and histochemistry by summarizing research data from various published articles. The structure of the adrenal gland has been morphologically investigated in clinically healthy chickens, ducks, geese, and quails. Data from the anatomical level of the adrenal gland have indicated that the shape of this organ in poultry of different species is not the same. In most cases, the shape of the adrenal gland of poultry is close to an oval, triangle, or pyramid. The color of the adrenal gland of poultry varies from gray to brown, which depends on the tissue saturation of this organ with carotenoids. The mass of the adrenal glands of poultry correlates with their age. The left adrenal gland has higher mass, volume, and length indicators than the right gland. The microscopic structure of the adrenal gland corresponds to the general laws of the structure and function of endocrine organs. However, the adrenal glands of poultry are characterized by class features of its histoarchitectonics. The adrenal capsule contains ganglia of the autonomic nervous system, the cell strands of cortical and medullary tissues are intertwined, and the configuration of these cell strands determines the formation of two or three zones of the adrenal gland. Studies of the adrenal glands of poultry at the cellular level have indicated that cortical tissue is represented by acidophilic cells and medullary tissue by basophilic cells. Depending on the shape and electron density of secretory granules, medullary tissue cells are divided into epinephrine and norepinephrine. Data on morphometric parameters (capsule thickness, area of cortical and medullary tissues, cortical-medullary ratio) of the adrenal gland are not the same and depend on the type, age, gender, and sexual activity of poultry. In conclusion, morphologists have paid great

attention to studying the features of the anatomy, microscopy, morphometry, and histochemistry of the adrenal gland in clinically healthy poultry. Therefore, the presented data can be used to assess deviations in the morphofunctional state of the adrenal gland in poultry under the influence of various factors and pathology.
Keywords: Adrenal gland, Anatomy, Morphological features, Histological and cellular levels, Poultry

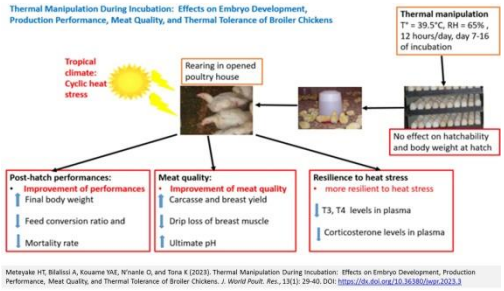
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Thermal Manipulation During Incubation: Effects on Embryo Development, Production Performance, Meat Quality, and Thermal Tolerance of Broiler Chickens

Meteyake HT, Bilalissi A, Kouame YAE, N'nanle O, and Tona K.

J. World Poult. Res. 13(1): 29-40, 2023; pii: S2322455X2300003-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.3



ABSTRACT: Thermal manipulations during the embryonic period have positive effects on thermotolerance and the productive performance of broilers subjected to acute heat stress. This study aimed to investigate the potential effects of Thermal manipulation during incubation (TMI) on productive performances and thermotolerance of broiler chickens growing in tropical climates. A total of 900 Cobb 500 broiler chicken eggs from a 35-week-old breeder flock were incubated in standard incubation conditions (37.8°C, 60% relative humidity) until day 7, when they were divided into 3 groups (300 eggs per group). The control group (C) was incubated at standard incubation conditions while T6 and T12 groups were subjected to, respectively, 6 hours/day and 12 hours/day of TMI (T° = 39.5°C, relative humidity = 65%, Embryonic day = 7-16). The relative embryo and albumen weight were determined from 10 to 18 days of incubation. The hatching event was checked between 450 and 504 hours of incubation, and egg hatchability, chick quality, and cloacal temperature were also determined. One hundred and twenty-five chicks from each incubation group were transferred to the farm and raised for 6 weeks. During this period, their post-hatch performances were determined. At week 6, blood samples were collected to measure T3, T4, and corticosterone hormone levels. Then, the 6-week-old broilers were slaughtered to determine meat yield and quality. Results showed that the chick's rectal temperature was significantly reduced in T6 and T12 groups compared to the C group, while hatchability and one-day-old chick weight were not affected. Final body weight and feed conversion ratio were significantly improved in the T12 group, compared to other groups. Thermal manipulation during incubation for 6 and 12 hours significantly reduced mortality rate and pectoralis major muscle drip loss while it increased muscle pH at 24 hours post-mortem (pH24). Corticosterone, T3, and T4 plasma hormone levels at week 6 were also significantly reduced by TMI. Therefore, exposing hatching eggs to 39.5°C and 65% of relative humidity from days 7 to 16 of incubation for 12 hours/day is recommended for the poultry industry in tropical climates.
Keywords: Chronic heat stress, Fast-growing broilers, Hatching and post-hatch performances, Thermal manipulation, Thermotolerance, Meat quality

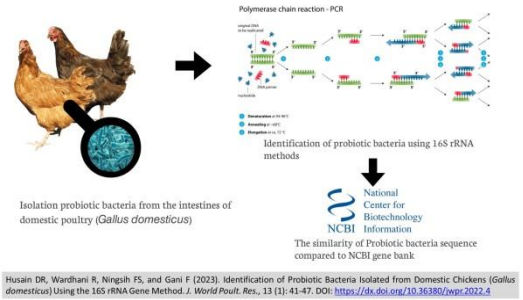
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Identification of Probiotic Bacteria Isolated from Domestic Chickens (*Gallus domesticus*) Using the 16S rRNA Gene Method

Husain DR, Wardhani R, Ningsih FS, and Gani F.

J. World Poult. Res. 13(1): 41-47, 2023; pii: S2322455X2300004-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.4



ABSTRACT: The intestines of domestic poultry (Gallus domesticus) are one of the potential sources of probiotic bacteria that can produce antibacterial agents. The objective of this study was to identify the types of probiotic bacteria obtained from the digestion of domestic poultry using the molecular analysis method of 16S rRNA gene sequencing. Observations were conducted on colony morphology, gram staining, biochemical tests, and antibacterial activity using the diffusion agar method. Molecular analysis of DNA extraction was carried out, followed by the amplification of samples using a 16S rRNA universal primer. Dielectrophoresis and sequencing were performed on the 16S rRNA gene. The identification of morphological observations, gram staining, and biochemical tests showed that probiotic bacteria isolates, including Gram-positive, rod-shaped, rounded colony form, flat elevation, entire nonmotile edge, and catalase-negative, could ferment all carbohydrate content in the TSIA medium. The antibacterial potential was also found in probiotic bacteria, as evidenced by the inhibition zone formed in the test. The results of the bacterial gene sequences of PaTa5 probiotic bacterial isolates had a

similarity of 98.37% with Lactobacillus plantarum. These findings indicated the presence of some bacteria species that have antibacterial activity in the intestines of domestic chickens (Gallus domesticus).
Keywords: Lactobacillus plantarum, Native chicken, Probiotic, 16S rRNA

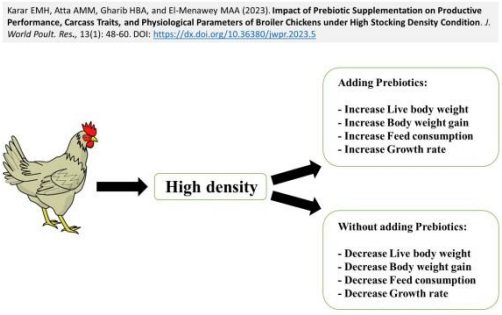
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Impact of Prebiotic Supplementation on Productive Performance, Carcass Traits, and Physiological Parameters of Broiler Chickens under High Stocking Density Condition

Karar EMH, Atta AMM, Gharib HBA, and El-Menawey MAA.

J. World Poult. Res. 13(1): 48-60, 2023; pii: S2322455X2300005-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.5



ABSTRACT: The present study was performed to investigate the effect of increasing stocking density, prebiotic supplementation, and the interactions on broiler chicken performance and some physiological parameters. A total of 912 one-day-old chickens were used in this study, and they were randomly divided into six groups with 4 replicates each. The experiment included three levels of stocking densities (10, 13, and 15 broiler chicken/m2) in 6 groups. Groups 1, 3, and 5 were maintained without prebiotic supplementation, while groups 2, 4, and 6 received a diet supplemented with prebiotics in water (1cm/liter). Reducing stocking densities and adding prebiotics improved body weight, feed consumption, feed conversion ratio, hemoglobin, packed cell volume, oxidative stress parameters (total antioxidant capacity), and European production efficiency factor, while decreasing malondialdehyde levels. On the other hand, stocking density and prebiotic supplementation did not affect dressing percentage, the relative weight of giblet parts, hind part, front part, and lymphoid organs (thymus and bursa of Fabricius). In conclusion, adding prebiotics at 1 cm/liter (Mannan-oligo saccharide and B-Glucan) can partially mitigate the negative effects of high stocking density on production performance, physiological and oxidative stress parameters, and European production efficiency factor.
Keywords: Antioxidant biomarkers, Broiler chicken, β-glucan, Mannan oligosaccharide, Oxidant, Prebiotic, Stocking density

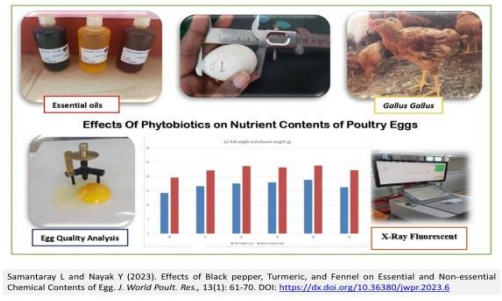
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Effects of Black pepper, Turmeric, and Fennel on Essential and Non-essential Chemical Contents of Egg

Samantaray L and Nayak Y.

J. World Poult. Res. 13(1): 61-70, 2023; pii: S2322455X2300006-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.6



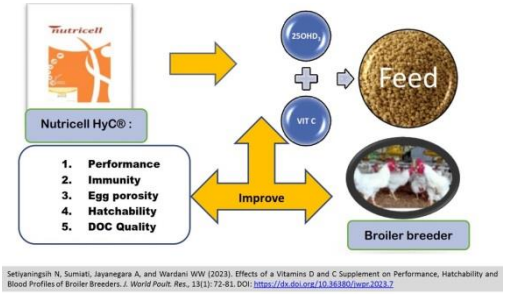
ABSTRACT: The use of essential oils (EOs) in animal feeding has gained attention as a potential antibiotic growth promoter replacement over the past two decades. The current study aimed to determine the impact of three feed additives, namely black pepper, turmeric, and fennel, on the productivity of laying hens, the chemical composition, and macro- and microelement content in layer eggs. A total of 280 chicks aged 75 days were randomly divided into 7 treatments (5 replicates of 8 chicks). One treatment group was provided as the unsupplemented control. The other six treatment groups, namely D0 (basal diet [BD] control), D1 (BD + 1% of black pepper), D2 (BD + 1% of turmeric), D3 (BD + 1% of fennel), D4 (BD + 0.5% of black pepper + 0.5% of turmeric), D5 (BD + 0.5% of black pepper + 0.5% of fennel), D6 (BD + 0.5% of turmeric + 0.5% of fennel) were supplemented with varying levels of phytobiotics. The result of the study indicated that the egg weight, Hen-day-production (HDP), egg quality, and haugh unit significantly improved with a combined supplementation of phytobiotics (D4, D5, and D6 diets) when compared with the control. However, there were no significant differences in the chemical composition of eggs. The X-ray fluorescence spectrometer analysis of eggs revealed the presence of 17 significant elements, including phosphorous, sulfur, chlorine, potassium, calcium, manganese, iron, copper, zinc, and bromine. The study findings showed that the combined supplementation of phytobiotics lowered K and Cl, whereas Zn, Ca, S, and Cu contents positively increased in hen eggs by including phytobiotic in the diet. In conclusion, the EOs of phytobiotics as dietary supplementation at 1% and 0.5% could improve the HDP, egg weight, and egg mass, including nutrient elements in the egg.
Keywords: Egg, Essential oil, Hen, Mineral, Phytobiotic
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Effects of a Vitamins D and C Supplement on Performance, Hatchability and Blood Profiles of Broiler Breeders

Setiyaningsih N, Sumiati, Jayanegara A, and Wardani WW.

J. World Poult. Res. 13(1): 71-80, 2023; pii: S2322455X2300007-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.7



ABSTRACT: Vitamin D is a fat-soluble vitamin that plays a crucial role in controlling Calcium and Phosphor homeostasis, bone mineralization, and modulation of immune responses. Vitamin C is a cofactor of enzymatic reactions with anti-inflammatory and antioxidant properties to prevent and repair damage to cells in the body from exposure to free radicals and the immune system. The current study aimed to investigate the effects of dietary supplementation of 25(OH)D3 with vitamin C at different doses on broiler breeders' blood profile, egg quality, and hatchability. The adaptation process before collecting the data was 2 weeks. A total of 6200 females and 620 male broiler breeders in the laying period aged 32-46 weeks were divided into 4 treatment groups with 5 replicates (each peach contained 310 female and 31 male breeders). The treatments included control as T0 (0 g/ton Nutricell HyC®), T1 (100 g/ton Nutricell HyC®), T2 (200 g/ton Nutricell HyC®), T3 (400 g/ton Nutricell HyC®) supplemented in feed. The observed variables were performance in breeding farms and hatcheries. The treatments with experimental doses indicated significant differences in the performance of broiler breeders, including feed intake, body weight, egg weight, egg mass, hen day production, hen house production, feed conversion ratio, and parameters of blood profile. The results showed a significant difference between the treatments and the control group in terms of hatch performance, clear eggs, exploding eggs, hatchability eggs, fertile eggs, salable chicks, and hatching of fertile eggs. However, no significant effects on fertility, culling of chicks, and embryonic mortality in the treatment groups were indicated. In conclusion, Nutricell HyC® with a dose of 400 g/ton in feed has indicated the best result in breeding farm and hatchery performance of broiler breeders in the laying period.
Keywords: Blood profile, Broiler breeder, Calcidiol (25(OH)D3), Nutricell HyC®, Performance, Vitamin C

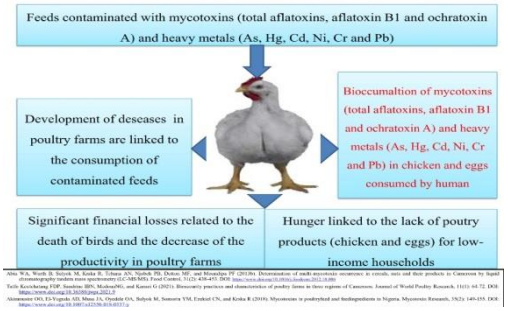
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Mycotoxins and Heavy Metals of Poultry Feeds from the Centre, Littoral, and Western Regions of Cameroon

Keutchatang FDPT, Mafogang B, Kamgain ADT, Nguegwouo E, Tene HM, Ntsama ISB, Nama GM, and Kansci G.

J. World Poult. Res. 13(1): 81-88, 2023; pii: S2322455X2300008-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.8



ABSTRACT: Heavy metals are a group of elements that could be found in poultry feeds and influence poultry production. Poultry feed generally consists of agricultural products, such as maize, groundnuts, and wheat, which may also be contaminated by mycotoxins. The use of mycotoxins and heavy metals contaminated feed in the poultry sector might represent a potential source of cross-contamination for humans. This study aimed to analyze total aflatoxins (AFs), aflatoxin B1 (AFB1), ochratoxin A (OTA), chromium, copper, nickel, zinc, arsenic, cadmium, lead, and mercury in poultry feed from the Centre, Littoral, and Western regions of Cameroon. In this order, six local broiler feeds, six local layer feeds, and three imported layer feeds were randomly collected from each region and analyzed using inductively coupled plasma spectrometry for heavy metals and competitive indirect ELISA for mycotoxins. The results indicated that all feed samples contained the analyzed mycotoxins and heavy metals. The ranges for the mean concentrations of mycotoxins were 3.5-19.7, 2.7-19.3, 0.8-1.1 µg/kg for AFs, AFB1, and OTA, respectively. They were globally below the established regulated limits (20 µg/kg for AFs, 10 µg/kg for AFB1 and 5 µg/kg for OTA). The bulk layer feed from the Littoral region had the highest lead (995.8 ± 0.4 µg/kg) and cadmium (3.3 ± 0.0 µg/kg) concentrations. The average concentration of lead was above the permissible limit (10 µg/kg). Bulk broiler feed from the Littoral region scored the highest concentration of arsenic (2819.4 ± 0.1 µg/kg) above the permissible limit (500 µg/kg). Bulk broiler feed from the Centre region showed the highest concentration of mercury (5.6 ± 0.0 µg/kg) although lower than the permissible limit of 100 µg/kg. This study demonstrates that there are potential safety issues associated to poultry feeds used in some regions of Cameroon. It suggests a possible low productivity of poultry and health issues for consumers.
Keywords: Aflatoxin, feed, Contamination, Heavy metals, Ochratoxin A, Poultry

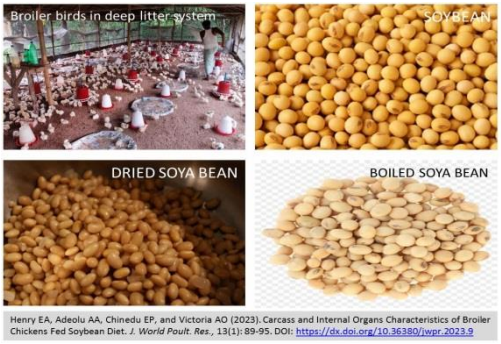
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Carcass and Internal Organs Characteristics of Broiler Chickens Fed Soybean Diet

Ekeocha AH, Aganga A A , Emerue P C, and Akinsoyinu OV.

J. World Poult. Res. 13(1): 89-95, 2023; pii: S2322455X2300009-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.9



ABSTRACT: Soybean (Glycine max) is a principal vegetable protein source in the animal feed industry in Nigeria, including the poultry feed industry, but because of the fact that it contains various anti-nutritional factors, the raw full-fat cannot be used in poultry nutrition. The present study was carried out to examine the carcass, and internal organ characteristics of broiler chickens fed a soybean diet. A total of eight hundred and four unsexed one-day-old broiler chickens (Arbor Acre) with an average weight of 45 ± 1.1 g were used in 56 days feeding trial to observe the effect of different processing methods of soybean on broiler chicken carcass and organs characteristics including; liver, spleen, lungs, crop, bile, pancreas, heart, intestine and empty gizzard. There were four dietary treatments (T1-T4), each containing soybean meal as the control diet, dried-boiled soybean, roasted soybean and dried-fermented soybean. Each treatment was replicated three times with 67 broiler chickens per replicate, giving 201 broiler chickens per treatment. The experiment was arranged in a completely randomized design. The starter diet was fed for 4 weeks and the finisher diet for 4 weeks. At the end of the feeding trial, 15 broiler chickens were randomly selected for sampling and collecting the data. The weight of the internal organs and carcass characteristics showed no significant difference in the treatment groups, but the fermented method showed the highest value in the live weight (2075.00 g), eviscerated weight (1532.46 g), and breast weight (483.72 g) compared to other carcass parameters in other treatments including the control diet. It is, therefore, concluded that for optimal broiler growth, the fermented processing method of soybean is recommended.
Keywords: Broilers chicken, Carcass characteristic, Diet, Internal organ, Processed soybean

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

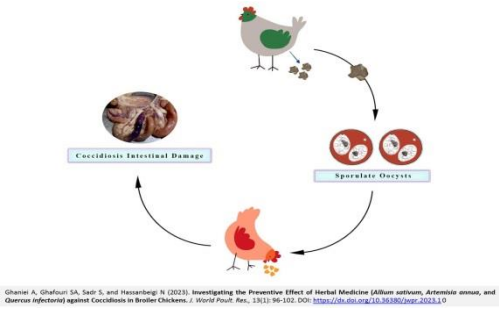
Research Paper

Investigating the Preventive Effect of Herbal Medicine (*Allium sativum*, *Artemisia annua*, and *Quercus infectoria*) against Coccidiosis in Broiler Chickens

Ghaniei A, Ghafouri SA, Sadr S, and Hassanbeigi N.

J. World Poult. Res. 13(1): 96-102, 2023; pii: S2322455X2300010-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.10



ABSTRACT: Coccidiosis is a critical disease in the poultry industry worldwide. Producers apply different strategies to control and prevent this disease. Herbal drugs are suitable remedies for reducing losses associated with coccidiosis in poultry. The present study aimed to evaluate the effectiveness of an herbal mixture in preventing coccidiosis. A total of 160 broiler chickens were divided into four treatment groups, with five replicates for each. Experimental infection of all groups, except group D, was carried out with mixed *Eimeria* species (*E. tenella*, *E. necatrix*, *E. brunetti,* and *E. maxima*) on day 14. Broiler chickens in group A were given an herbal mixture (75% *Quercus infectoria*, 16% *Artemisia annua*, and 9% *Allium sativum*) as feed additives during the rearing period, and group B was treated with Monensin. No treatment was applied to group C after the chickens were experimentally infected with mixed *Eimeria* spp. Group D was used as the negative control since chickens in this group were not infected or sick during the experiment. Body weight gain, feed conversion ratio (FCR), mortality rate, intestinal lesion scoring, and oocyst count per gram (OPG) were evaluated in this study. The results of the present study revealed that the highest mean body weight was gained in group D, followed by chickens in group A. The best FCR results were attributed to chickens in group D, followed by group B. In this study, both drugs decreased mortality rate, intestinal lesion scores, and OPG in the treated chickens. In conclusion, this herbal mixture can reduce coccidial lesions.
Keywords: Broiler, Coccidiosis, Herbal mixture, Prevention

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

Processing of *Sargassum binderi* Seaweed for Supplementation in Poultry Diet

Dewi YL, Yuniza A, Nuraini, Sayuti K, and Mahata ME.

J. World Poult. Res. 13(1): 103-111, 2023; pii: S2322455X2300011-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.11

Dewi YL, Yuniza A, Nuraini, Sayuti K, and Mahata ME (2023). Processing of *Sargassum binderi* Seaweed for Supplementation in Poultry Diet. *J. World Poult. Res.*, 13(1): 103-111. DOI: https://dx.doi.org/10.36380/jwpr.2023.11

ABSTRACT: *Sargassum binderi* has been potentially used as laying hen feed since it contains bioactive compounds useful for poultry health. In addition, the high alginate content of *S. binderi* has made it inappropriate for the poultry diet. Therefore, the alginate content should be reduced before its use in poultry feed. This study aimed to reduce the alginate of *S. binderi* for use as laying hen feed. The experiment was performed in two phases in a completely randomized design. The first phase included heated *S. binderi* in the autoclave and the second phase entailed the immersion of *S. binderi* in whiting filtrate. The treatments in the physical method contained a control group, and four treatment groups heating for 15, 30, 45, and 60 minutes. The treatments in the chemical method had a control group and four treatment groups with immersion periods of 1, 2, 3, and 4 hours. Each treatment was repeated five times, and the investigated parameters were crude protein, total dry matter, organic matter, ash, and alginate, respectively. The heating durations of *S. binderi* in an autoclave and different immersion periods of *S. binderi* in whiting filtrate did not significantly affect total dry matter, organic matter, ash, alginate, and crude protein. The results of this study showed that physical treatment (heat treatment) and chemical treatments (whiting filtrate immersion) did not have a significant effect on the alginate content, crude protein, ash, dry matter, and organic matter.
Keywords: Alginate, Heating, Laying hen, *Sargassum binderi*, Whiting filtrate

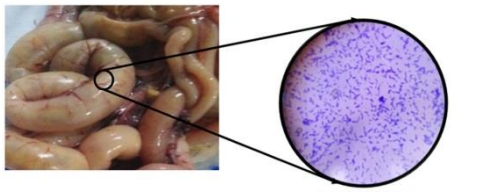
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Clostridium perfringens in Broiler Chickens: Isolation, Identification, Typing, and Antimicrobial Susceptibility

Eid NM, Ahmed EF, Shany SAS, Dahshan AM, and Ali A.

J. World Poult. Res. 13(1): 112-119, 2023; pii: S2322455X2300012-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.12



Eid NM, Ahmed EF, Shany SAS, Dahshan AM, and Ali A (2023). *Clostridium perfringens* in Broiler Chickens: Isolation, Identification, Typing, and Antimicrobial Susceptibility. *J. World Poult. Res.*, 13(1): 112-119. DOI: https://dx.doi.org/10.36380/jwpr.2023.12

ABSTRACT: Necrotic enteritis (NE) is a common worldwide poultry disease caused by the bacterium of *Clostridium perfringens* (*C. perfringens*) which has significant economic losses in the poultry industry as well as the cost of treatment and preventive measures. The current study was conducted to evaluate the incidence of NetB toxin positive in *C. perfringens* on different farms in Egypt. In the years 2020 and 2021, on industrial broiler farms (15-45 days- old), 100 intestinal samples were collected consisting of 30 healthy Ross broiler chickens and 70 unhealthy Ross broiler chickens. Culture and biochemical characterization (Catalase, urease, sugar fermentation, gelatin liquefaction, nitrate reduction, and lecithinase reaction tests) confirmed that *C. perfringens* was isolated at a rate of 10% (3/30) from apparently healthy broiler chickens and 40% from unhealthy broiler chickens. Thirty-one isolates were tested for toxigenicity and typing by ELISA kits and the results showed that 80% of the isolates from unhealthy broiler chickens were *C perfringens* type A alpha-toxin (toxigenic), 20% were non-toxigenic, and 66.7% isolates from apparently normal broiler chickens were toxigenic. The same thirty-one (44%) *C. perfringens* isolates were detected by PCR to investigate the presence of the NetB toxin gene in apparently healthy and unhealthy broilers and subsequently detect the role of NetB toxin in inducing NE. Of the samples, 82% of the isolates from unhealthy chicks were found to incode NetB gene, while none of the isolates from healthy broiler chickens had NetB. *Clostridium perfringens* showed sensitivity to amoxicillin, amoxicillin with clavulanic acid and ampiclox, intermediate for ofloxacin, and high resistance to cephalexin, streptomycin, colistin sulfate, erythromycin, sulfa trimethoprim, gentamycin, and oxytetracycline. The present study revealed the importance of NetB gen in the appearance of clinical signs of NE in broiler chickens.

Keywords: Alpha toxin, Antibiotic sensitivity test, *Clostridium perfringens*, Necrotic enteritis, NetB

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

Research Paper

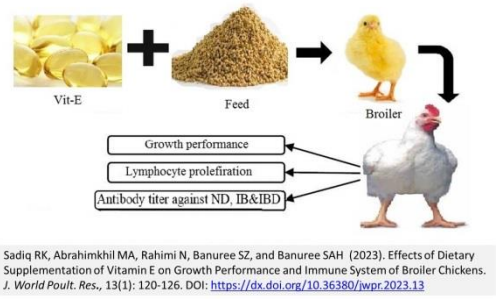
Effects of Dietary Supplementation of Vitamin E on Growth Performance and Immune System of Broiler Chickens

Sadiq RK, Abrahimkhil MA, Rahimi N, Banuree SZ, and Banuree SAH.

J. World Poult. Res. 13(1): 120-126, 2023; pii: S2322455X2300013-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.13

ABSTRACT: As a potent antioxidant, Vitamin E may lessen the potentially harmful consequences of such oxidative stress to protect broilers against immune-pathological damage. Broiler chicken growth and viability are enhanced by Vitamin E supplementation. The present study aimed to investigate the effects of Vitamin E dietary supplementation on broiler chickens' growth performance and health status. A total of 48 one-day-old Ross chicks were randomly divided into two groups of control and treatment (supplementation of Vitamin E at a dose of 300 mg/kg diet) with three replicates per group. The study included an equal number of Ross breed chicks and Vitamin E dosage in two trials on two different dates (January and March, 2022). In both trials, the obtained results indicated no significant changes in weight gain in the control and treatment groups. In both trials, there were no significant differences in the spleen weight of the control and treated groups; however, from day 1 to 28 of the second trial, the bursa of Fabricius was heavier in the treated group than in the control group. Additionally, Vitamin E had no significant effects on the mitogenic responses to phytohemagglutinin (PHA) and Concanavalin A (Con A). Dosages of 20 and 10 µl for both PHA and Con A did not significantly affect the rate of pure lymphocyte proliferation in chicks fed 300 mg Vitamin E /kg feed. Cell-mediated immunity did not differ significantly between the two trials. The percentages of CD4, CD8, Bu1, and MHCII molecules in the spleen and cecal tonsil of the chicks that received Vitamin E 300 mg/kg feed did not change significantly. The antibody titers against infectious bronchitis and infectious bursal disease vaccines showed no significant differences. On day 42, there was a trend toward an increase in antibody titer in the case of the Newcastle disease vaccine. In conclusion, 300 mg/kg of Vitamin E added to the diet did not improve growth performance and immunity in broiler chicks.



Sadiq RK, Abrahimkhil MA, Rahimi N, Banuree SZ, and Banuree SAH (2023). Effects of Dietary Supplementation of Vitamin E on Growth Performance and Immune System of Broiler Chickens. *J. World Poult. Res.*, 13(1): 120-126. DOI: https://dx.doi.org/10.36380/jwpr.2023.13

Keywords: Broiler chicken, Growth performance, Immune system, Vitamin E

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

Research Paper

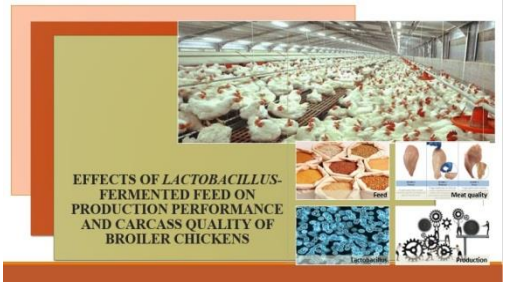
Effects of Lactobacillus-Fermented Feed on Production Performance and Carcass Quality of Broiler Chickens

Palupi R, Lubis FNL, Pratama ANT, and Muhakka.

J. World Poult. Res. 13(1): 127-135, 2023; pii: S2322455X2300014-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.14

ABSTRACT: The quality of broiler chicken carcasses is greatly influenced by feed management and the number of nutrients digested in the digestive tract that will be utilized for optimal meat production. The study aimed to determine the effect of feeding fermented feed at different times on the production performance and quality of broiler chicken carcasses. The number of day-old chicks used in this study was 180 broiler chickens strain Cobb. This study was carried out experimentally using a complete randomized design consisting of four treatments and five replications. Each treatment carried out in this experiment consisted of a different length of time, namely feeding for 2, 3, 4, and 5 weeks. Experimental parameters included feed consumption, weight gain, and ration conversion. In addition, the carcass quality was investigated as live weight, carcass percentage, and percentage of broiler chicken abdominal fat. The results showed that the longer the time of fermentation feed significantly increased feed consumption by 40.07% and increased 13.77% weight gain, as well as decreased ration conversion by 25.33%. Furthermore, the same results were also obtained regarding live weight by 17.80% and increased percentage of the carcass by 8.84%, while the percentage of abdominal fat decreased by 12.90%. It can be concluded that the provision of fermented feed for 5 weeks can improve the production performance and carcass quality of broiler chickens.



Keywords: Broiler chicken, Carcass quality, Fermented feed, Performance

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

Effects of Sex and Rearing Season on Body Weight Gain and Growth Curve Parameters of Local Chickens in Niger

Guisso Taffa A, Hamani B, Moula N, Issa S, Mahamadou Ch, and Detilleux J.

J. World Poult. Res. 13(1): 136-142, 2023; pii: S2322455X2300015-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.15

ABSTRACT: Local chicken breeding is widespread in Niger, a country with harsh environmental conditions. This study aimed to investigate the effects of sex, temperature, and hygrometry variations on the body weight gain and growth curve of local Nigerien chickens. Two groups of local chickens were followed from hatching to 20 weeks of age. The first and second groups consisted of 96 and 124 chickens, respectively. Three seasons were identified based on continuously recording ambient temperature and humidity over a year. The dry and warm seasons (February, March, April, and May), the wet and warm seasons (June, July, August, and September), and the dry and cold seasons (October, November, December, and January). The average hatch weight was about 24 g, and monthly body weight gains ranged from 100 to 360 g. Asymptotic weights were 2214.02 ± 69.94 g and 1776.93 ± 63.57 g for roosters and 1380.25 ± 25.96 g and 1433.08 ± 71.24 g for hens. The sexual maturity rates indicate that hens are more precocious than roosters. Sex and season had significant impacts on the growth performance of the chickens. In conclusion, the results of the present study indicated that the optimal time to raise local chickens in rural Niger is from June to January, and males are better candidates for meat production.

Keywords: Growth curve, Hygrometry, Local chicken, Temperature, Weight gain

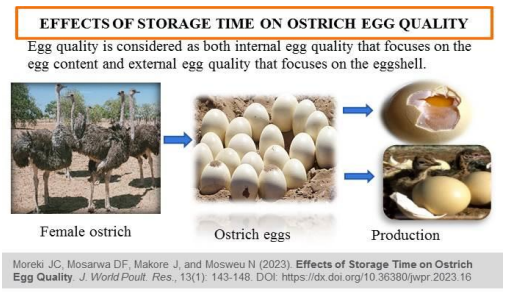
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Effects of Storage Time on Ostrich Egg Quality

Moreki JC, Mosarwa DF, Makore J, and Mosweu N.

J. World Poult. Res. 13(1): 143-148, 2023; pii: S2322455X2300016-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.16



ABSTRACT: Egg quality is considered as both internal egg quality that focuses on the egg content and external egg quality that focuses on the eggshell. This study investigated the effect of storage time on ostrich egg quality. A total of 15 ostrich eggs were obtained from Dibete Ostrich Multiplication Unit and subjected to five storage periods (0, 3, 6, 9, and 12 days) at room temperature (18-25oC). The measured parameters were egg weight, egg length, egg width, yolk weight, albumen weight, albumen height, yolk height, shell weight, egg specific gravity (ESG), egg surface area, Haugh Unit (HU), egg shape index, albumen ratio, shell ratio, yolk ratio and weight of egg contents. Results showed that storage time did not influence egg weight for eggs stored for 0, 3, and 6 days. On the other hand, storage time significantly affected egg weight for eggs stored at 9 and 12 days. The albumin ratio for egg storage duration had no significant impact on eggs held for 0, 3, or 6 days. However, the albumen ratios of eggs held for 9 and 12 days were impacted by the storage period compared to those stored for 0, 3, and 6 days. The HU for the eggs stored for 0, 3, and 6 days was not affected by storage time. On the contrary, the HU for eggs stored for 9 and 12 days was significantly impacted by storage time as the HU decreased with the prolonged storage time, compared to those stored for 0, 3, and 6 days. The results of this study suggest that ostrich eggs should not be stored for more than 6 days at ambient temperature to avoid egg quality degradation.
Keywords: Cuticle, Egg quality, Ostrich eggs, Storage time

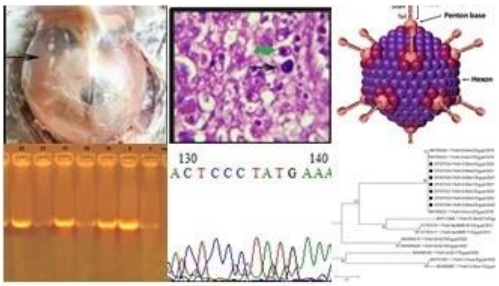
[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Isolation and Characterization of Fowl Adenoviruses Associated with Hydro-pericardium Syndrome from Broiler Chickens in Egypt

Hussein E, Anwar NF, ElsebaeyHSh, Abdelmagid MA, Abo Elkhair M, and Mahana O.

J. World Poult. Res. 13(1): 149-160, 2023; pii: S2322455X2300017-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.17



Fowl Adenovirus Species D in Egypt

ABSTRACT: One of the most prominent viral diseases affecting the poultry industry is hydropericardium syndrome caused by fowl adenoviruses. Hydropericardium syndrome has recently led to significant economic losses in the Egyptian poultry sector. Many outbreaks of hydropericardium syndrome have been documented across the country in the last few years. This research examined the epidemiology and molecular characterization of fowl adenoviruses in broiler chickens in Egypt. Samples were taken from 26 outbreaks of commercial broiler chicken farms in the Beheira and Menofia governorates, Egypt, from January 2021 to March 2022. Adenoviruses genomes were detected in cloacal swabs of 10 flocks using polymerase chain reaction. Clinically, infected broiler chickens (Cobb, Ross, Indian River, Modified-Avian, and Arbor Acres) showed depression, ruffled feathers, retarded growth, and ascites, with mortality rates of 10-28%. The most common postmortem lesions were hydropericardium, yellowish enlarged liver with ecchymotic hemorrhages, pancreatitis, and enteritis. Histopathologically, intranuclear inclusion bodies, commonly basophilic type, were scattered in the hepatocyte, proventriculus, duodenum, kidney, pancreas, and spleen. In addition, depletion of lymphocytes in the bursa of Fabricius and the thymus was observed. Seven samples were selected for gene sequencing of the loop 1 region of the hexon gene. The sequence analysis revealed that all samples were identical and similar to fowl adenoviruses species D serotype 2/11, suggesting that this serotype was the predominant fowl adenoviruses circulating in the study location in the last two years. Further studies are required to address the pathogenicity of isolated fowl adenoviruses and evaluate the vaccine used to control fowl adenoviruses in Egypt.
Keywords: Fowl adenoviruses, Hexon, Hydropericardium syndrome, Phylogenetic analysis, Polymerase chain reaction

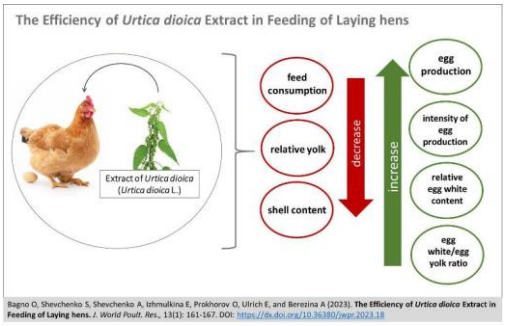
---

The Efficiency of *Urtica dioica* Extract in Feeding of Laying Hens

Bagno O, Shevchenko S, Shevchenko A, Izhmulkina E, Prokhorov O, Ulrich E, and Berezina A.

J. World Poult. Res. 13(1): 161-167, 2023; pii: S2322455X2300018-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.18

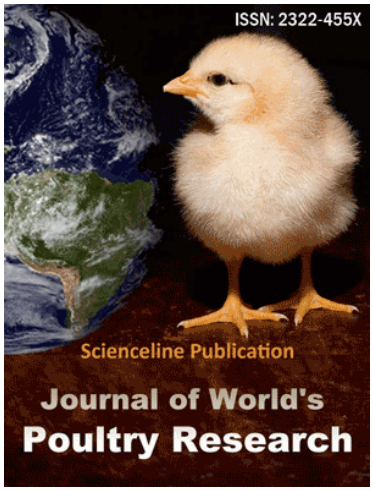


ABSTRACT: Currently, poultry specialists are working hard to find feed additives of natural origin. Medicinal plants are a source of a wide range of biologically active compounds with multifunctional effects, including antimicrobial ones. To understand the potential use of various medicinal plants and their extracts in poultry farming, the current study aimed to investigate the effect of feeding different doses of water-ethanol extract of *Urtica dioica* (*Urtica dioica* L.) on the egg productivity of laying hens. A total of 300 laying hens were divided to control and five experimental groups of chickens, each with 5 replicates. During the entire experiment, the laying hens of the control group were fed complete compound feeds according to the egg-laying phase, and the chickens of the experimental groups were additionally fed *Urtica dioica* extract in different doses. The results indicated that feeding laying hens with *Urtica dioica* extract in doses of 5, 10, 15, 20, and 25 mg/kg of body weight had a positive effect on their egg productivity. An increase in egg production per average laying hen in the experimental groups was 2.6-6.1%, and the intensity of egg production was 2.1-5.4%, compared to the control. However, the feed consumption in all experimental groups decreased. When introducing Urtica dioica extract into full-fledged compound feeds for laying hens, there was an increase in the relative egg white content, egg white/egg yolk ratio, and a decrease in relative yolk and shell content. Accordingly, it is suggested to include Urtica dioica extract at a dose of 15 mg/kg in the diet of laying hens which can improve economic efficiency and egg parameters.
Keywords: Chemical composition, Egg morphology, Egg production, Feed conversion, Medicinal plant

---

Previous issue | Archive

Journal of World's Poultry Research



ISSN: 2322-455X

Frequency: Quarterly

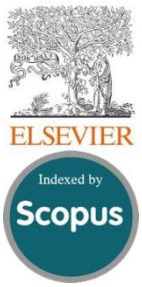
Current Issue: 2023, Vol: 13, Issue: 1 (March 25)

Publisher: SCIENCELINE

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

www.jwpr.science-line.com

» Indexed/covered by SCOPUS (CiteScore=0.9), NLM Catalog (NLM ID: 101681042), NAAS (Score: 4.79), CIARDRING, Ulrich's™/ ProQuest, PUBDB, ICV 2021= 128.45, TOCs, TIB, WorldCat, EZB, Google Scholar...full index information



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

» Digital Archiving: Journal Repository (ePrints)

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

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

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

» High visibility of articles over the internet.

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

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

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 1-19, March 25, 2023

Review Paper, PII: S2322455X2300001-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.1

Poultry Management Strategies to Alleviate Heat Stress in Hot Climates: A Review

Shame Bhawa, John Cassius Morêki, and James Butti Machete

Department of Animal Sciences, Faculty of Animal and Veterinary Sciences Botswana University of Agriculture and Natural Resources, Private Bag 0027, Gaborone, Botswana

Corresponding author's E-mail: jmoreki@buan.ac.bw

Received: 08 January 2023
Accepted: 03 March 2023

ABSTRACT

Heat stress remains a major challenge affecting poultry production in sub-tropical and tropical environments; hence it continues to receive attention. The present study aimed to discuss heat stress and its effects on poultry production and suggests mitigation strategies to combat the effects of increased environmental temperature on poultry performance. Poultry raised in hot climates suffers from heat stress, which reduces meat and egg production, reproductive performance, feed intake, and feed conversion efficiency leading to poor growth rates. Reduced feed intake results in a reduction in meat quality, growth, egg yield, and quality. A decrease in feed utilization efficiency is the major cause of poor growth performance in hot environments. To counteract the negative impacts of high ambient temperatures on the performance of poultry, a wide range of management practices are widely used, including nutrient manipulations (particularly protein and energy), electrolyte and vitamin supplementation, feed form (especially particle size and moisture content), choice feeding, controlled feeding, time of feeding, wet feeding, water management, and use of new breeds that thrive well in hot environments. These management practices help lower heat load and facilitate evaporative cooling, all of which may positively impact poultry performance and health.

Keywords: Choice feeding, Feed conversion efficiency, Heat stress, Poultry production.

INTRODUCTION

There has been a significant increase in global average temperatures recently, which has affected the farming sector in the tropics (Barrett et al., 2019; Sohsuebngarm et al., 2019; Kennedy et al., 2022). High temperature above 32^{o}C depresses feed intake, leading to poor performance in poultry (Cassuce et al., 2013; Sohsuebngarm et al., 2019). In addition, the increase in temperature results in the number of etiologically harmful microorganisms in the environment around the animals increasing. Due to an increase in parasites and microorganisms, climate change influences disease emergence and transmission (Ranjan et al., 2019). When the temperature in a living organism exceeds the threshold limit (i.e., thermo-neutral zone), it disrupts normal physiological functions and causes cell damage (Mack et al., 2013; Kennedy et al., 2022). High environmental temperatures typically cause stress-related issues such as output losses, metabolic alterations, poor development, and inefficiency (Dayyani and Bakhtiari, 2013; Afsal et al., 2018). At high temperatures, feed intake decreases while water intake increases (Mottet and Tempio, 2017).

Due to their insulating feathers, the absence of sweat glands on the skin, and the significantly high mass-to-body surface area ratio, broiler chicken strains are highly susceptible to rising temperatures (Scanes, 2015; Sejian et al., 2018; Bernabucci, 2019) compared to laying hens. In broiler chickens, for instance, rigorous genetic selection has enhanced metabolic activity in the pursuit of a higher development rate, further eroding the potential of a modern bird to withstand heat (Tamzil, 2014; Bohler et al., 2021). The broiler sector is confronted with heat stress, which raises production costs and degrades meat quality. This is attributable to the vulnerability of poultry to heat stress given the rapid metabolic and faster growth rates. In chickens, notably broilers, grown in hot climates, metabolic changes occur, resulting in a significant

reduction in breast muscle growth (Safdar and Maghami, 2014).

Heat stress is divided into two types: acute heat stress (AHS), which is characterized by exposure to high temperatures for a short time, and chronic heat stress (CHS), which is characterized by exposure to high temperatures for a longer time (Lara and Rostagno, 2013; Pawar et al., 2016). In contrast to acute heat stress, chronic heat stress can increase fat content while destroying the muscles (Song and King, 2015; Adu-Asiamah et al., 2021). Besides the duration of excessive heat, the degree of heat stress influences the level of production (Adu-Asiamah et al., 2021). Both AHS and CHS have the potential to produce a significant decrease in poultry metabolism, which could lead to substantial issues with broiler growth performance and carcass characteristics which include meat color change, water holding capacity, muscular pH, and meat juiciness (Song and King, 2015; Gonzalez-Rivas et al., 2020).

Understanding the basic aspects underlying the causes, and impacts of heat stress, as well as, the approaches that can be used to mitigate or control such a widespread threat, will help solve worldwide food security challenges. Despite the ongoing debate in the literature on heat exposure, a synthesis of knowledge on such systems in terms of elevated ambient temperature exposure is still yet to be published. Therefore, this review aimed to discuss the management strategies that poultry producers can utilize to boost production in hot places around the world.

EFFECTS OF HEAT STRESS ON POULTRY

Heat stress (also referred to as hyperthermia) is a result of global warming and is considered one of the crucial factors that negatively influence poultry production (Vandana and Sejian, 2018). Excessive heat depresses feed intake, feed conversion efficiency, growth, meat and egg output, and reproductive function (Alverdy and Luo, 2017; Quinteiro-Filho et al., 2017; Rostagno, 2020). The reduced feed intake due to high temperatures has a negative effect on semen quality and fertility, thus leading to poor hatchability rates (Nawab et al., 2018; Nyoni et al., 2019). In addition, heat stress affects a poultry's production performance, digestive health, body temperature, immunological responses, hunger hormone modulation, and oxidative properties (Goel, 2021). Recently, Nawaz et al. (2021) observed that heat stress degrades meat quality by altering the pH, water-holding capacity, and drip loss in the meat leading to changes in the normal meat color, flavor, and texture of chicken meat. Moreover, the effects of heat stress on meat quality include a reduction in protein synthesis and an increase in unfavorable fat (Kadykalo et al., 2018). By adjusting to changing climatic conditions, poultry frequently sacrifices their production capacity (Slawinska et al., 2019; Smith et al., 2019). However, poultry breeds are more resilient to climate change which continues to influence egg and meat production (Farag and Alagawany, 2018; Liverpool-Tasie et al., 2019).

Overcrowding and high outside temperatures contribute to the development of heat stress. However, by increasing cooling options, which include using the fogging system, use of a wet pad system, and micro-sprinklers, the heat load may be reduced by lowering the heat production level or changing the pattern of thermal production throughout the day (Gicheha, 2021). Commercial broilers' growth rate and meat yield are known to be slowed by high ambient temperatures (Zhang et al., 2017). In addition to poorer weight gain, high mortality rate, and reduced feed consumption, high temperatures negatively affect intestinal development (Rostagno, 2020). Furthermore, high temperatures disrupt broilers' acid-base balance and increase respiratory rate which can contribute to respiratory alkalosis (Scanes, 2015).

Heat stress can have a substantial influence on layer flocks, but some precautions can be done to keep hens healthy and produce eggs. For instance, the lighting schedule should be changed to provide more light hours during the colder hours of the day to promote feed consumption during cooler times of the day. In addition, when it is hot outside, it is best to lower stocking density (Reddy and Ramya, 2015; Abbas et al., 2021). High stocking rates during the hot season can lead to inadequate ventilation. Early heat conditioning also appears to be an effective method for boosting the heat tolerance of some chicken breeds (Saeed et al., 2019). Layer flocks can be kept calm by starving or fasting during hot hours (Saeed et al., 2019; Bilal et al., 2021; Shakeri and Le, 2022). Therefore, egg producers must be prepared when summer temperatures rise as egg yield will decrease and flock mortality increases (Yahav, 2015; Sinha et al., 2018).

During the chicks' first few days of life, chickens cannot regulate its heat production in response to the environmental temperature, therefore a decrease in environmental temperature leads to a reduction in body temperature (Ranjan et al., 2019). However, after 21 days, chicks start to develop additional homeothermic traits, such as the capacity to match their heat production to the

surrounding temperature, allowing them to endure the lowering effect that a decrease in ambient temperature has on their body temperature (Ranjan et al., 2019; Saeed et al., 2019). The normal body temperature of an adult chicken is 40.6-41.7^{o}C (Ranjan et al., 2019). The comfortable ambient temperature for adult poultry is 18-24^{o}C, whereas chicks require higher temperatures of around 32^{o}C in their first week of life which decreases over time (Scanes, 2015). Above 32^{o}C, poultry fails to maintain their normal internal body temperature, due to the absence of sweat glands and the presence of complete feather coverage of the body (Hu et al., 2016). When the ambient temperature rises above 24^{o}C, the internal body temperature of the chicken also rises, which causes it to consume less feed (Cassuce et al., 2013). Heat stress, panting, and prostration results at a temperature above 35^{o}C (Hu et al., 2016). When a chicken's core body temperature reaches a critical level of 47°C, sometimes known as the upper lethal temperature, chickens may die from heat prostration (Reddy and Ramya, 2015; Scanes, 2015). In laying hens, heat stress causes low egg production and an increased number of hatching egg rejects in breeder hens (Abbas et al., 2021). Heat stress is less likely similar to affect younger and lighter chicks than older and heavier chickens (Farag and Alagawany, 2018). Therefore, heat stress can be alleviated by modifying the macro and microenvironments in which chickens are kept. High humidity and high environmental temperatures adversely affect poultry production (Saeed et al., 2019; Yousaf et al., 2019).

POULTRY RESPONSES TO HEAT STRESS

The susceptibility of broiler chickens to heat stress increases as air relative humidity and ambient temperature values are above the thermal comfort zone (16-23^{o}C and 50-70% relative humidity), making it hard for birds to release heat (Gamba et al., 2015). This results in their body temperature rising, which harms their growth performance. Hot weather causes poultry to perform poorly as it results in decreased feed intake and increased water intake (Saeed et al., 2019; Rahman and Hidayat, 2020). At high temperatures, laying hens lay fewer eggs, watery eggs, and eggs with thin shells or even shell-less eggs due to lack of calcium; grow slower; and are more likely to become sick due to their compromised nutritional requirement as protein digestibility is reduced up to 9.7% (Habashy et al., 2017; Nawaz et al., 2021). In broiler chickens, decreases in growth rates, feed efficiency, immunity, and carcass quality were observed at high ambient temperatures (Dayyani and Bakhtiyari, 2013). Aswathi et al. (2019) reported a reduction in fertility percentage (-7.22%) and hatchability of fertile egg sets (-2.51%) in breeders. Heat stress has a negative effect on not just feed intake and utilization, but also carcass quality (Rath et al., 2015, Aswathi et al., 2019; Rahman and Hidayat, 2020) due to the unfavorable partitioning of metabolizable energy consumed, with a large proportion of it being stored as fat and the remainder as muscle (Rahman and Hidayat, 2020). The signs of a heat-stressed chicken include panting, extending the wings, holding the wings slightly apart from the body, standing or lying down, and closing the eyes (Dayyani and Bakhtiyari, 2013). A study by Altan et al. (2003) reported that heat stress increases fearfulness, induces oxidative stress, and initiates significant physiological responses in broiler chickens. Birds can survive a gradual increase in temperature, but a rapid increase in temperature will result in higher mortality rates (Rostagno, 2020). Figure 1 illustrates the responses of chickens to heat stress.

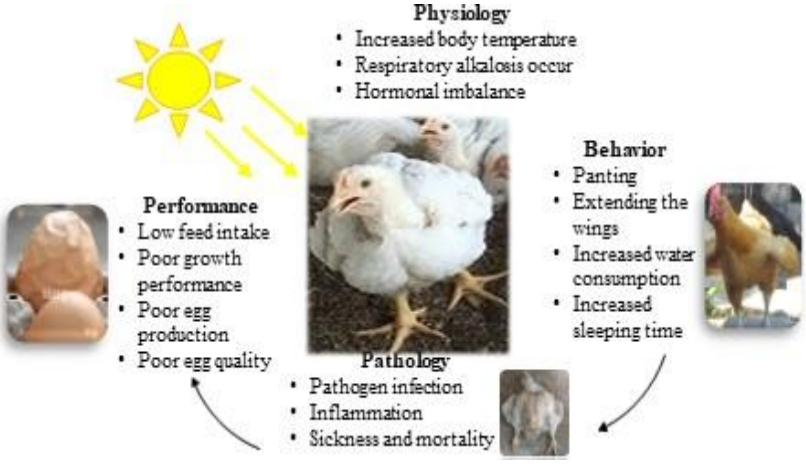


Figure 1. Poultry responses to heat stress

BIOLOGICAL CHANGES IN CHICKENS DUE TO HEAT STRESS

Heat stress in poultry results in several behavioral, physiological, and neuroendocrine changes that influence health and performance (Ahmad et al., 2022). The major physiological changes that occur in heat-stressed poultry are discussed briefly below.

Oxidative stress

Reactive oxygen species (ROS) are peroxyl radicals produced by cells during normal metabolism and are required for physiological functions such as ion transport, immunomodulation, and cytokine production (Wasti et al., 2020). Extra ROS is removed from cells through physiological detoxification processes. The Nrf2, a transcriptional factor when activated, under thermoneutral conditions, causes an increase in the production of a collection of antioxidant molecules that deal with the elevated ROS generated within the cell (Surai et al., 2019). Since the mitochondria create a significant amount of ROS, excessive mitochondrial ROS production may be a key factor in oxidative stress. Acute heat stress increases the formation of ROS from mitochondria, harming birds' skeletal muscles by oxidation (Akbarian et al., 2016). Acute heat stress causes an increase in the activity of the electron transport chain and mitochondrial substrate oxidation, which results in an excessive generation of superoxide (Akbarian et al., 2016).

Heat stress has been linked to cellular oxidative stress in chickens (Estévez, 2015; Surai et al., 2019). Down-regulation of chicken uncoupling protein exacerbates the oxidative stress situation during the later stages of acute heat stress, leading to mitochondrial malfunction and tissue damage (Mishra and Jha, 2019). Constant heat stress reduces the mitochondria's ability to create oxidative energy and consequently increases the chicken's uncoupling protein, this significantly alters the pattern of antioxidant enzyme activities, leading to the depletion of antioxidant reserves (Sahin et al., 2016). Oxidative stress has been linked to reduced growth rates, biological defects, loss of income, and severe health concerns in poultry (Estévez, 2015; Zaboli et al., 2019). While the chicken's physiology struggles to maintain thermal homeostasis, elevated ROS concentrations increase in stressful environmental situations (Sahin et al., 2016). In an effort to defend itself from the damaging effects of ROS on cells, the body undergoes an oxidative stress state and starts to manufacture and release heat shock proteins (HSP, Archana et al., 2017). Studies by Arnal and Lallès (2016) and Hao et al. (2017) have demonstrated that when exposed to heat stress, laying hens and broilers have higher *HSP70* levels.

Role of genes in heat stress

The global poultry industry has difficulty with genetic screening for high-temperature tolerant broilers (Zeferino et al. 2016). Therefore, crossing commercial chickens with strains that are highly tolerant to high temperatures can also be used to integrate heat stress resistance into the genome. The most common breeding approach for generating a commercial hybrid robust to tropical conditions and capable of producing a respectable amount of eggs and meat is a crossbreeding program between indigenous and foreign breeds (Duangjinda et al., 2017; Abd El-Hack et al., 2018).

The introduction of genetics from high-temperature tolerant strains into grandparental stock is a useful technique for speeding up the genetic advancement of commercial strains that can withstand heat stress. In chickens, heat-tolerant genes such as dwarfism (Vandana et al., 2021), naked-neck (Desta, 2021), slow/rapid feathering (Wells et al., 2012), and frizzle genes (Fathi et al., 2013; 2019), have been extensively studied. In every case, the chickens' appearance and performance indicators which include body weight gain, body weight, and feed conversion ratio (FCR), were influenced by their genes (Nawaz et al., 2021). Another downside of temperature control is immune inhibition (Goel et al., 2021). When exposed to high ambient temperature, differences in the levels of several immunological marker genes including interleukins (ILs), tumor necrosis factors (TNF), and toll-like receptors (TLRs) had a more pronounced increase in the spleen and intestine of chicks (Varasteh et al., 2015; Moraes et al., 2019).

The response of prokaryotic and eukaryotic cells to potentially harmful stimulations like heat stress induces the synthesis of stress proteins which are referred to as heat shock proteins (Efeoğlu, 2009). Many strategies, including the development of thermotolerance, modification of apoptotic and anti-apoptotic signaling pathways, and control of cellular redox conditions, are used by heat-shock proteins to provide protection against heat stress to cells (Shehaha et al., 2020). The *HSP70* and *HSP90* relate to families of HSP that are around 70 and 90 kilo Daltons, respectively (Datta et al., 2017). The *HSP70* gene is thought to protect the body from the harmful consequences of oxidative stress (Xie et al., 2015),

whereas *HSP90* engages with client proteins during the last stages of folding and changes their shape (Kumbhar et al., 2018). The *HSP70* is a chaperone polypeptide that successfully protects a variety of proteins and cell components from stress (Habashy et al., 2017; Perin et al., 2021). In chickens, Cedraz et al. (2017) found a nucleotide polymorphism in the coding area of *HSP70*. Hyperthermia causes oxidative stress and promotes the formation of ROS, resulting in the stimulation of *HSP70* expression (Robert et al. 2017).

Acid-base balance

As the ambient temperature rises, birds must release heat through panting as thermoregulation is difficult (Wasti et al., 2020). Panting is a behavior in which chickens open their beaks to increase their rate of breathing such that the respiratory tract will provide the evaporative cooling effect (Park and Kim, 2016). When panting occurs, CO_2 is excreted faster than it is produced by the cells, causing the blood's regular bicarbonate buffer system to be disrupted. Carbonic acids (H_2CO_3) and hydrogen ions (H^+) concentrations decrease when CO_2 levels are reduced (Hamm et al., 2015). On the other hand, the concentration of H_2CO_3 is raised leading to an increase in the blood pH, and the blood becomes alkaline. To cope with this situation and maintain a normal blood pH, chickens will begin to expel more H_2CO_3 and retain H^+ from the kidneys (Saeed et al., 2019). The increased H^+ disrupts the acid-base balance, resulting in respiratory alkalosis and metabolic acidosis, as well as, a reduction in poultry production (Zaboli et al., 2017).

Suppressed immune-competence

Chickens pant to expel heat and reduce body temperature, but they frequently experience instabilities in their overall energy balance because of insufficient feed consumption under heat stress (Hirakawa et al, 2020). In broilers, the weights of the main organs such as the liver and pectoral muscle do not improve as expected under the heat stress situation, in addition to impairment of broiler growth performance (Piestun et al., 2017; Hirakawa et al., 2020; Tang et al., 2022). Decreased humoral immunity is one of the most common forms of immunodeficiency in heat-stressed chickens, which might increase the risks of secondary infections that restrict vaccination efficacy (Lara and Rostagno, 2013).

Bursa of Fabricius is a fundamental immunological tissue unique to birds that are connected to the cloaca and it is necessary for B cell development and antibody competence diversification brought on by gene conversion and V(D)J recombination that causes B cell exportation to the lower limbs (Ratcliffe et al., 2014; Monson et al., 2018). Continuing heat stress accelerates the rate of the bursa of Fabricius atrophy and adds to the atrophy of the other immune components in hens intensively selected for muscle yield and growth (Jahanian and Rasouli, 2015; Campbell et al., 2019). Reduced intestinal integrity, which increases exposure to pathogens and antigenic compounds such as lipopolysaccharides (LPS), or systemic stress responses such as circulatory cytokines and acute-phase proteins, could affect the bursa of Fabricius during heat stress (Nochi et al., 2018). These factors influence the formation, survival, and motility of the bursa of Fabricius (Calefi et al., 2016).

The hypothalamic-pituitary-adrenal and sympathetic adrenal medullar axis are the main mechanisms by which the body's immune response can be altered (Herman et al., 2016; Goel et al., 2021). Neuroendocrine products of both the hypothalamic-pituitary-adrenal and sympathetic adrenal medulla axes including cortisol and catecholamines have been shown to have receptors on monocytes, lymphocytes, and granulocytes, which might affect proliferation, cytokine production, cellular trafficking, cytolytic activity, and antibody production (Bohler et al., 2021). Heat stress affects the microbiome's makeup and abundance in addition to causing oxidative stress in the gut epithelium, which impairs permeability and increases susceptibility to infection and inflammation (Cao et al., 2021).

It has been shown that broilers that have been exposed to heat stress had decreased concentrations of free circulating antibodies and specific IgG and IgM, along with lower levels of general and humoral reactivity (Van Goor et al., 2017). The weights of the bursa, thymus, liver, and spleen were also observed to be drastically lowered. Similarly, Cantet et al. (2021) reported reduced bursa weight and lymphocyte numbers in the medulla and the cortex regions of the bursa in broilers exposed to heat stress. Faud et al. (2016) also reported that heat stress was associated with a decrease in spleen and thymus size in laying chickens. Heat stress has also been shown in recent research to change the number of circulating cells (Santos et al., 2015). Due to lower quantities of circulatory lymphocytes and greater concentrations of heterophils, heat stress leads to a significant increase in the heterophil: lymphocyte ratio which is an indication of chronic stress (Santos et al., 2015; McGregor et al., 2016). Consequent to this, communicable and infectious poultry diseases such as Newcastle and infectious bursal disease become more prevalent in tropical environments throughout the summer

(Badruzzaman et al., 2015; Saelao et al., 2021). In another study, Hirakawa et al. (2020) reported lowered levels of antibodies in heat-stressed birds (Hirakawa et al., 2020).

Neuroendocrine changes

During heat stress, the neuroendocrine system is critical for the maintenance of homeostasis and proper physiological functioning in poultry (Jessop et al., 2016). The sympathetic nerves detect a rise in ambient temperature and send an impulse to the adrenal medulla (Kumari and Nath, 2018), which enhances catecholamine secretion in response to stress (Ruuskanen et al., 2021), resulting in elevated blood glucose levels, exhaustion of liver glycogen, loss of muscle glycogen, accelerated respiration rate, peripheral blood vessel vasodilation, and heightened neurological responsiveness (Kumari and Nath, 2018; Beckford et al., 2020). In response to stress, the hypothalamus releases a corticotrophin-releasing hormone (CRH), which induces the pituitary to release adrenocorticotrophic hormone (ACTH, Wasti et al., 2020). Corticosteroids are produced and released by the adrenal glands in response to ACTH (Souza et al., 2016). Corticosteroids increase plasma glucose levels by stimulating gluconeogenesis (Kumari and Nath, 2018). The thyroid thyroxine and hormones triiodothyronine are also crucial in maintaining a consistent metabolic rate by playing a pivotal role in digestion, and heart and muscle function (Cioff et al., 2013; Wasti et al., 2020).

HEAT STRESS MITIGATION STRATEGIES

The strategies to combat heat stress are categorized broadly as genetic approach, managerial practices, and nutritional manipulation.

A genetic approach to mitigate heat stress

Current developments in genetics and biotechnology may pave the way for the investigation of changes to the chicken gene to assist reduce heat stress (Cedraz et al., 2017). The increased metabolic rate of improved broiler lines makes them more sensitive to heat stress. Therefore, improving the production qualities of these breeds in hot and arid environments may require creating poultry lines that incorporate some of the genes that reduce heat stress (Wasti et al., 2020).

A single dominant autosomal gene called "naked neck" enables chicken necks to have less plumage, which helps the neck to dissipate heat (Tóth et al., 2021). In heterozygous necked neck (*Na*/*na*) and homozygous necked neck (*Na*/*Na*), the naked neck gene reduces the neck plumage cover by 20% and 40%, respectively, in comparison to normal siblings (*na*/*na*, Rajkumar et al., 2010). In broilers, the *Na* gene is associated with an increase in body weight and breast muscle, a decrease in abdominal fat, and an increase in body temperature (Wang et al., 2018). It was found that the heterophil to lymphocyte (H/L) ratio and total plasma cholesterol levels of the naked-necked chickens were much lower throughout the hot season compared to normal chickens (Wasti et al., 2020). Under high temperatures, laying hens with the bare neck gene also demonstrated improvements in egg weight, number, and quality (Azhar et al., 2019). These experiments show that it is possible to use these genes to create a breed of chicken that can withstand heat stress.

The *frizzle* (*F*) gene enables the feather's edge to curve, which decreases the feather's weight, enhances heat radiation from the body, and improves the feather's ability to act as an insulator (Nawaz et al., 2021). Relative to heterozygous carriers and regular feathered hens, laying hens with the homozygous frizzle gene had increased egg production and quality features by enhancing the extent of heat dissipation (Kumari and Nath, 2018). Except for sexual development under heat stress, there is a positive interaction between the feathering genotype (*FF*) and ambient temperature for all reproductive variables, including egg production, hatchability, and chick production (Dong et al., 2018).

In poultry, a sex-linked recessive gene called the *dwarf* gene (*dw*) causes homozygous females and males to weigh about 30% and 40% less than normal, respectively (Zerjal et al., 2013). The benefit of the *dw* gene in heat-stressed laying hens has been the subject of some debate (Wasti et al., 2020). However, Fathi et al. (2022) recommended the development and commercialization of *frizzled* and *naked-necked*, and *dwarf* genes in poultry.

Managerial practices

Housing

In the tropics, poultry houses are predominantly naturally ventilated open-sided (Alchalabi, 2013). With rising air velocity, heat loss via radiation and convection can increase significantly (Saleeva et al., 2019; Elbaz et al., 2021). Therefore, it is best to allow natural airflow from the north and south sides while also shielding birds from direct sunlight throughout the day; thus, the shed's longitudinal direction should be from east to west (Oloyo and Ojerinde, 2019). To maintain their internal temperature, poultry houses should be designed with optimal insulation (Scanes, 2015).

The roof of the poultry shed should be at a 45^{o} angle which will be able to maximize the distance of the poultry from the heat accumulated under the roof (Oloyo and Ojerinde, 2019). Furthermore, water sprinkling can keep the roof cool at high temperatures (Saeed et al., 2019). The heat that is gained or lost from the building is significantly influenced by the size, pitch, the roof's color, reflectivity, and direction, as well as, the structure's ventilation system (Wang et al., 2018). According to Saleeva et al. (2019), the reflectivity of the roof can be increased by adding an aluminum roof or painting it with metallic zinc. Evaporative cooling technologies with cooling pads and sprinklers inside the chicken house can be used in farms with extreme outside temperatures and low relative humidity (Saeed et al., 2019). Glass wool is currently used as an insulating material in the ceiling of environmentally controlled chicken houses (Alchalabi, 2013).

Stocking density is another factor that contributes to heat stress. A study by Moreki et al. (2020) in Botswana showed that the stocking density of 10-12 birds/m^2 was ideal for open-sided poultry sheds in summer. The authors concluded that broiler chicken growth performance was negatively impacted by stock densities of more than 12 birds/m^2. In another study, Gholami et al. (2020) reared broilers at four different stocking densities (10, 15, 17, and 20 birds/m^2) under hot and dry conditions and observed that the stocking density of 10 birds/m^2 resulted in lower FCR, higher body weights, weight gains, and feed intake compared to those reared at 15, 17 and 20 birds/m^2. The higher metabolic rate of chickens during the summer increases heat generation inside the poultry house and slows heat loss during hot and humid weather giving rise to an increase in the poultry house's total temperature (Nilsson et al., 2016; Donald, 2018).

The use of corrugated iron sheets and walls which are painted white to reflect heat is encouraged in sub-tropical and tropical regions (Oloyo and Ojerinde, 2019). Furthermore, grass can be used as a roofing material which can also serve as an insulation material. Sidewalls should have roll-down reinforced curtains that can be adjusted for use in cold weather and at night (Bhadauria, 2017). A sidewall's height should be between 25 and 70 cm high to allow natural airflow during the hot period as side wall curtains will be rolled down (Oloyo and Ojerinde, 2019). The open space between the sidewall and the roof gable will be closed with a 25 mm wire mesh (Alchalabi, 2013). However, as technology progresses, the use of a closed housing system for the intensification of agricultural operations has increased significantly (Donald, 2018). Climate-controlled housing systems (also referred to as closed buildings) with exhaust fans, air conditioning, cooling pads, and cool perches are beneficial in assisting chickens in dealing with the negative consequences of heat stress (Bhadauria, 2017). Closed buildings, on the other hand, are costly to construct and maintain (Glatz and Pym, 2013).

Feeding strategies

Only feeding methods can lessen heat exhaustion if the animal generates less heat or dissipates heat from the body through radiation during tunnel ventilation, where air velocity is higher. Lower heat production can be realized by a reduced heat increment, catabolism of fewer nutrients above requirements, or more efficient nutrient digestion (Barrett et al., 2019). Broiler chickens compared to laying hens appear to need more attention to feeding schedules. Many of the difficulties related to heat exhaustion in broilers can be alleviated simply by feeding at the right time (Syafwan et al., 2011; Kennedy et al., 2022). To address heat stress, coarser meals, diurnal feeding patterns, self-selection procedures, and wet feeding are all viable options. The feed should be well processed into mash, crumb, or pellets, and supplementary feeders should be available on hot days to increase appetite (Rahman and Hidayat, 2020).

The use of low-beam lights may also minimize activity, thus lessening the heat burden on the birds (Bhadauria et al., 2016). Lighting schedules are utilized for broiler chickens to control feed intake (Wu et al., 2022) and provide access to feed and water, especially during the cooler parts of the day (De Oliveira and Lara, 2016). The length of the photoperiod can be altered as an alternate strategy to enhance the well-being, immune response (Riber, 2015), and ultimately the performance of birds that are under heat stress (Parvin et al., 2014). Using low-intensity lighting when the temperature is high (for example 180 Lux) can prevent broilers from moving around and agitating, which can lead to them to be heavier (Mousa-Balabel et al., 2021; Wu et al., 2022). Mousa-Babel et al. (2021) compared the performance of broiler chickens reared at low-beam blue light intensity (5 Lux), medium blue light (20 Lux), and high blue light intensity (320 Lux) and found that broiler chickens raised under low-beam blue light intensity had significantly higher body weight, body weight gain, antibody titers against the Newcastle disease virus, and foot pad dermatitis compared to their counterparts in high blue light intensity. In addition, chickens on low-beam blue light intensity had lower activity levels and heterophil/lymphocyte ratios, and FCR.

Feeding time is a significant component in reducing the effects of heat stress on feed intake and utilization (Farghly et al., 2018, Kennedy et al., 2022). Therefore, during the time of low temperatures, for example, in the early hours of the day and late evening, a significant portion of the feed should be supplied to the poultry, with the remaining amount available *ad libitum*. According to Daghir (2009), chickens that are feed-starved produce less heat than those that are fed; hence removing feed on hot days has some ameliorating benefits on performance. Farghly et al. (2018) reported that feed withdrawal involves alterations in intestinal morphology and depletion of intestinal mucosa due to fasting which may damage the intestinal cells.

A study by Zaboli et al. (2019) reported that a rise in the room temperature from 21.1°C to 32.2°C leads to a decline in feed intake of around 9.5% /bird/day from 1 to 6 weeks of age. In another study, He et al. (2019) reported that a rise in environmental temperature from 32.2°C to 37.8°C results in a 9.9% decrease in feed intake per bird/day. It is, however, not recommended to allow birds to go for a long time without a feed as this will have an impact on growth and may increase skin scratches at feeding time resulting in downgraded carcasses (Suganya et al., 2015; Vandana et al., 2021).

The form in which the feed is presented to the birds affects the consumption of poultry exposed to high environmental temperatures. In warmer conditions, poultry, particularly broilers, prefer eating larger particles (Ranjan et al., 2019; Massuquetto et al., 2020). According to Smalling et al. (2019), when broilers are fed pelleted feed, the energy required for feeding is reduced by 67%, allowing that energy to be channeled toward more productive applications. Khalil et al. (2021) reported that feeding pellets to laying hens during high ambient temperatures contributes to higher feed efficiency, egg production, and water intake compared to mash feeding. The physical feature of the pellets enables the birds to ingest feed with less wasted energy, therefore the pellets' quality and durability are extremely important. The FCR can be altered by 0.01 points if the pelleted feed contains 10% fine particles (Ahmed and Abbas, 2013).

The quantity of coarse particles in droppings is adversely correlated to the water in the droppings. The higher retention duration of coarse particles inside the gastrointestinal tract (GIT) is responsible for this association (Smalling et al., 2019; Abdel-Moneim et al., 2021). In comparison to fine diets, coarse diets can enable more retention of water from GIT (Smalling et al., 2019) and this may aid to release the heat burden. More heat loss by evaporative cooling, on the other hand, emphasizes the importance of increased water intake in heat-stressed birds (Lara and Rostagno, 2013). Therefore, the provision of high-physical-quality feed will minimize energy expended and heat generated during feeding (Mir et al., 2018).

Choice feeding encourages chickens to select a meal and reduce the heat burden associated with the metabolic process in hot environments. It could also help the chickens to better match their nutrient intake to their needs. When given a choice of diet, chickens are reported to select a variety of food items to meet their nutrient needs (Sinha et al., 2018). It has been observed that chickens that are choice fed choose feed ingredients with lower heat increments to minimize excess heat during the harshest times of the day, thus enhancing their heat tolerance (Diarra et al., 2014). Suganya et al. (2015) reported that choice-fed broilers ingest less protein and much more energy at high temperatures than those feeding on a complete diet, presumably to limit body heat output from protein-high heat increment. Similarly, De Almeida et al. (2012) observed that when Japanese quails were kept at temperatures ranging from 20 to 35^{o}C, they chose to eat more calories and less protein when given a choice diet vs. a single complete diet.

Diet management changes, including rehydrating feed, have long been known to improve poultry performance (Rahman and Hidayat, 2020). Relative to broiler chickens consuming dry feed, this technique enhances weight gain, feed intake, FCR, and the weight of the gut in broilers at ordinary temperatures (Kaldhusdal et al., 2016; Rostagno., 2020). In another study, it was reported that even though the weight of the digesta across the entire digestive system of chickens was lower while the feed intake was higher, wet feeding has been associated with a quicker rate of passage through the gut (Calefi et al., 2016).

A previous study by Calefi et al. (2014) reported advancements in digestive efficiency which were assumed to be due to a higher empty weight, a longer gut length, and greater gut wall thickness in some areas of the digestive tract with wet feeding. Farghly et al. (2018) and Kadykalo et al. (2018) observed that wet feeding increased the ingesta's fluidity, possibly indicating a faster digesta transit rate. Additionally, a thicker intestinal wall could help with digestion. Farghly et al. (2018), compared rehydrating to dry feed and found that rehydrating feed lowers digesta fluidity to a similar degree and promotes pre-digestion and absorption, presumably due to faster digestion enzyme penetration into feed particles. This may result in increased nutrient digestibility. In addition,

external enzyme inclusion in the wet feed may have an additional potential influence on absorption, since they may promote substrate accessibility for enzymes, hence, increasing nutrient absorption (Holtmann et al., 2017). Saleh et al. (2021) reported that wet feeding may improve performance because it increases dry matter (DM) intake at high temperatures. Egg weight and egg production could be boosted in this manner under high temperatures. Waiz et al. (2016) observed that compared to dry feeding, moistening laying hen's feed at a 1:1 (feed: water) ratio in hot environments improves laying performance. High performance in hot conditions is predominantly caused by an increase in DM intake on wet feed, which enhances the intake of micronutrients (Afsharmanesh et al., 2016).

At high temperatures birds eat less, thus failing to meet their nutrient needs (Rath et al., 2015). Therefore, heat stress can be alleviated by increasing the nutrient density of the diet. During summer, especially for broiler hens, adding fat to the diet should be taken into consideration to keep their daily energy requirement in line with their needs for growth (Diarra and Tabuaciri, 2014; Teyssier et al., 2022). Due to fat's lower heat increment when compared to alternative energy sources like carbohydrates or proteins, the inclusion of fat in diets for broilers that are under heat stress improves their feed intake and performance (Rath et al., 2015; Pursey et al., 2017). However, to achieve a balanced meal and hence optimize utilization, the content of other nutrients, notably proteins, must be appropriately adjusted whenever the energy density is raised by added fat (Rahman and Hidayat, 2020). Heat-stressed chickens have a strong urge to reduce feed intake to lower their body temperature (Wasti et al., 2020). Low-digestible energy and protein-rich diets are favorable when heat stress is moderate (Lemme et al., 2019). In addition, it was reported that fat in the diet increases nutrient utilization by slowing feed passage through the GIT (Jha and Mishra, 2021).

According to a previous study, polyunsaturated fatty acid-rich fat sources, such as soybean oil, fish oil, canola oil, flaxseed oil, and walnuts must be avoided or be used in moderation, indicating that caution must be exercised when choosing a fat source to include in a diet (Seifi et al., 2020). According to Surai et al. (2019), such sources are deficient in antioxidants and are vulnerable to oxidative rancidity, which results in the degradation of vitamins A and E and the taste of poultry meat being altered. Moreover, soybean oil has a high concentration of polyunsaturated fatty acids that frequently result in the creation of excess visceral and breast intramuscular fat, which lowers the quality of the carcass (Abdel-Moneim et al., 2021). However, if the energy density of the diet is to be increased, the levels of all nutrients must be adjusted to maintain optimal intake (Pawar et al., 2016).

It was previously noted, poultry limit feed consumption in hot weather which results in nutrient deficiencies (Teyssier et al., 2022). Due to a reduction in consumption, there is a decrease in the intake of essential nutrients, such as protein, essential amino acids, minerals, and vitamins (Rath et al., 2015). In this case, it is preferable to improve and balance vital amino acids because increasing protein levels can increase heat production during protein metabolism (Teyssier et al., 2022). Bird performance is unaffected even if the diet is lacking in protein but contains a balanced amino acid content (Kumar et al., 2016). If protein levels must be increased, vegetable-derived proteins such as soy, sesame, and sunflower are excellent choices since animal-source proteins will produce more heat during metabolism (Tari et al., 2020). Vegetable proteins are rich in arginine, an essential amino acid required during heat stress. Dao et al. (2021) reported that the role of arginine aids in protein synthesis and immunity. At the macrophage level, arginine is transformed into nitric oxide (Rath et al., 2014), a mediating component in vasodilation and increased peripheral blood flow which are significant thermoregulatory responses to heat stress (Liu et al., 2019).

When feed intake is lowered due to heat stress, it was normally advised that dietary protein levels be raised to maintain a steady protein intake (Liu et al., 2019). However, studies conducted over time suggest that birds under heat stress may not always require more protein (Suganya et al., 2015). A recent study reported that feeding broilers high-protein diets at high environmental temperatures result in their growth being inhibited (Qaid and AlGaradi, 2021). It was indicated that hens' growth performance at 3 to 6 weeks of age under hot temperatures of 32^{o}C was not improved by raising protein content from 17 to 23% (Awad et al., 2019). This was primarily caused by the increased nitrogen excretion and reduced efficacy of the high-protein diet compared to the low-protein diet (Kidd et al., 2021). Previous investigations demonstrated that higher body heat generation due to increased feed intake contributed to poor performance (Diarra et al., 2014). The mentioned authors reported that birds on low-protein diets consumed more protein, possibly due to a physiological shift that allows them to use the protein more efficiently when it is scarce. On the other hand, prolonged heat-stress exposure affects the reaction of poultry to dietary protein levels, therefore lowering crude

protein levels as a strategy for mitigating heat stress is not justified (Bohler et al., 2021).

In a separate study, it was found that using protein sources that provide the appropriate amounts and proportions of methionine and lysine can lower 2-4% of dietary protein without compromising weight increase or feed conversion (Attia et al., 2020). It was reported that adding 0.05% methionine to water boosted feed efficiency considerably in heat-stressed chickens (Cadirci and Koncagul, 2014). Any loss in amino acids will result in their insufficiency, making protein non-ideal irrespective of the protein amount (Kumar et al., 2016). Therefore, supplementing low-protein meals with essential amino acids has been shown to help heat-stressed chickens perform better (Lemme et al., 2019). Heat intensity and duration, breed, age of birds, the quantity of amino acid supplementation, and diet composition could all influence how heat-stressed chicken responds to low-protein diets. Under hyper thermoneutral conditions compared to thermoneutral conditions, the total sulfur amino acids (TSAA) demand would be higher (Babazadeh and Ahmadi, 2022). In addition, it takes more TSAA to attain optimal growth performance when broiler chicks are raised at high temperatures (Del Vesco et al., 2013; Zarghi et al., 2020). When adding methionine supplements, factors such as age and production parameters must be considered to mitigate the harmful effects of heat stress (de Freitas Dionizio et al., 2021). Supplementing with methionine is also useful for lowering immunological stress and can change how the immune system responds (Pacheco et al., 2018).

Feed as a source of calcium carbonate

Calcium is supplied to commercial breeders in many ways which include using grower diets that contain 0.9 to 1.0% calcium supplemented with up to 5% egg production or using classical pre-breeder diets that allow for the development of greater medullary bone reservoirs without using the diets that contain 2-2.5% calcium (Bryden et al., 2021). Heat stress causes poultry to consume less than 3.5 grams of calcium each day (Abbas et al., 2021). In addition, heat stress decreases the production of calbindin, a calcium-binding protein required for calcium absorption in the intestine (Ebeid et al., 2012). Ranjan et al., (2019) it is reported that feeding laying hens in the evening improves their laying rate and eggshell quality by increasing calcium intake. A decrease in egg production is directly linked to a reduction in calcium intake (Bryden et al., 2021).

During heat stress, reduced calcium intake and poor absorption result in lower plasma calcium levels, leading to less calcium being available for eggshell formation in laying hens. This results in lower egg output, smaller eggs, or thin-shelled eggs, and poor skeletal development, causing economic losses to producers (Allahverdi et al., 2013; Ventura and Matias da Silva, 2019). As poultry's DM intake is already low due to heat stress, adding large amounts of calcium supplements may not be viable. However, a larger particle-size calcium source including limestone or oyster shells is retained in the gizzard for a longer period and is released slowly into the duodenum for eventual absorption into circulation (Mir et al., 2018).

Electrolytes and vitamins

The main causes of poor performance in heat-stressed chickens have been identified by the alteration of the acid-base balance and lowered feed intake (Sugiharto et al., 2017). The minerals potassium (K), sodium (Na), and chlorine (Cl) are essential for maintaining the acid-base balance of bodily fluids (Popoola et al., 2019). As a result, adding minerals such as ammonium chloride (NH_4Cl), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), potassium chloride (KCl), and potassium sulfate (K_2SO_4) to the diet or drinking water of heat-stressed chickens will assist to mitigate the negative effects of heat stress (Diarra and Tabuaciri, 2014; Pawar et al., 2016).

At high ambient temperatures water intake increases while feed intake decreases. Chickens drink four times more at 38°C compared to 21°C (Orakpoghenor et al., 2020), indicating that water must be available all the time during this period. Increased water intake, which improves heat dissipation and cools down the body provides relief from the detrimental effects of heat exhaustion by supplementing the drinking water with Na+, K+, and Cl^- salts (Gamba et al., 2015). Bryden et al. (2021) found that heat-stressed laying hens treated with 0.5% hydrochloric acid in drinking water had significant gains in egg production and egg quality. Gamba et al. (2015) observed that excreta and litter moisture rise due to increased water intake caused by elevated Na^+ and K^+ levels.

In another study, Cherian (2015) found that supplementing drinking water with vitamins A, D, E, and B complex increased broiler performance and immune function. Additionally, supplementation of vitamin C (ascorbic acid) has been found to improve performance through improved feed consumption and nutritional intake in heat-stressed birds. Furthermore, Asensio et al. (2020) observed that supplementing broilers with ascorbic acid enhanced the weight and protein content of the carcass

while lowering carcass fat content. Daghir (2009) recommended 1 g of vitamin C per liter of drinking water and 20 mg per liter of water for broilers and laying hens, respectively. A study by Wang et al. (2011) in laying hens found that vitamin C does not affect egg weight or egg production. However, Skřivan et al. (2013) reported that 50 and 100 mg/kg vitamin C supplementation significantly increased fertility and hen-day egg production of broiler breeders.

Since poultry cannot synthesize vitamin E, they must be supplemented (Attia et al., 2016). The hormone levels of catecholamine and corticosterone rise in response to stress, particularly heat stress, and lipid peroxidation in cell membranes begins (Abd El-Hack et al., 2018). Vitamin E has also been proven to safeguard macrophages, lymphocytes, and plasma cells from oxidative stress while also enhancing their viability, propagation, and functionality (Shakeri et al., 2020). Therefore, supplementing with vitamin E in the diet during times of stress improves the immunological response of poultry. According to new research, adding vitamin E at a dosage of 250 mg/kg to broiler chickens is a viable protective approach for reducing the severity of heat stress and it may result in optimal performance and enhanced meat quality (Saeed et al., 2019). For layers, however, the dosage is 125-250 mg/kg has been found to result in an improved immunological response, egg production, and feed utilization (Shakeri et al., 2020). Heat stress raises the levels of malondialdehyde in the blood and liver, whereas vitamin E inhibits the formation of malondialdehyde in the liver by preventing lipid peroxidation and cell damage (McDowell, 2012), resulting in improved chicken performance.

Supply of cool water

Water consumption and balance are linked to evaporative heat dissipation and calories dissipated every breath (Chikumba and Chimonyo, 2013; Abdel-Moneim et al., 2021). Reduced water temperature encourages water consumption, which increases evaporative cooling and heat dissipation for each breath (McCreery, 2015). Furthermore, a 20% water consumption increase can result in a 30% increase in heat loss in each breath, with a corresponding performance improvement (Abdel-Moneim et al., 2021).

Water temperature, height, and the shape of drinkers affect poultry performance during heat stress (Orakpoghenor et al., 2020). Water consumption is high in nipple drinkers that are slightly above chick eye than in lower nipple drinkers as chickens find it difficult to bend down and drink from lower nipples (Quilumba et al., 2015; Ranjan et al., 2019). Daghir (2009) recommended the use of wider and deeper drinkers during heat stress as they will permit immersion of not only the beak but the whole face and help dissipate more heat. Cool water at 10-12^{o}C is helpful to poultry, therefore, there is a need to protect water tanks and pipes from the direct sun because birds will not drink warm water (Park et al., 2015). Poultry should always have access to cool, clean water that is below 25^{o}C and has ice in it so that their body temperatures can remain steady during times of heat stress (Park et al., 2015).

Use of phytochemicals in mitigating heat stress

To reduce heat stress in poultry, various phytochemical supplements have been added to the diet.

Resveratrol

Natural bioactive polyphenols called resveratrol are mostly found in peanuts, grapes, turmeric, and berries (Saeed et al., 2017). Resveratrol supplementation (400 mg/kg of feed) has been found in previous studies to boost the antioxidant capacity in broilers under heat stress (Hu et al., 2019). In yellow-feather broilers under heat stress, resveratrol supplementation at 300 or 500 mg/kg of feed increased average daily growth, decreased rectal temperature, and decreased the levels of adrenocorticotropin hormone, malondialdehyde (MDA), corticosterone, and cholesterol (He et al., 2019). Resveratrol supplementation of 200 mg/kg of feed increased egg production in laying hens, whereas resveratrol supplementation of 400 mg/kg of feed decreased total blood cholesterol and triglycerides, decreased egg cholesterol content, increased antioxidant activity, and increased egg sensory scores (Zhang et al., 2017).

Lycopene

The carotenoid lycopene, which is mostly present in tomatoes and tomato-based products, is known to increase the synthesis of antioxidant enzymes by activating the DNA's antioxidant response element (Wasti et al., 2020). Heat-stressed broilers' total feed intake, weight gain, and FCR were all improved when lycopene (200 or 400 mg/kg of feed) was added (Sahin et al., 2016). Lycopene has been reported to increase the levels of antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in broilers (Arain et al., 2018). Lycopene administration increased vitamin levels,

improved oxidative stability, and the yolk color of eggs in laying hens (Sahin et al., 2016; Arain et al., 2018).

Epigallocatechin gallate

Green tea extract contains the polyphenol epigallocatechin gallate (EGCG), which has strong anti-inflammatory and antioxidant effects (Hu et al., 2019). When Luo et al. (2018) fed heat-stressed broiler birds at three EGCG dosages (0, 300, and 600 mg/kg), they observed a linear increase in feed intake, body weight, and levels of blood total protein, glucose, and alkaline phosphatase activity. In a related study, Xue et al. (2017) found that feeding EGCG improved body weight and antioxidant enzyme levels (catalase, GSH-Px, and SOD) in heat-stressed broiler chicks' liver and serum.

Curcumin

The main polyphenols extracted from turmeric are called curcumin, which has anti-inflammatory and antioxidant properties (Attia et al., 2017; Wasti et al., 2020). Even though curcumin is easily absorbed by animals, more recent studies have concentrated on its potential application as a compound to reduce heat stress in chickens (Wasti et al., 2020). It was reported that adding curcumin to feed at a rate of 100 mg/kg significantly increased broiler body weight during heat stress (Zhang et al., 2017). Furthermore, the inclusion of 150 mg/kg of curcumin in the diet of laying hens enhanced egg quality, laying efficiency, antioxidant enzyme activity, and immunological response to heat stress (Liu et al., 2020).

Mitigation of heat stress by use of probiotics and betaine

Betaine is widely distributed in plants, animals, microbes, and its rich food sources include fish, spinach, and wheat bran (Saeed et al., 2017). Betaine plays an essential role in sustaining the basic functions of poultry, including osmoregulation, fat distribution, methionine sparing, immunity, and the bird's ability to withstand heat stress (Attia et al., 2016; Saeed et al., 2019). The performance of chickens kept under heat stress can be greatly improved by including betaine in their diets (Hao et al., 2017; Saeed et al., 2017). In addition, betaine also functions as a methyl donor, which enables feed cost reductions by substituting methionine and choline supplements (Gholami et al., 2015). Betaine supports a variety of intestinal bacteria in their defense against osmotic changes, improving microbial fermentation activity (Abd El-Ghany and Babazadeh, 2022).

The term "probiotics" refers to feed additives that contain live beneficial microorganisms such as Bifidobacterium, Streptococci, and Lactobacillus, yeast cultures with Saccharomyces and candida strains, and fungi (*Aspergillus awamori*, *A. niger*, and *A. oryza*), which may improve poultry performance, intestinal microbiota, and immune system (Abd El-Hack, et al., 2018; El-Moneim et al., 2020). Probiotics have received a lot of attention lately for reducing the oxidative damage brought about by heat stress in chickens (Ahmad et al., 2022). It has been shown that the addition of probiotics to the diet of broilers increased their growth performance, FCR, and immunological response (Wang et al., 2018).

A symbiotic relationship occurs when prebiotics and probiotics are combined to have a positive effect on poultry raised in hot environments (Lara and Rostagno, 2013). It has been suggested that incorporating synbiotics in the diet may benefit chickens kept in areas that experience high levels of heat stress by minimizing the negative effects of heat and possibly improving their welfare and performance (Mohammed et al., 2018). Probiotic supplements have been shown to have favorable benefits on the health and productivity of chickens in tropical climates (Ahossi et al., 2016; Deraz, 2018). It was reported that the performance, intestinal morphology, and immunological response of heat-stressed chickens were all improved by consuming mannan-oligosaccharides, prebiotics, and a probiotic combination (Jahromi et al., 2015).

CONCLUSION

Heat stress has a negative impact on the health and productivity of poultry and is a significant challenge in poultry production in the tropics. Heat stress results from a combination of many factors including high ambient temperature, radiant heat, humidity, and airspeed. Due to heat stress many behavioral, neuroendocrinal, and physiological changes occur. Gene screening for higher growth rates to meet the ever-increasing food requirement has made poultry susceptible to heat stress. In birds raised for egg and meat production, an increase in the ambient temperature induces decreases in body weight gain, feed intake, eggshell weight, higher FCR, and increases in body temperature. These negative effects can be addressed by strategic managerial enhancements. Several approaches are used worldwide to combat the severe impacts of heat stress, including the selection of rearing systems with better ventilation, suitable housing conditions, and recommended correct stocking densities, all of which are crucial for enhancing performance at high temperatures.

Given that there is no single strategy for heat stress, a variety of strategies will help to reduce it. Further research on new innovative strategies which include utilizing heat tolerance genes and selecting genotypes with higher heat tolerance using genetic markers should be carried out.

DECLARATIONS

Acknowledgments

The researchers would like to thank the reviewers for their constructive criticisms that helped to improve the quality of this manuscript.

Funding

None.

Authors' contributions

Shame Bhawa and John Cassius Moreki conceptualized this study. Shame Bhawa surveyed the literature, and drafted and revised the manuscript while John Moreki edited and suggested changes to the manuscript. James Butti Machete also surveyed and played a part in drafting the manuscript. All authors checked and approved the final version of the manuscript for publication in this journal.

Competing interests

The authors declare no existence of competing for interests.

Ethical considerations

The authors have examined ethical issues, such as plagiarism, permissions to publish, misconduct, and duplicate publishing.

REFERENCES

Abbas G, Arshad M, Tanveer A, Jabbar M, Mahmood A, Khan M, Konca Y, Sultan Z, Qureshi R, Iqbal A et al. (2021). Combating heat stress in laying hens a review. Pakistan Journal of Science, 73(4): 633-655. Available at: http://pjosr.com/index.php/pjs/article/view/365/211

Abd El-Ghany WA and Babazadeh D (2022). Betaine: A potential nutritional metabolite in the poultry industry. Animals, 12(19): 2624. DOI: https://www.doi.org/10.3390/ani12192624

Abd El-Hack A, Mohamed E, Alagawany M, and Noreldin AE (2018). Managerial and nutritional trends to mitigate heat stress risks in poultry farms. In: A. Negm and M. Abu-hashim (Editros). Sustainability of agricultural environment in Egypt: Part II. The handbook of environmental chemistry. Springer., Cham. 77: 325-338. DOI: https://www.doi.org/10.1007/698_2018_290

Abdel-Moneim AE, Shehata AM, Khidr RE, Paswan VK, Ibrahim NS, El-Ghoul AA, Aldhumri SA, Gabr SA, Mesalam NM, Elbaz AM et al. (2021). Nutritional manipulation to combat heat stress in poultry – A comprehensive review. Journal of Thermal Biology, 98: 102915. DOI: https://www.doi.org/10.1016/j.jtherbio.2021.102915

Adu-Asiamah P, Zhang Y, Amoah K, Leng QY, Zheng JH, Yang H, Zhang WL, and Zhang L (2021). Evaluation of physiological and molecular responses to acute heat stress in two chicken breeds. Animal, 15(2): 100106. DOI: https://www.doi.org/10.1016/j.animal.2020.100106

Afsal A, Sejian V, Bagath M, Devaraj C, and Bhatta R (2018). Heat stress and livestock adaptation: Neuro-endocrine regulation. International Journal of Veterinary and Animal Medicine, 1(2): 1-7. DOI: https://www.doi.org/10.31021/ijvam.20181108

Afsharmanesh M, Lofti M, and Mehdipour Z (2016). Effects of wet feeding and early feed restriction on blood parameters and growth performance of broiler chickens. Animal Nutrition, 2(3): 168-172. DOI: https://www.doi.org/10.1016/j.aninu.2016.04.002

Ahmad R, Yu YH, Hsiao FSH, Su CH, Liu HC, Tobin I, Zhang G, and Cheng YH (2022). Influence of heat stress on poultry growth performance, intestinal inflammation, and immune function and potential mitigation by probiotics. Animals, 12(7): 2297. DOI: https://www.doi.org/10.3390/ani12172297

Ahmed ME and Abbas TE (2013). The effect of feeding pellets versus mash on performance and carcass characteristics of broiler chicks. Bulletin of Environment, Pharmacology and Life Sciences, 2(2): 31-34. Available at: https://bepls.com/jan_2013/8.pdf

Ahossi PK, Dougnon JT, Kiki PS, and Houessionon JM (2016). Effects of Tridax procumbens powder on zootechnical, biochemical parameters and carcass characteristics of Hubbard broiler chicken. Journal of Animal Health and Production, 4(1): 15-21. Available at: http://www.nexusacademicpublishers.com/uploads/files/JAHP_MH20151110121122_%20Ahossi%20et%20al.pdf

Akbarian A, Michiels J, Degroote J, Majdeddin M, Golian A, and De Smet S (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunctionand dietary interventions with phytochemicals. Journal of Animal Science and Biotechnology, 7: 37. Available at: https://jasbsci.biomedcentral.com/articles/10.1186/s40104-016-0097-5?report=reader

Alchalabi AD (2013). Poultry housing design. Chapter 3. Pp. 1-8. DOI: https://www.doi.org/10.13140/2.1.2729.7280

Allahverdi A, Feizi A, Takhtfooladi HA, and Nikpiran H (2013). Effects of heat stress on acid-base imbalance, plasma calcium concentration, egg production and egg quality in commercial layers. Global Veterinaria, 10(2): 203-207.

Altan O, Pabuccuoglu A, Altan A, Konyalioglu S, and Bayraktar H (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. British Poultry Science, 44: 545-550. DOI: doi:https://www.doi.org/10.1080/00071660310001618334

Alverdy JC and Luo JN (2017). The influence of host stress on the mechanism of infection: Lost microbiomes, emergent pathobiomes, and the role of interkingdom signalling. Frontiers in Microbiology, 8: 322. DOI: https://www.doi.org/10.3389/fmicb.2017.00322

Arain MA, Mei Z, Hassan FU, Saeed M, Alagawany M, Shar AH, and Rajput IR (2018). Lycopene: A natural antioxidant for prevention of heat-induced oxidative stress in poultry. World's Poultry Science Journal, 74(1): 89-100. DOI: https://www.doi.org/10.1017/S0043933917001040

Archana PR, Aleena J, Pragna P, Vidya MK, Abdul Niyas PA, Bagath M, Krishnan G, Manimaran A, Beena V, Kurien EK et al. (2017). Role of heat shock proteins in livestock adaptation to heat stress. Journal of Dairy Veterinary and Animal Research, 5(1): 13-19. DOI: https://www.doi.org/10.15406/jdvar.2017.05.00127

Arnal M E and Lallès JP (2016). Gut epithelial inducible heat-shock proteins and their modulation by diet and the microbiota. Nutrition Reviews, 74(3): 181-197. DOI: https://www.doi.org/10.1093/nutrit/nuv104

Asensio X, Abdelli N, Piedrafita J, Soler MD, and Barroeta AC (2020). Effect of fibrous diet and vitamin C inclusion on uniformity, carcass traits, skeletal strength, and behaviour of broiler breeder pullets. Poultry Science, 99(5): 2633-2644. DOI: https://www.doi.org/10.1016/j.psj.2020.01.015

Aswathi PB, Bhanja SK, Kumar P, Shyamkumar TS, Mehra M, Bhaisare DB, and Rath PK (2019). Effect of acute heat stress on the physiological and reproductive parameters of broiler breeder hens–A study under controlled thermal stress. Indian Journal of Animal Research, 53(9): 1150-1155. Available at:

https://arccjournals.com/journal/indian-journal-of-animal-research/B-3641

Attia YA, Abd El-Hamid AEHE, Abedalla AA, Berika MA, Al-Harthi MA, Kucuk O, Sahin K, and Abou-Shehema BM (2016). Laying performance, digestibility and plasma hormones in laying hens exposed to chronic heat stress as an ected by betaine, vitamin C, and/or vitamin E supplementation. SpringerPlus, 5: 1619. DOI: https://www.doi.org/10.1186%2Fs40064-016-3304-0

Attia YA, Al-Harthi MA, and Hassan SS (2017). Turmeric (Curcuma longa Linn.) as a phytogenic growth promoter alternative for antibiotic and comparable to mannan oligosaccharides for broiler chicks. Revista Mexicana de Ciencias Pecuarias, 8: 11-21. DOI: https://www.doi.org/10.22319/rmcp.v8i1.4309

Attia YA, Bovera F, Wang J, Al-Harthi MA, and Kim WK (2020). Multiple amino acid supplementations to low- protein diets: Effect on performance, carcass yield, meat quality and nitrogen excretion of g finishing broilers under hot climate conditions. Animals, 10(6): 973. DOI: https://www.doi.org/10.3390/ani10060973

Awad E, Zulkifli I, Soleimani A, Law F, Ramiah S, Yousif MIM, Hussein EA, and Khalil ES (2019). Response of broilers to reduced protein diets under heat stress conditions. World's Poultry Science Journal, 75(4): 583-598. DOI: https://www.doi.org/10.1017/S0043933919000576

Azhar M, Mahmud A, Usman M, Javed K, Ishaq H, Mehmood S, Ahmad S, Hussain J, Ghayas A, and Abbas M (2019). Effect of breeder age on the progeny performance of three naked-neck chicken phenotypes. Brazilian Journal of Poultry Science, 21(3): 1-6. DOI: https://www.doi.org/10.1590/1806-9061-2018-0729

Babazadeh D and Ahmadi Simab P (2022). Methionine in poultry nutrition: A review. Journal of World's Poultry Science, 1(1): 1-11. Available at: https://jwps.rovedar.com/article_138282.html

Badruzzaman ATM, Noor M, AL Mamun M, Husna A, Islam KM, and Rahman MM (2015). Prevalence of diseases in commercial chickens at Sylhet division of Bangladesh. International Clinical Pathology Journal, 1(5): 104-108. DOI: https://www.doi.org/10.15406/icpjl.2015.01.00023

Barrett NW, Rowland K, Schmidt CJ, Lamont SJ, Rothschild MF, Ashwell CM, and Persia ME (2019). Effects of acute and chronic heat stress on the performance, egg quality, body temperature, and blood gas parameters of laying hens. Poultry Science, 98(12): 6684-6692. DOI: https://www.doi.org/10.3382/ps/pez541

Beckford RC, Ellestad LE, Weglarz MP, Farley L, Brady K, Angel R, Liu HC, and Porter TE (2020). Effects of heat stress on performance, blood chemistry, and hypothalamic and pituitary mRNA expression in broiler chickens. Poultry Science, 99(12): 6317-6325. DOI: https://www.doi.org/10.1016/j.psj.2020.09.052

Bernabucci U (2019). Climate change: Impact on livestock and how can we adapt. Animal Frontiers, 9(1): 3-5. DOI: https://www.doi.org/10.1093/af/vfz022

Bhadauria P (2017). Different types of poultry housing system for tropical climate. Indian Council of Agricultural Research, pp. 1-68.

Bhadauria P, Keshava MP, Murai A, and Jadoun YS (2016). Management of heat stress in poultry production system. ICAR Agricultural Technology Application Research Institute, Zone-1, Ludhiana 141: 4.

Bilal RM, Hassan F, Farag RM, Nasir TA, Ragni M, Mahgoub HAM, and Alagawany M (2021). Thermal stress and high stocking densities in poultry farms: Potential effects and mitigation strategies. Journal of Thermal Biology, 99: 102944. DOI: https://www.doi.org/10.1016/j.jtherbio.2021.102944

Bohler MW, Chowdhury VS, Cline MA, and Gilbert ERR (2021). Heat stress responses in birds: A review of the neural components. Biology, 10(11): 1095. DOI: https://www.doi.org/10.3390/biology10111095

Bryden WL, Li X, Ruhnke I, Zhang D, and Shini S (2021). Nutrition, feeding and laying hen welfare. Animal Production Science, 61(10): 893-914. DOI: https://www.doi.org/10.1071/AN20396

Cadirci S and Koncagul S (2014). Possible effects of delivering methionine to broilers in drinking water at constant low and high environmental temperatures. Italian Journal of Animal Science, 13(1): 3013. DOI: https://www.doi.org/10.4081/ijas.2014.3013

Calefi AS, de Siqueira A, Namazu LB, Costola-de-Souza C, Honda BBT, Ferreira AJP, Quinteiro-Filho W, Fonseca JGS, and Palermo-Neto J (2016). Effects of heat stress on the formation of splenic germinal centres and immunoglobulins in broilers infected by Clostridium perfringens type A. Veterinary Immunology and Immunopathology, 171: 38-46. DOI: https://www.doi.org/10.1016/j.vetimm.2016.02.004

Calefi AS, Honda BT, Costola-de-Souza C, de Siqueira A, Namazu LB, Quinteiro-Filho WM, Fonseca JG, Aloia TP, Ferreira AJP, and Palermo-Neto J (2014). Effects of long term heat stress in an experimental model of avian necrotic enteritis. Poultry Science, 93(6): 1344-1353. DOI: https://www.doi.org/10.3382/ps.2013-03829

Campbell ZA, Otieno L, Shirima GM, Marsh TL, and Palmer GH (2019). Drivers of vaccination preferences to protect a low-value livestock resource: Willingness to pay for Newcastle disease vaccines by smallholder households. Vaccine, 37(1): 11-8. DOI: https://www.doi.org/10.1016/j.vaccine.2018.11.058

Cantet JM, Yu Z, and Ríus AG (2021). Heat stress- mediated activation of immune–inflammatory pathways. Antibiotic, 10(11): 1285. DOI: https://www.doi.org/10.3390/antibiotics10111285

Cao C, Chowdhury VS, Cline MA, and Gilbert ER (2021). The microbiota-gut-brain axis during heat stress in chickens: A review. Frontiers in Physiology, 12: 752265. DOI: https://www.doi.ord/10.3389/fphys.2021.752265

Cassuce D, Tinôco I, Baêta F, Zolnier S, Cecon P, and Vieira MF (2013). Thermal comfort temperature update for broiler chickens up to 21 days of age. Engenharia Agrícola, 33(1): 28-36. DOI: https://www.doi.org/10.1590/S0100-69162013000100004

Cedraz H, Gromboni JGG, Garcia AAP Junior, Farias Filho RV, Souza TM, de Oliveira ER, de Oliverira EB, do uscimento ES, Meneghetti C, and Wanceslau AA (2017). Heat stress induces expression of HSP genes in genetically divergent chickens. PloS ONE, 12(10): e0186083. DOI: https://www.doi.org/10.1371/journal.pone.0186083

Cherian G (2015). Nutrition and metabolism in poultry: Role of lipids in the early diet. Journal of Animal Science and Biotechnology, 6: 28. DOI: https://www.doi.org/10.1186/s40104-015-0029-9

Chikumba N and Chimonyo M (2013). Effects of water restriction on the growth performance, carcass characteristics and organ weights of Naked Neck and Ovambo chickens of Southern Africa. Asian-Australasian Journal of Animal Sciences, 27(7): 974-980. DOI: https://www.doi.org/10.5713%2Fajas.2013.13383

Cioffi F, Senese R, Lanni A, and Goglia F (2013). Thyroid hormones and mitochondria: with a brief look at derivatives and analogues. Molecullar and Cellular Endocrinology, 379(1-2): 51-61. DOI: https://www.doi.org/10.1016/j.mce.2013

Daghir NJ (2009). Nutritional strategies to reduce heat stress in broilers and broiler breeders. Lohmann Information, 44(1): 6-15. Available at: http://www.lohmann-information.com/co Accessed 19/10/2020

Dao HT, Sharma NK, Bradbury EJ, and Swick RA (2021). Effects of L-arginine and L-citrulline supplementation in reduced protein diets for broilers under normal and cyclic warm temperature. Animal Nutrition, 7(4): 927-938. DOI: https://www.doi.org/10.1016/j.aninu.2020.12.010

Datta K, Rahalkar K, and Dinesh DK (2017). Heat shock proteins (HSP): Classifications and its involvement in health and disease. Journal of Pharmaceutical Care & Health Systems, 4(2): 1000175. DOI: https://www.doi.org/10.4172/2376-0419.1000175

Dayyani N and Bakhtiyari H (2013). Heat stress in poultry: Background and affective factors. International Journal of Advanced Biological and Biomedical Research, 1(11): 1409-1413. Available at: http://www.ijabbr.com/article_7968_f56af19891048da54f54040274e4cd73.pdf

De Almeida A, Dias A, Bueno CFD, Couto FAP, Rodrigues PA, Nogueira WCL, and Emygdio de Faria Filho D (2012). Crude protein and metabolizable energy levels for layers reared in hot climates. Revista Brasileira de Ciência Avícola, 14: 203-208. DOI: https://www.doi.org/10.1590/S1516-635X2012000300007

de Freitas Dionizio A, de Souza Khatlab A, Alcalde CR, Gasparino E, and Feihrmann AC (2021). Supplementation with free methionine or methionine dipeptide improves meat quality in broilers exposed to

heat stress. Journal of Food Science and Technology, 58(1): 205-215. DOI: https://www.doi.org/10.1007/s13197-020-04530-2

De Oliveira RG and Lara LJC (2016). Lighting programmes and its implications for broiler chickens. World's Poultry Science Journal, 72(4): 735-742. DOI: https://www.doi.org/10.1017/S0043933916000702

Del Vesco A, Gasparino PE, Neto ARO, Rossi RM, Soares MAM, and da Silva SCC (2013). Effect of Methionine supplementation on mitochondrial genes expression in the breast muscle and liver of broilers. Livestock Science, 151(2-3): 284-291. Available at: https://www.infona.pl/resource/bwmeta1.element.elsevier-9c387820-6b46-316a-a936-58722a56f0cb

Deraz SF (2018). Synergetic effects of multispecies probiotic supplementation on certain blood parameters and serum biochemical profile of broiler chickens. Journal of Animal Health and Production, 6(1): 27-34. DOI: https://www.doi.org/10.17582/journal.jahp/2018/6.1.27.34

Desta TT (2021). The genetic basis and robustness of naked neck mutation in chicken. Tropical Animal Health and Production, 53: 95. DOI: https://www.doi.org/10.1007/s11250-020-02505-1

Diarra SS and Tabuaciri P (2014). Feeding management of poultry in high environmental temperatures. International Journal of Poultry Science, 13(11): 657-661. DOI: https://www.doi.org/10.3923/ijps.2014.657.661

Diarra SS, Sandakabatu D, Perera D, Tabuaciri P, and Mohammed U (2014). Growth performance and carcass yield of broiler chickens fed commercial finisher and cassava copra meal-based diets. Journal of Applied Animal Research, 43(3): 352-356. DOI: https://www.doi.org/10.1080/09712119.2014.978774

Donald J (2018). How poultry housing can optimise performance. Biomin, Auburn University, in Alabama, USA. https://www2.biomin.net/hr/articles/how-poultry-housing-can-reduce-stress-and-optimize-performance/

Dong J, Chuan H, Zhibing W, Yanqing L, Shanshan L, Lin T, Jiebo C, Donghua, L, Fenxia Y, Naibin L et al. (2018). A novel deletion in KRT75L4 mediates the frizzle trait in a Chinese indigenous chicken. Genetics Selection Evolution, 50: 68. DOI: https://www.doi.org/10.1186/s12711-018-0441-7

Duangjinda MI, Tunim S, Duangdaen CI, and Boonkum W (2017). HSP70 genotypes and heat tolerance of commercial and native chickens reared in hot and humid conditions. Brazilian Journal of Poultry Science, 19(1): 7-18. DOI: http://www.doi.org/10.1590/1806-9061-2016-024

Ebeid TA, Suzuki T, and Sugiyama T (2012). High ambient temperature influences eggshell quality and calbindin D28k localization of eggshell gland and all intestinal segments of laying hens. Poultry Science, 91(9): 2282-2287. DOI: https://www.doi.org/10.3382/ps.2011-01898

Efeoğlu B (2009). Heat shock proteins and heat shock response in plants. Ghazi University Journal of Science, 22(2): 67-75. Available at: https://www.acarindex.com/pdfler/acarindex-43ec2602-cd1b.pdf

Elbaz AM, Ibrahim NS, Shehata AM, Mohamed NG, and Abdel-Moneim A-ME (2021). Impact of multi-strain probiotic, citric acid, garlic powder or their combinations on performance, ileal histomorphometry, microbial enumeration and humoral immunity of broiler chickens. Tropical Animal Health and Production, 53: 115. DOI: https://www.doi.org/10.1007/s11250-021-02554-0

Estévez M (2015). Oxidative damage to poultry: From farm to fork. Poultry Science, 94(6): 1368-1378. DOI: https://www.doi.org/10.3382/ps/pev094

Farag MR and Alagawany M (2018). Physiological alterations of poultry to the high environmental temperature. Journal of Thermal Biology, 76(1): 101-106. DOI: https://www.doi.org/10.1016/j.jtherbio.2018.07.012

Farghly MFA, Abd El-Hack ME, Alagawany M, Saadeldin IM, and Swelum AA (2018). Wet feed and cold water as heat stress modulators in growing Muscovy ducklings. Poultry Science, 97(5): 1588-1594. DOI: https://www.doi.org/10.3382/ps/pey006

Fathi M, Al-Homidan I, Rayan G, El-Safty S, Ebeid T, and Abou-Emera O (2019). Laying performance, immune response and antioxidant properties of hens segregating for naked neck and frizzle genes under low ambient temperature. Czech Journal of Animal Science, 64(5): 216-225. DOI: http://www.doi.org/10.17221/221/2018-CJAS

Fathi M, Galal AA, El-Saft SAE, and Mahrous M (2013). Naked neck and frizzle genes for improving chickens raised under high ambient temperature: I. Growth performance and egg production. World's Poultry Science Journal, 69(4): 813-832. DOI: http://www.doi.org/10.1017/S0043933913000834

Fathi MM, Galal A, Radwan LM, Abou-Emera OK, and Al-Homidan IH (2022). Using major genes to mitigate the deleterious effects of heat stress in poultry: An updated review. Poultry Science, 101(11): 102157. DOI: https://www.doi.org/10.1016/j.psj.2022.102157

Faud AM, Chen W, Ruan D, Wang S, Xia WG, and Zheng CT (2016). Impact of heat stress on meat, egg quality, immunity and fertility in poultry and nutritional factors that overcome these effects: A review. International Journal of Poultry Science, 15(3): 81-95. DOI: http://www.doi.org/10.3923/ijps.2016.81.95

Gamba JP, Rodrigues MM, Garcia Neto M, Perri SH, Faria de Júnior MJA, and Pinto MF (2015). The strategic application of electrolyte balance to minimize heat stress in broilers. Brazilian Journal of Poultry Science, 17(2): 237-246. DOI: http://www.doi.org/10.1590/1516-635x1702237-246

Gholami J, Qotbi AAA, Seidavi A, Meluzzi A, Tavaniello S, and Maiorano G (2015). Effects of *in ovo* administration of betaine and choline on hatchability results, growth and carcass characteristics and immune response of broiler chickens. Italian Journal of Animal Science, 14(2): 3694. DOI: https://www.doi.org/10.4081/ijas.2015.3694

Gholami M, Chamani M, Seidavi A, Asgha S, and Aminafshar M (2020). Effects of stocking density and climate region on performance, immunity, carcass characteristics, blood constituency, and economical parameters of broilers. Revista Brasileira de Zootecnia, 49: e20190049. DOI: https://www.doi.org/10.37496/rbz4920190049

Gicheha MG (2021). The effects of heat stress on production, reproduction, health in chicken and its dietary amelioration. In (Ed.), Advances in poultry research, IntechOpen. DOI: https://www.doi.org/10.5772/intechopen.97284

Glatz P and Pym R (2013). Poultry Development Review, poultry housing and management in developing countries. FAO., Rome, Italy. pp. 24-28. Available at: https://www.fao.org/3/i3531e/i3531e.pdf#page=30

Goel A (2021). Heat stress management in poultry. Journal of Animal Physiology and Animal Nutrition, 105(6): 1136-1145. DOI: https://www.doi.org/10.1111/jpn.13496

Gonzalez-Rivas PA, Chauhan SS, Ha M, Fegan N, Dunshea FR, and Warner RD (2020). Effects of heat stress on animal physiology, metabolism, and meat quality: A review. Meat Science, 162: 108025. DOI: https://www.doi.org/10.1016/j.meatsci.2019.108025

Habashy WS, Milfort MC, Fuller AL, Attia YA, Rekaya R, and Aggrey SE (2017). Effect of heat stress on protein utilization and nutrient transporters in meat-type chickens. International Journal of Biometeorology, 61: 2111-2118. DOI: https://www.doi.org/10.1007/s00484-017-1414-1

Hamm LL, Nakhoul N, and Hering-Smith KS (2015). Acid-Base base homeostasis. Clinical journal of the American Society of Nephrology, 10(12): 2232-2242. DOI: https://www.doi.org/10.2215/CJN.07400715

Hao S, Liu L, Wang G, Xian GU, and Pan F (2017). Effects of dietary betaine on performance, egg quality and serum biochemical parameters of laying hens under heat stress condition. Chinese Journal of Animal Nutrition, 29(1): 184-192. Available at: http://www.chinajan.com/CN/abstract/html/20170121.htm

He S, Li S, Arowolo MA, Yu Q, Chen F, Hu R, and He J (2019). Effect of resveratrol on growth performance, rectal temperature, and serum parameters of yellow-feather broilers under heat stress. Animal Science Journal, 90(3): 401-411. DOI: https://www.doi.org/10.1111/asj.13161

Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, Scheimann J, and Myers B (2016). Regulation of the hypothalamic-pituitary- adrenocortical stress response. Comprehensive Physiology, 6(2): 603-621. DOI: https://www.doi.org/10.1002/cphy.c150015

Hirakawa R, Nurjanah S, Furukawa K, Murai A, Kikusato M, Nochi T, and Toyomizu M (2020). Heat stress causes Iimmune abnormalities via massive damage to effect proliferation and differentiation of lymphocytes in broiler chickens. Frontiers in Veterinary Science, 7: 46. DOI: https://www.doi.org/10.3389/fvets.2020.00046

Holtmann G, Shah A, and Morrison M (2017). Pathophysiology of functional gastrointestinal disorders: A holistic overview. Digestive Diseases, 35(Suppl 1): 5-13. DOI: https://www.doi.org/10.1159/000485409

Hu JY, Hester PY, Makagon MM, Vezzoli G, Gates RS, Xiong YJ, and Cheng HW (2016). Cooled perch effects on performance and well-being traits in caged white leghorn hens. Poultry Science, 95(12): 2737-2746. DOI: https://www.doi.org/10.3382/ps/pew248

Hu R, He Y, Arowolo MA, Wu S, and He J (2019). Polyphenols as potential attenuators of heat stress in poultry production. Antioxidants, 8(3): 67. DOI: https://www.doi.org/10.3390/antiox8030067

Jahanian R and Rasouli E (2015). Dietary chromium methionine supplementation could alleviate immunosuppressive effects of heat stress in broiler chicks. Journal of Animal Science, 93(7): 3355-3363. DOI: https://www.doi.org/10.2527/jas.2014-8807

Jahromi MF, Altaher YW, Shokryazdan P, Ebrahimi R, Ebrahimi M, Idrus Z, Tufarlli V, and Lian JB (2015). Dietary supplementation of a mixture of Lactobacillus strains enhances performance of broiler chickens raised under heat stress conditions. International Journal of Biometeorology, 60(7): 1099-1110. DOI: https://www.doi.org/10.1007/s00484-015-1103-x

Jessop TS, Meagan LL, Teasdale L, Stuart-Fox D, Willson RS Careau V, and Moore IT (2016). Multiscale evaluation of thermal dependence in the glucocorticoid response of vertebrates. The American Naturalist, 188(3): 342-356. DOI: https://www.doi.org/10.1086/687588

Jha R and Mishra P (2021). Dietary fibre in poultry nutrition and their effects on nutrient utilisation, performance, gut health, and on the environment: A review. Journal of Animal Science and Biotechnology, 12: 51. DOI: https://www.doi.org/10.1186/s40104-021-00576-0

Kadykalo S, Roberts T, Thompson M, Wilson J, Lang M, and Espeisse O (2018). The value of anticoccidials for sustainable global poultry production. International Journal of Antimicrobial Agents, 51(3): 304-310. DOI: https://www.doi.org/10.1016/j.ijantimicag.2017.09.004

Kaldhusdal M, Benestad SL, and Løvland A (2016). Epidemiologic aspects of necrotic enteritis in broiler chickens – disease occurrence and production performance. Avian Pathology, 45(3): 271-274. DOI: https://www.doi.org/10.1080/03079457.2016.1163521

Kennedy GM, Lichoti KJ, and Ommeh SC (2022). Review article: Heat stress and poultry: Adaptation to climate change, challenges, and opportunities for genetic breeding in Kenya. Journal of Agriculture Science and Technology, 21(1): 49-61. DOI: https://www.doi.org/10.4314/jagst.v21i1.6

Khalil MM, Abdollahi MR, Zaefarian F, and Ravindran V (2021). Influence of feed form on the apparent metabolisable energy of feed ingredients for broiler chickens. Animal Feed Science and Technology, 271: 114754. DOI: https://www.doi.org/10.1016/j.anifeedsci.2020.114754

Kidd MT, Maynard CW, and Mullenix GJ (2021). Progress of amino acid nutrition for diet protein reduction in poultry. Journal of Animal Science and Biotechnolology, 12: 45. DOI: https://www.doi.org/10.1186/s40104-021-00568-0

Kumar BC, Gloridoss RG, Singh KC, Prabhu TM, and Suresh BN (2016). Performance of broiler chickens fed low protein, limiting amino acid supplemented diets formulated either on total or standardized ileal digestible amino acid basis. Asian Australasian Journal of Animal Science, 29(11): 1616-1624. DOI: http://www.doi.org/10.5713/ajas.15.0648

Kumari NRK and Nath ND (2018). Ameliorative measures to counter heat stress in poultry. World's Poultry Science Journal, 74(1): 117-130. DOI: https://www.doi.org/10.1017/S0043933917001003

Kumbhar S, Khan AZ, Parveen F, Nizamani ZA, Siyal FA, Mohamed E, Abd El-Hack ME, Gan F, Liu Y, Hamid M et al. (2018). Impacts of selenium and vitamin E supplementation on mRNA of heat shock proteins, selenoproteins and antioxidants in broilers exposed to high temperature. AMB Express, 8: 112. DOI: https://www.doi.org/10.1186/s13568-018-0641-0

Lara LJ and Rostagno MH (2013). Impact of heat stress on poultry production. Animals, 3(2): 356-369. DOI: https://www.doi.org/10.3390/ani3020356

Lemme A, Hiller P, Klahsen M, Taube V, Stegemann J, and Simon I (2019). Reduction of dietary protein in broiler diets not only reduces n-emissions but is also accompanied by several further benefits. Journal of Applied Poultry Research, 28(4): 867-880. DOI: https://www.doi.org/10.3382/japr/pfz045

Liu F, de Ruyter EM, Athorn RZ, Brewster CJ, Henman DJ, Morrison RS, Smits RJ, Cottrell JJ, and Dunshea FR (2019). Effects of L-citrulline supplementation on heat stress physiology, lactation performance and subsequent reproductive performance of sows in summer. Journal of Animal Physiology and Animal Nutrition, 103(1): 251-257. DOI: https://www.doi.org/10.1111/jpn.13028

Liu M, Lu Y, Gao P, Xie X, Li D, Yu D, and Yu M (2020). Effect of curcumin on laying performance, egg quality, endocrine hormones, and immune activity in heat-stressed hens. Poultry Science, 99(4): 2196-2202. DOI: https://www.doi.org/10.1016/j.psj.2019.12.001

Liverpool-Tasie LSO, Sanou A, and Tambo JA (2019). Climate change adaptation among poultry farmers: Evidence from Nigeria. Climate Change, 157(1): 527-544. DOI: https://www.doi.org/10.1007/s10584-019-02574-8

Luo J, Song J, Liu L, Xue B, Tian G, and Yang Y (2018). Effect of epigallocatechin gallate on growth performance and serum biochemical metabolites in heat-stressed broilers. Poultry Science, 97(2): 599-606. DOI: https://www.doi.org/10.3382/ps/pex353

Mack LA, Felver-Gant JN, Dennis RL, and Cheng HW (2013). Genetic variations alter production and behavioural responses following heat stress in 2 strains of laying hens. Poultry Science, 92(2): 285-294. DOI: https://www.doi.org/10.3382/ps.2012-02589

Massuquetto A, Durau J, Barrilli L, Santos R, Krabbe E, and Maiorka A (2020). Thermal processing of corn and physical form of broiler diets. Poultry Science, 99(6): 3188-3195. DOI: https://www.doi.org/10.1016/j.psj.2020.01.027

McCreery DH (2015). Water consumption behaviour in broilers. Graduate Theses and Dissertations. Available at: https://scholarworks.uark.edu/etd/1301

McDowell LR (2012). Vitamins in animal nutrition: Comparative aspects to human nutrition. Elsevier., Amsterdam, The Netherlands.

McGregor BA, Murphy KM, Albano DL, and Ceballos RM (2016). Stress, cortisol, and B lymphocytes: A novel approach to understanding academic stress and immune function. Stress, 19(2): 185-191. DOI: https://www.doi.org/10.3109/10253890.2015.1127913

Mir SH, Pal RP, Mani V, Malik TA, Ojha L, and Yadav S (2018). Role of dietary minerals in heat-stressed poultry: A review. Journal of Entomology and Zoology Studies, 6(5): 820-826.

Mishra B and Jha R (2019). Oxidative stress in the poultry gut: Potential challenges and interventions. Frontiers in Veterinary Science, 6(1): 60. DOI: http://www.doi.org/10.3389/fvets.2019.00060

Mohammed AA, Jacobs JA, Murugesan GR, and Cheng HW (2018). Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. Poultry Science, 97(4): 1101–1108. DOI: https://www.doi.org/10.3382/ps/pex421

Monson MS, Van Goor AG, Ashwell CM, Persia ME, Rothschild MF, Schmidt CJ, and Lamont SJ (2018). Immunomodulatory effects of heat stress and lipopolysaccharide on the bursal transcriptome in two distinct chicken lines. BMC genomics, 19(1): 643. DOI: https://www.doi.org/10.1186/s12864-018-5033-y

Moraes PO, Andretta I, Cardinal KM, Ceron M, Vilella L, Borille R, Frazzon AP, Frazzon J, Santin E, and Ribeiro AML (2019). Effect of functional oils on the immune response of broilers challenged with Eimeria spp., Animal, 13(10): 2190-2198. DOI: https://www.doi.org/10.1017/S1751731119000600

Moreki JC, Magapatona S, and Manyeula F (2020). Effect of stocking density on performance of broiler chickens. International Journal of

Agriculture and Rural Development, 23(2): 5367-5372. Available at: http://researchhub.buan.ac.bw/bitstream/handle/13049/40/IJARD%202020.pdf?sequence=1&isAllowed=y

Mottet A and Tempio G (2017). Global poultry production: Current state and future outlook and challenges. Worlds Poultry Science Journal, 73(2): 245-56. DOI: https://www.doi.org/10.1017/S0043933917000071

Mousa-Balabel T, Al-Midany S, and Algazzar W (2021). Dim blue light colour reduces the activities and improves the performance of Indian river broilers under Egyptian conditions. Journal of the Hellenic Veterinary Medical Society, 72(3): 3171-3178. DOI: https://www.doi.org/10.12681/jhvms.28511

Nawab A, Ibtisham F, Li G, Kieser B, Wu J, Liu W, Zhao Y, Nawab Y, Li K, Xiao M, et al. (2018). Heat stress in poultry production: Mitigation strategies to overcome the future challenges facing the global poultry industry. Journal of Thermal Biology, 78: 131-139. DOI: https://www.doi.org/10.1016/j.jtherbio.2018.08.010

Nawaz AH, Amoah K, Leng QY, Zheng JH, Zhang WL, and Zhang L (2021). Poultry response to heat stress: Its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. Frontiers in Veterinary Science, 8: 699081. DOI: https://www.doi.org/10.3389/fvets.2021.699081

Nilsson JA, Molokwu MN, and Olsson O (2016). Body temperature regulation in hot environments. PLoS One, 11(8): e0161481. DOI: https://www.doi.org/10.1371/journal.pone.0161481

Nochi T, Jansen CA, Toyomizu M, and van Eden W (2018). The well-developed mucosal immune systems of birds and mammals allow for similar approaches of mucosal vaccination in both types of animals. Frontiers in Nutrition, 5: 60. DOI: https://www.doi.org/10.3389/fnut.2018.00060

Nyoni NMB, Grab S, and Archer ERM (2019). Heat stress and chickens: climate risk effects on rural poultry farming in low-income countries. Climate and Development, 11(1): 83-90. DOI: https://doi.org/10.1080/17565529.2018.1442792

Oloyo A and Ojerinde A (2019). Poultry Housing and Management. In Poultry; IntechOpen., London, UK. Available at: https://cdn.intechopen.com/pdfs/65864.pdf

Orakpoghenor O, Ogbuagu NE, and Sa'Idu L (2020). Effect of environmental temperature on water intake in poultry. In: A. Kumar Patra (Editor), Advances in poultry nutrition research, IntechOpen., pp. 1-8. DOI: https://www.doi.org/10.5772/intechopen.95695

Pacheco LG, Sakomura NK, Suzuki RM, Dorigam JCP, Viana GS, Van Milgen J, and Denadai JC (2018). Methionine to cystine ratio in the total sulfur amino acid requirements and sulfur amino acid metabolism using labelled amino acid approach for broilers. BMC Veterinary Research, 14(1): 364. DOI: https://www.doi.org/10.1186/s12917-018-1677-8

Park S, Park B, and Hwangbo J (2015). Effect of cold water and inverse lighting on growth performance of broiler chickens under extreme heat stress. Journal of Environmental Biology, 36(4): 865-873. Available at: https://www.cabdirect.org/cabdirect/abstract/20153404891

Park SO and Kim WK (2016). Effects of betaine on biological functions in meat-type ducks exposed to heat stress. Poultry Science, 96(5): 1212-1218. DOI: https://www.doi.org/10.3382/ps/pew359

Parvin R, Mushtaq MMH, Kim MJ, and Choi HC (2014). Light emitting diode (LED) as a source of monochromatic light: A novel lighting approach for behaviour, physiology, and welfare of poultry. World's Poultry Science Journal, 70(3): 543-556. DOI: https://www.doi.org/doi:10.1017/S0043933914000609

Pawar S, Sajjanar B, Lonkar V, Kurade N, Kadam A, Nirmal A, Brahmane M, and Bal S (2016). Assessing and mitigating the impact of heat stress on poultry. Advances in Animal and Veterinary Sciences, 4: 332-341. DOI: http://www.doi.org/10.14737/journal.aavs/2016/4.6.332.341

Piestun Y, Patael T, Yahav S, Velleman SG, and Halevy O (2017). Early post-hatch thermal stress affects breast muscle development and satellite cell growth and characteristics in broilers. Poultry Science, 96(8): 2877-2888. DOI: https://www.doi.org/10.3382/ps/pex065

Popoola IO, Popoola OR, Ojeniyi MO, Olajide OO, and Iyayi EA (2019). The roles of key electrolytes in balancing blood acid-base and nutrient in broiler chickens reared under tropical conditions. Natural Science, 12(1): 4-11. DOI: https://www.doi.org/10.4236/ns.2020.121002

Pursey KM, Davis C, and Burrows TL (2017). Nutritional aspects of food addiction. Current Addiction Reports, 4: 142-150. DOI: https://www.doi.org/10.1007/s40429-017-0139-x

Qaid MM and AlGaradi MA (2021). Protein and amino acid metabolism in poultry during and after heat stress: A review. Animals, 11(4): 1167. DOI: https://www.doi.org/10.3390/ani11041167

Quilumba C, Quijia E, Gernat A, Murillo G, and Grimes J (2015). Evaluation of different water flow rates of nipple drinkers on broiler productivity. Journal of Applied Poultry Research, 24(1): 58-65. DOI: https://www.doi.org/10.3382/japr/pfv005

Quinteiro-Filho WM, Calefi AS, Cruz DSG, Aloia TPA, Zager A, Astolfi-Ferreira CS, Piantino FJA, Sharif S, and Palermo-Neto J (2017). Heat stress decreases the expression of the cytokines, avian β-defensins 4 and 6 and Toll-like receptor 2 in broiler chickens infected with *Salmonella enteritidis*. Veterinary Immunology and Immunopathology, 186(1): 19-28. DOI: https://www.doi.org/10.1016/j.vetimm.2017.02.006

Rahman H and Hidayat C (2020). Reducing the negative effect of heat stress in broiler through nutritional and feeding strategy. IOP Conference Series: Earth Environmental Science, 465: 012034. DOI: https://www.doi.org/10.1088/1755-1315/465/1/012034

Rajkumar U, Reddy BLN, Rajaravindra K, Niranjan M, Tarun B, Chatterjee RN, Arun P, Reddy RM, and Sharma RP (2010). Effect of naked neck gene on immune competence, serum biochemical and carcass traits in chickens under a tropical climate. Asian-Australasian Journal of Animal Sciences, 23(7): 867-872. DOI: https://www.doi.org/10.5713/ajas.2010.90548

Ranjan A, Sinha R, Devi I, Rahim A, and Tiwari S (2019). Effect of heat stress on poultry production and their management approaches. International Journal of Current Microbiology and Applied Sciences, 8(2): 1548-1555. DOI: https://www.doi.org/10.20546/ijcmas.2019.802.181

Ratcliffe MJ and Härtle S (2014). B cells, the bursa of Fabricius and the generation of antibody repertoires. In: K. A. Schat, B. Kaspers, P. Kaiser (Editors), Avian immunology, 2nd Edition. Academic Press., San Diego. pp. 65-89.

Rath KP, Behura CN, Sahoo PS, Panda P, Mandal DK, and Panigrahi NP (2015). Amelioration of heat stress for poultry welfare: A strategic approach. International Journal of Livestock Research, 5(3): 1-9. DOI: https://www.doi.org/10.5455/IJLR.20150330093915

Rath M, Muller I, Kropf P, Closs EL, and Munder M (2014). Metabolism viaarginase or nitric oxide synthase: Two competing arginine pathways in macrophages. Frontiers in Immunology Inflammation, 5: 532. DOI: https://www.doi.org/10.3389/fimmu.2014.00532

Reddy TE and Ramya P (2015). Heat stress strategies for layers in hot climates. Available at: https://www.wattagnet.com/articles/22314-heat-stress-strategies-for-layers-in-hot-climates

Riber AB (2015). Effects of color of light on preferences, performance, and welfare in broilers. Poultry Science, 94(8): 1767-1775. DOI: https://www.doi.org/10.3382/ps/pev174

Robert L, Francisco JT, Ulf T, and Ingrid H (2017). Nanoparticle‑based hyperthermia distinctly impacts the production of ROS, expression of Ki‑67, TOP2A, and TPX2, and induction of apoptosis in pancreatic cancer. International Journal of Nanomedicine, 12: 1009-1018. DOI: http://www.doi.org/10.2147/IJN.S108577

Rostagno HM (2020). Effects of heat stress on the gut health of poultry. Journal of Animal Science, 98(4): skaa090. DOI: https://www.doi.org/10.1093/jas/skaa090

Ruuskanen S, Hsu YB, and Nord A (2021). Endocrinology and thermoregulation of birds in changing climate. Molecular and Cellular Endocrinology, 519: 111088. DOI: https://www.doi.org/10.1016/j.mce.2020.111088

Saeed M, Abbas G, Alagawany M, Kamboh AA, Abd El-Hack ME, Khafaga AF, and Chao S (2019). Heat stress management in poultry farms: A comprehensive overview. Journal of Thermal Biology, 84: 414-425. DOI: https://www.doi.org/10.1016/j.jtherbio.2019.07.025

Saeed M, Babazadeh D, Naveed M, Arain MA, Hassan FU, and Chao S (2017). Reconsidering betaine as a natural anti-heat stress agent in poultry industry: a review. Tropical Animal Health and Production, 49(7): 1329-1338. Available at: https://europepmc.org/article/med/28733762

Saelao P, Wang Y, Chanthavixay G, Yu V, Gallardo RA, Dekkers JCM, Lamont SJ, Kelly T, and Zhou H (2021). Distinct transcriptomic response to Newcastle disease virus infection during heat stress in chicken tracheal epithelial tissue. Scientific Reports, 11(1): 7450. DOI: https://www.doi.org/10.1038/s41598-021-86795-x

Safdar AHA and Maghami SPMG (2014). Heat stress in poultry: Practical tips. European Journal of Experimental Biology, 4: 625-631.

Sahin K, Orhan C, Tuzcu M, Sahin N, Hayirli A, Bilgili S, and Kucuk O (2016). Lycopene activates antioxidant enzymes and nuclear transcription factor systems in heat-stressed broilers. Poultry Science, 95(5): 1088-1095. DOI: https://www.doi.org/10.3382/ps/pew012

Saleeva IP, Sklyar AV, Marinchenko TE, Postnova MV, Ivanov AV, and Tikhomirov AI (2019). Feasibility study on innovative energy-saving technologies in poultry farming. E3S Web of Conferences, 124: 05070. DOI: https://www.doi.org/10.1051/e3sconf/201912405070

Saleh AA, Shurky M, Farrag F, Soliman MM, and Moneim AMEA (2021). Effect of feeding wet feed or wet feed fermented by *Bacillus licheniformis* on growth performance, histopathology growth and lipid metabolism marker genes in broiler chickens. Animals, 11(1): 83. DOI: https://www.doi.org/10.3390/ani11010083

Santos RR, Awati A, Roubos-van den Hil PJ, Tersteeg-Zijderveld MHG, Koolmees PA, and Fink-Gremmels J (2015). Quantitative histomorphometric analysis of heat-stress-related damage in the small intestines of broiler chickens. Avian Pathology, 44(1): 19-22. DOI: https://www.doi.org/10.1080/03079457.2014.988122

Scanes CG (2015). Sturkie's avian physiology. Regulation of body temperature: Strategies and mechanisms, 6th Edition. Academic Press., USA. Chapter 37, pp. 869-905.

Seifi K, Rezaei M, Yansari AT, Zamiri MJ, Riazi GH, and Heidari R (2020). Short chain fatty acids may improve hepatic mitochondrial energy efficiency in heat stressed-broilers. Journal of Thermal Biology, 89: 102520. DOI: https://www.doi.org/10.1016/j.jtherbio.2020.102520

Sejian V, Bhatta R, Gaughan JB, Dunshea FR, and Lacetera N (2018). Review: Adaptation of animals to heat stress. Animal, 12(2): 431-44. DOI: https://www.doi.org/10.1017/S1751731118001945

Shakeri M and Le HH (2022). Deleterious effects of heat stress on poultry production: Unveiling the benefits of betaine and polyphenols. Poultry, 1(3): 147-156. DOI: https://www.doi.org/10.3390/poultry1030013

Shakeri M, Oskoueian E, Le HH, and Shakeri M (2020). Strategies to combat heat stress in broiler chickens: Unveiling the roles of selenium, vitamin E and vitamin C. Veterinary Sciences, 7(2): 71. DOI: https://www.doi.org/10.3390/vetsci7020071

Shehata AM, Saadeldin IM, Tukur HA, and Habashy WS (2020). Modulation of heat-shock proteins mediates chicken cell survival against thermal stress. Animals, 10(12): 2407. DOI: https://www.doi.org/10.3390/ani10122407

Sinha B, Mandal K, Kumari R, and Kumari T (2018). Estimate and effect of breeds on egg quality traits of poultry - A review. International Journal of Livestock Research, 8: 8-21. DOI: https://www.doi.org/10.5455/ijlr.20170812102444

Skřivan M, Milan M, Michaela E, and Skřivanová V (2013). Influence of dietary vitamin C and selenium, alone and in combination, on the performance of laying hens and quality of eggs. Czech Journal of Animal Science, 58: 91-97. DOI: http://www.doi.org/10.17221/6619-CJAS

Slawinska A, Mendes S, Dunislawska A, Siwek M, Zampiga M, Sirri F, Meluzzi A, Tavaniello S, and Maiorano G (2019). Avian model to mitigate gut-derived immune response and oxidative stress during the heat. BioSystems, 178: 10-15. DOI: https://www.doi.org/10.1016/j.biosystems.2019.01.007

Smalling S, Diarra SS, and Amosa F (2019). Effect of feed form and water addition on growth performance of finishing broilers in a hot humid environment. Pakistan Journal of Nutrition, 18(4): 339-345. DOI: https://www.doi.org/10.3923/pjn.2019.339.345

Smith BA, Meadows S, Meyers R, Parmley EJ, and Fazil A (2019). Seasonality and zoonotic foodborne pathogens in Canada: Relationships between climate and Campylobacter, E. coli and Salmonella in meat products. Epidemiology and Infection, 147: e190. DOI: https://www.doi.org/10.1017/S0950268819000797

Sohsuebngarm D, Kongpechr S, and Sukon P (2019). Microclimate, body weight uniformity, body temperature, and footpad dermatitis in broiler chickens reared in commercial poultry houses in hot and humid tropical climates. World's Veterinary Journal, 9(4): 241-248. DOI: https://www.doi.org/10.36380/scil.2019.wvj30

Song DJ and King AJ (2015). Effects of heat stress on broiler meat quality. Worlds Poultry Science Journal, 71(4): 701-709. DOI: https://www.doi.org/10.1017/S0043933915002421

Souza LFA, de Espinha LP, de Almeida EA, Lunedo RA, Furlan RL, and Macari M (2016). How heat stress (continuous or cyclical) interferes with nutrient digestibility, energy and nitrogen balances and performance in broilers. Livestock Science, 192: 39-43. DOI: http://www.doi.org/10.1016/j.livsci.2016.08.014

Suganya T, Senthilkumar S, Deepa K, and Amutha R (2015). Nutritional management to alleviate heat stress in broilers. International Journal of Science, Environment and Technology, 4(3): 661-666. Available at: https://www.ijset.net/journal/683.pdf

Sugiharto S, Turrini Y, Isroli, I, Endang W, and Endang K (2017). Dietary supplementation of probiotics in poultry exposed to heat stress—A review. Annals for Animal Science, 17(3): 591-604. DOI: https://www.doi.org/10.1515/aoas-2016-0062

Surai PF, Kochish II, Fisinin VI, and Kidd MT (2019). Antioxidant defence systems and oxidative stress in poultry biology: An update. Antioxidants, 8(7): 235. DOI: http://www.doi.org/10.3390/antiox8070235

Syafwan S, Kwakkel R, and Verstegen M (2011). Heat Stress and feeding strategies in meat-type chickens. World's Poultry Science Journal, 67(4): 653-674. DOI: https://www.doi.org/10.1017/S0043933911000742

Tamzil MH (2014). Heat stress on poultry: Metabolism, effects and efforts to overcome. Indonesian Bulletin of Animal and Veterinary Sciences, 24(2): 57-66. DOI: https://www.doi.org/10.14334/wartazoa.v24i2.1049

Tang LP, Liu YL, Zhang JX, Ding KN, Lu MH, and He YM (2022). Heat stress in broilers of liver injury effects of heat stress on oxidative stress and autophagy in liver of broilers. Poultry Science, 101(10): 102085. DOI: https://www.doi.org/10.1016/j.psj.2022.102085

Tari AA, Sadeghi AA, and Mousavi NS (2020). Dietary vegetable oils inclusion on the performance, hormonal levels and HSP 70 gene expression in broilers under heat stress. Acta Scientiarum Animal Sciences, 42(1): e45517 DOI: https://www.doi.org/10.4025/actascianimsci.v42i1.45517

Teyssier JR, Brugaletta G, Sirri F, Dridi S, and Rochell SJ (2022). A review of heat stress in chickens. Part II: Insights into protein and energy utilization and feeding. Frontiers in Physiology, 13: 943612. DOI: https://www.doi.org/10.3389/fphys.2022.943612

Tóth R, Szabadi TN, Lázár B, Buda K, Végi B, Barna J, Patakiné Várkonyi PE, Liptói K, Pain B, and Gócza E (2021). Effect of post-hatch heat-treatment in heat-stressed Transylvanian naked neck chicken. Animals, 11(6): 1575. DOI: https://www.doi.org/10.3390/ani11061575

Van Goor A, Ashwell CM, Persia ME, Rothschild MF, Schmidt CJ, and Lamont SJ (2017). Unique genetic responses were revealed in RNA-seq of the spleen of chickens stimulated with lipopolysaccharide and short-term heat. PLoSOne, 12(2): e0171414. DOI: https://www.doi.org/10.1371/journal.pone.0171414

Vandana GD and Sejian V (2018). Towards identifying climate-resilient poultry birds. Journal of Dairy, Veterinary and Animal Research, 7(3): 84-85. DOI: http://www.doi.org/10.15406/jdvar.2018.07.00195

Vandana GD, Sejian V, Lees AM, Pragna P, Silpa MV, and Maloney SK (2021). Heat stress and poultry production: Impact and amelioration. International Journal of Biometeorology, 65(2): 163-179. DOI: https://www.doi.org/10.1007/s00484-020-02023-7

Varasteh S, Braber S, Akbari P, Garssen J, and Fink-Gremmels J (2015). Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galactooligosaccharides. PLoS One, 10: e0138975. DOI: https://www.doi.org/10.1371/journal.pone.0138975

Ventura MVA and Matias da Silva R (2019). Bone problems are caused by the deficiency of calcium and phosphorus in the feeding of broilers. Biomedical Journal of Scientific and Technical Research, 16(4): 12223-12229. DOI: https://www.doi.org/10.26717/BJSTR.2019.16.002886

Waiz AH, Gautam L, Nagada R, and Bhat AG (2016). Effect of wet feeding on feed conversion efficiency in laying hens during the summer season. Iranian Journal of Applied Animal Science, 6(2): 383-387. Available at: https://ijas.rasht.iau.ir/article_522858.html

Wang JP, Lee JH, Jang HD, Yan L, Cho JH, and Kim IH (2011). Effects of delta-aminolevulinic acid and vitamin C supplementation on iron status, production performance, blood characteristics and egg quality of laying hens. Journal of Animal Physiology and Animal Nutrition, 95(4): 417-423. DOI: http://www.doi.org/10.1111/j.1439-0396.2010.01067.x

Wang W, Yan F, Hu J, Amen O, and Cheng H (2018). Supplementation of Bacillus subtilis-based probiotic reduces heat stress-related behaviors and inflammatory response in broiler chickens. Journal of Animal Science, 96(5): 1654-1666. DOI: https://www.doi.org/10.1093/jas/sky092

Wang Y, Saelao P, Chanthavixay K, Gallardo R, Bunn D, Lamont SJ, Dekkers JM, Kelly T, and Zhou H (2018). Physiological responses to heat stress in two genetically distinct chicken inbred lines. Poultry Science, 97(3): 770-780. DOI: https://www.doi.org/10.3382/ps/pex363

Wang Y, WeiChao Z, HaiPeng S, Jiang T, and BaoMing L (2018). Roof insulation improving thermal environment and laying performance of poultry houses in summer. Transactions of the Chinese Society of Agricultural Engineering, 34(17): 207-213. Available at: https://www.cabdirect.org/cabdirect/abstract/20183344426

Wasti S, Sah N, and Mishra B (2020). Impact of heat stress on poultry health and performances, and potential mitigation strategies. Animals, 10(8): 1266. DOI: https://www.doi.org/10.3390/ani10081266

Wells KL, Hadad Y, Ben-Avraham D, Hillel J, Cahaner A, and Headon DJ (2012). Genome-wide SNP scan of pooled DNA reveals a nonsense mutation in FGF20 in the scaleless line of featherless chickens. BMC Genomics, 13: 257. DOI: https://www.doi.org/10.1186/1471-2164-13-257

Wu Y, Huang J, Quan S, and Yang Y (2022). Light regimen on health and growth of broilers: an update review. Poultry Science, 101(1): 101545. DOI: https://www.doi.org/10.1016/j.psj.2021.101545

Xie J, Tang L, Lu L, Zhang L, Lin X, Liu HC, Odle J, and Luo X (2015). Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. Poultry Science, 94(7): 1635-1644. DOI: https://www.doi.org/10.3382/ps/pev105

Xue B, Song J, Liu L, Luo J, Tian G, and Yang Y (2017). Effect of epigallocatechin gallate on growth performance and antioxidant capacity in heat-stressed broilers. Achieves of Animal Nutrition, 71(5): 362-372. DOI: https://www.doi.org/10.1080/1745039X.2017.1355129

Yahav S (2015). Regulation of body temperature: Strategies and mechanisms. G. Colin. Scanes, (Editor), Sturkie's avian physiology. Academic Press., London, UK. pp. 869-905.

Yousaf A, Jabbar A, Rajput N, Memon A, Shahnawaz R, Mukhtar N, Farooq F, Abbas M, and Khalil R (2019). Effect of environmental heat stress on performance and carcass yield of broiler chicks. World's Veterinary Journal, 9(1): 26-30. DOI: https://www.doi.org/10.36380/scil.2019.wvj4

Zaboli G, Huang X, Feng X, and Ahn DU (2019). How can heat stress affect chicken meat quality? - A review. Poultry Science, 98(3): 1551-1556. DOI: https://www.doi.org/10.3382/ps/pey399

Zaboli GR, Rahimi S, Shariatmadari F, Torshizi MAK, Baghbanzadeh A, and Mehri M (2017). Thermal manipulation during pre and post hatch on thermo-tolerance of male broiler chickens exposed to chronic heat stress. Poultry Science, 96(2): 478-485. DOI: https://www.doi.org/10.3382/ps/pew344

Zarghi H, Golian A, and Yazdi FT (2020). Effect of dietary sulphur amino acid levels and guanidinoacetic acid supplementation on performance, carcase yield and energetic molecular metabolites in broiler chickens fed wheat-soy diets. Italian Journal of Animal Science, 19(1): 951-959. DOI: https://www.doi.org/10.1080/1828051X.2020.1809537

Zeferino CP, Komiyama CM, Pelícia VC, Fascina VB, Aoyagi MM, Coutinho LL, Sartori JR, and Moura ASAMT (2016). Carcass and meat quality traits of chickens fed diets concurrently supplemented with vitamins C and E under constant heat stress. Animal, 10(1): 163-171. DOI: https://www.doi.org/10.1017/s1751731115001998

Zerjal T, Gourichon D, Rivet B, and André B (2013). Performance comparison of laying hens segregating for the frizzle gene under thermoneutral and high ambient temperatures. Poultry science, 92(6): 1474-1485. DOI: https://www.doi.org/10.3382/ps.2012-02840

Zhang C, Zhao XH, Yang L, Chen XY, Jiang RS, Jin SH, and Geng ZY (2017). Resveratrol alleviates heat stress-induced impairment of intestinal morphology, microflora, and barrier integrity in broilers. Poultry Science, 96(12): 4325-4332. DOI: https://www.doi.org/10.3382/ps/pex266

Zhang X, Owens CM, and Schilling MW (2017). Meat: the The edible flesh from mammals only or does it include poultry, fish, and seafood?. Animal Frontiers, 7(4): 12-8. DOI: https://www.doi.org/10.2527/af.2017.0437

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 20-28, March 25, 2023

Review Paper, PII: S2322455X2300002-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.2

Adrenal Gland of Poultry: Anatomy, Microscopy, Morphometry, and Histochemistry

Tetiana Kot[1], Svitlana Tkachuk[2], Svitlana Usenko[2], and Vladislav Prokopenko[1]

[1]*Faculty of Veterinary Medicine, Polissia National University, 10008, Zhytomyr, Ukraine*
[2]*Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine, 03041, Kyiv, Ukraine*

Corresponding author's E-mail: tkotvet@ukr.net

Received: 26 December 2022
Accepted: 12 February 2023

ABSTRACT

The adrenal gland plays a crucial role in poultry's body. Its hormones affect growth, tissue differentiation, and metabolism regulation, as well as the bird body's resistance to infections, intoxication, stress, and low temperature. For poultry farming, veterinary medicine, and ornithology, it is of scientific interest to study the morphological features of the adrenal gland of birds. This review aimed to assess poultry adrenal anatomy, microscopy, morphometry, and histochemistry by summarizing research data from various published articles. The structure of the adrenal gland has been morphologically investigated in clinically healthy chickens, ducks, geese, and quails. Data from the anatomical level of the adrenal gland have indicated that the shape of this organ in poultry of different species is not the same. In most cases, the shape of the adrenal gland of poultry is close to an oval, triangle, or pyramid. The color of the adrenal gland of poultry varies from gray to brown, which depends on the tissue saturation of this organ with carotenoids. The mass of the adrenal glands of poultry correlates with their age. The left adrenal gland has higher mass, volume, and length indicators than the right gland. The microscopic structure of the adrenal gland corresponds to the general laws of the structure and function of endocrine organs. However, the adrenal glands of poultry are characterized by class features of its histoarchitectonics. The adrenal capsule contains ganglia of the autonomic nervous system, the cell strands of cortical and medullary tissues are intertwined, and the configuration of these cell strands determines the formation of two or three zones of the adrenal gland. Studies of the adrenal glands of poultry at the cellular level have indicated that cortical tissue is represented by acidophilic cells and medullary tissue by basophilic cells. Depending on the shape and electron density of secretory granules, medullary tissue cells are divided into epinephrine and norepinephrine. Data on morphometric parameters (capsule thickness, area of cortical and medullary tissues, cortical-medullary ratio) of the adrenal gland are not the same and depend on the type, age, gender, and sexual activity of poultry. In conclusion, morphologists have paid great attention to studying the features of the anatomy, microscopy, morphometry, and histochemistry of the adrenal gland in clinically healthy poultry. Therefore, the presented data can be used to assess deviations in the morphofunctional state of the adrenal gland in poultry under the influence of various factors and pathology.

Keywords: Adrenal gland, Anatomy, Morphological features, Histological and cellular levels, Poultry

INTRODUCTION

Birds are a large class of vertebrates. There are about 10000 bird species from 27 genera and 170 families in the wildlife. A large number of bird species have been recorded in Colombia (1700), Ecuador (1357), and Brazil (1440). Other countries, such as Cameroon (670), the United States of America, and Canada (775), as well as Portugal (315) and Greece (339), are in the second and third places, respectively (Tobias et al., 2022). For many centuries, man has domesticated chicken, turkey, guinea fowl, quail, duck, goose, and pigeon. Canaries, cormorants, and parrots are bred in captivity (Domyan and Shapiro, 2017; Batool et al., 2020; Peng and Broom, 2021). Poultry farming occupies a leading position among the livestock industries (Hafez and Attia, 2020). Over the past half-century, poultry production has increased fivefold and continues to grow (OECD-FAO, 2016). According to the Organization for Economic Cooperation and Development/Food and Agriculture Organization agricultural forecast for 2019-2028, poultry is the most consumed animal protein in the world (OECD-FAO, 2019). When raising poultry, diseases that damage the endocrine system can cause many problems (Rudik et al.,

2021). Organs of the endocrine system are divided into central, peripheral, and mixed, including single endocrinocytes and endocrine organs, forming a dissociated endocrine system (Ritchie and Pilny, 2008; Scanes, 2015).

The adrenal gland is a peripheral organ of the endocrine system. The microscopic structure of the adrenal gland corresponds to the general laws of the structure and function of endocrine organs. It is known that there are no excretory ducts in the endocrine organs. The products of their activity (hormones) are released directly into the lymph and blood. Endocrine organs consist of the connective tissue stroma and parenchyma. The parenchyma forms follicles, strands, and islets, the cells of which are in close contact with the vessels of the microcirculatory bed. There are many blood capillaries between the structural components of the parenchymal adrenal gland, mainly of the sinusoid type (Scanes, 2015; Zakrevska and Tybinka, 2019; Scanes, 2020).

Adrenal hormones play a crucial role in the vital activity of the body by affecting the growth and differentiation of tissues, the development of reproductive organs, and the course of the sexual cycle (Kot et al., 2021; Gjuen and Jensen, 2022). Adrenal hormones regulate water, protein, carbohydrate, fat, and mineral metabolism (Manju Madhavan and Varghese, 2016; Lotfi et al., 2018; Di Lorenzo et al., 2020). The adrenal gland is sensitive to changes in the external and internal environment (Muller et al., 2015; Rudik et al., 2021). Morphological studies have indicated that the mass, size, vascularization of the adrenal gland, the ratio of cortical and cerebral zones, and their cytophysiological characteristics change in poultry affected by stress, hypothermia, and diseases (Scanes, 2016; Lotveld et al., 2017; Qureshi et al., 2020). Adrenal morphology in clinically healthy poultry is closely related to the anatomical, histological and cellular levels. The obtained data can be used to form a basis for the normal morphological characteristics of the adrenal gland in poultry. This makes it possible to assess the morphofunctional state of the adrenal glands of poultry in specific periods of their life, which is necessary for the scientific justification of technologies used in raising poultry, as well as for mastering the mechanisms of the development of adrenal diseases. This review aimed to assess features of the anatomy, microscopy, morphometry, and histochemistry of the adrenal glands of poultry by summarizing research data from various published articles.

ANATOMY

Topography, shape, and color

The adrenal gland of poultry is a paired organ. The left and right glands are situated cranio-medially to the kidneys on each side of the dorsal aorta and inferior vena cava (Figure 1A, B, C; Kober et al., 2012; Moawad and Randa, 2017; Prokopenko, 2022). Accessory adrenal glands in the chicken are near the main adrenal gland or in its capsule (Kot and Prokopenko, 2020). Accessory adrenal glands often develop in fish (Gaber and Abdel-maksoud, 2019) and mammals (Zakrevska and Tybinka, 2019). It has been claimed that accessory adrenal glands are the epicenter of neoplasms development (Afuwape et al., 2009). Kassaby et al. (2017) found adrenal tissue in a hernia sac of a human.

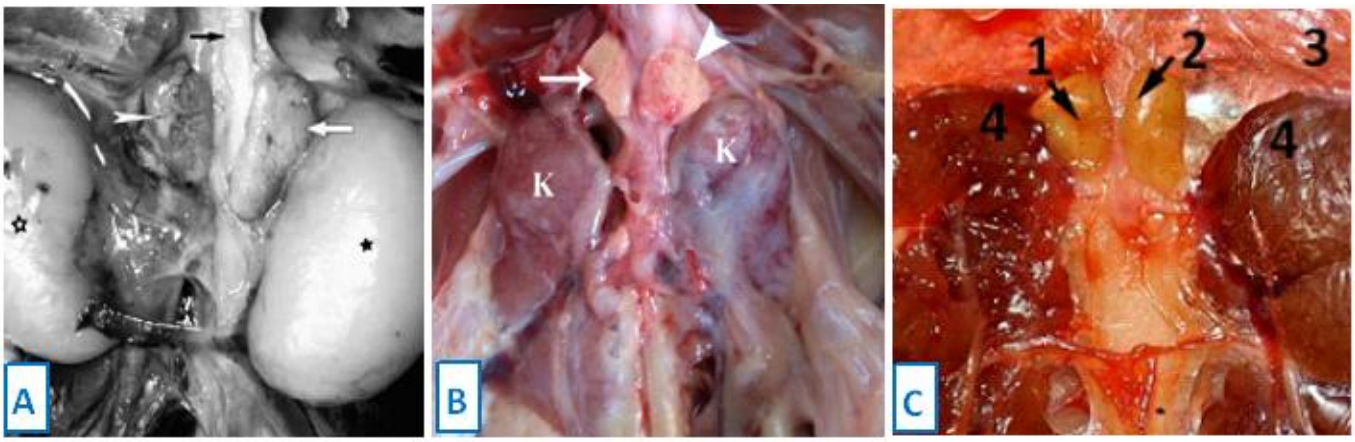


Figure 1. Anatomical position of the adrenal gland in chickens. (**A**): White arrowheads: Right and left adrenal gland, Black-and-white star: Right and left testis, Black arrow: Vena cava (Kober et al., 2012); in chicken (**B**): White arrows: Right and left adrenal gland, K: Kidneys (Moawad and Randa, 2017); in blue rock pigeon (**C**): 1: Right adrenal gland, 2: Left adrenal gland, 3: Left lung, 4: Superior kidney lobe (Prokopenko, 2022).

The shape of the adrenal gland in poultry is different (Figure 2A, B, C; Fathima and Lucy, 2014; Kober et al., 2012; Kot and Prokopenko, 2020). In a one-day-old duck, the adrenal gland has a spherical shape. By the age of 24 weeks, the right adrenal gland becomes pyramidal, and the left adrenal gland becomes oval in shape (Fathima and Lucy, 2014). The right adrenal gland of the chicken has an oval or triangular shape, and the left adrenal gland is elongated-oval in shape (Kober et al., 2012; Sarkar et al., 2014; Kot and Prokopenko, 2020), while in the ostrich they are ellipsoid and oblong in shape, respectively (Tang et al., 2009). The shape of the right gland of Japanese quails is triangular, while the left is elongated in shape (Rudik et al., 2021).

The color of the adrenal glands of poultry varies from gray, cream in pigeons, cream-yellow in quails (El-Desoky and El-Zahraa, 2021; Erdem et al., 2021) to yellow or yellow-red in guinea fowl (Prokopenko and Kot, 2021a). The duck is characterized by a change in the cream or yellow color of the adrenal gland in young animals to brown in adult birds (Fathima and Lucy, 2014). As reported by Scanes (2015), the intensity of the yellow color of the adrenal gland depends on the saturation of its tissues with carotenoids.

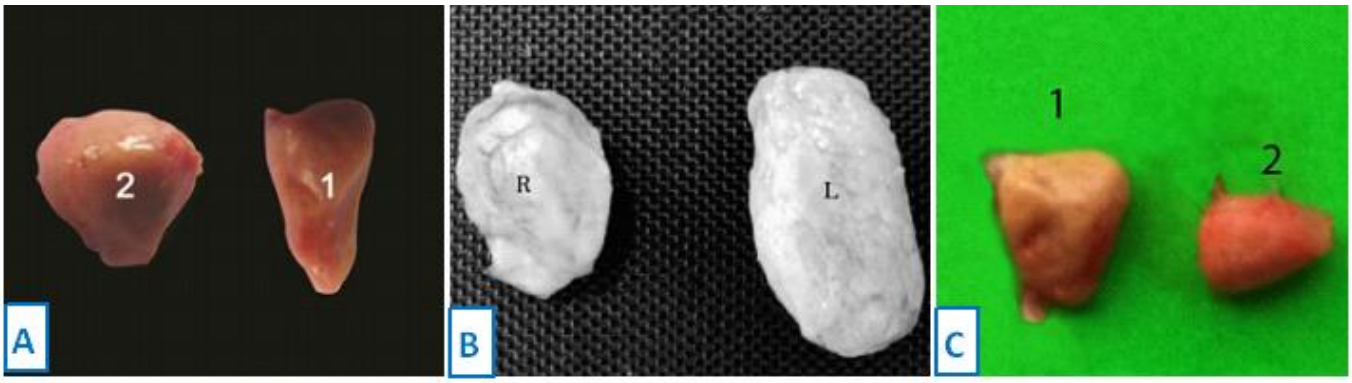


Figure 2. Shape of the adrenal gland in ducks. (**A**): 1: Left adrenal gland, 2: Right adrenal gland (Fathima and Lucy, 2014); in chicken, (**B**): L: Left adrenal gland, R: Right adrenal gland (Kober et al., 2012); in chicken (**C**): 1: Right adrenal gland, 2: Left adrenal gland (Kot and Prokopenko, 2020).

Organometry

The mass of the adrenal glands of poultry correlates with their age. In ducks, the adrenal mass increased from day-old (0.011 ± 0.001 g) to 12 weeks of age (0.093 ± 0.002 g), then decreased to 0.088 ± 0.003 g at week 16 (at the beginning of egg laying) and increased again to 0.137 ± 0.006 g at the age of 24 weeks (Fathima and Lucy, 2014). The relative mass of the duck's adrenal gland decreases intensively after hatching (Fathima and Lucy, 2014). Compared to the right adrenal gland, the left one has higher indicators of mass, volume, and length (Colcimen and Cakmak, 2021; Prokopenko and Kot, 2021a). In chickens, the masses of the left and right adrenal glands are 104.1 mg and 97.2 mg, with a length of 0.9 cm and 0.8 cm, respectively. The width and thickness of the left adrenal gland (0.5 and 0.4 cm) are less than those of the right in chickens (0.6 and 0.5 cm, Kober A et al., 2012). The weight and size of the adrenal gland of poultry change during their sexual activity, violation of the detention conditions, stress, and the use of pharmacological drugs (Vyas and Jacob, 1976; Muller et al., 2015).

MICROSCOPY

Capsule and trabeculae

The adrenal gland of poultry is covered on the outside by a capsule consisting of dense fibrous connective tissue, rich in collagen, reticular fibers, and blood vessels with few elastic elements (Figure 3A, B; Moawad and Randa, 2017; Kot and Prokopenko, 2020). In chickens, the thickness of the adrenal capsule of females (22.09 ± 2.17 µm) is greater than in males (18.14 ± 1.82 µm, Kot and Prokopenko, 2020). The thickness of the pigeon adrenal capsule does not exceed 13.46 ± 0.67 µm (Prokopenko, 2022). Ganglia of the autonomic nervous system and chromaffin cells are recorded in the adrenal capsule of quails (El-Desoky and El-Zahraa, 2021), geese (Prokopenko and Kot, 2021b), chickens (Figure 3B, C; Moawad and Randa, 2017; Kot and Prokopenko, 2020),

and guinea fowls (Moghada and Mohammadpour, 2017). Vascularization and encapsulation of the quail's adrenal glands are completed on day 10 of incubation (Basha et al., 2009). In the adrenal gland of chicken, guinea fowl, and geese, trabeculae are directed from the capsule to enter the parenchymal glandular tissues (Moawad and Randa, 2017; Moghadam and Mohammadpour, 2017; Prokopenko and Kot, 2021b). Capsule duck's adrenal glands consist of connective tissue fibers and flattened cells arranged in 2-3 rows with elongated nuclei. According to morphological features, these structures correspond to different fibroblastic cells (Plakhotniuk et al., 2021).

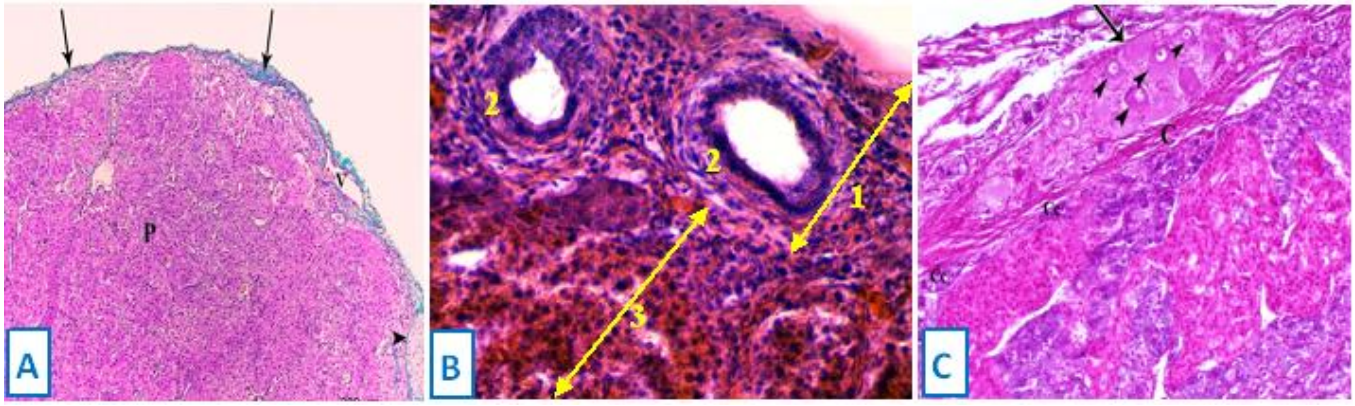


Figure 3. Photomicrographs of the adrenal gland of chickens. (**A**): Black arrows: Collagen fibers in capsule, V: Blood vessels, P: Parenchyma, Arrow head: Septum (Crossmon's trichrome stain, × 100, Moawad and Randa, 2017); (**B**): 1: Capsule, 2: Blood vessels, 3: Parenchyma (H&E, × 400, Kot and Prokopenko, 2020); (**C**): Black arrow: Autonomic ganglia, C: Capsule, Arrow heads: Nerve cells, Cc: Clusters of subcapsular chromaffin cells (H&E, × 200, Moawad and Randa, 2017).

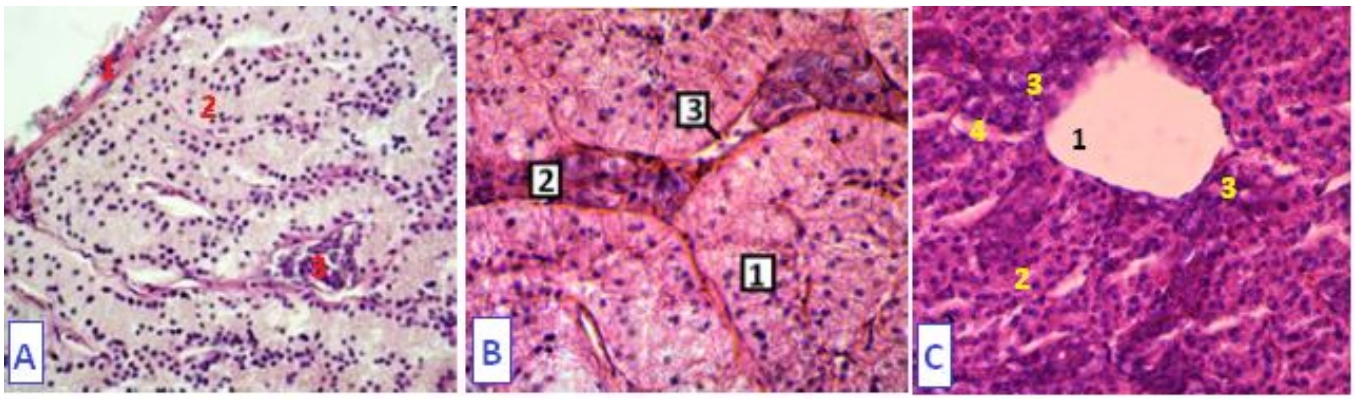


Figure 4. Photomicrographs of adrenal gland in ducks. (**A**): 1: Capsule, 2: Cortical tissues, 3: Medullary tissues (H&E, × 150, Plakhotniuk et al., 2021); in geese (**B**): 1: Cortical tissues, 2: Medullary tissues, 3: Hemocapillary (H&E, × 400, Prokopenko and Kot, 2021b); in chicken (**C**): 1: Lumen of the venous sinus, 2: Cortical tissues, 3: Medullary tissues, 4: Hemocapillary (H&E, × 100, Kot and Prokopenko, 2020).

Parenchima, cortical (interrenal), and medullary (chromaffin) tissue zones

The adrenal parenchyma of poultry consists of cortical and medullary tissues, the cellular strands of which are intertwined throughout the organ (Figures 4A, B; Plakhotniuk et al., 2021; Prokopenko and Kot, 2021b). Narrow gaps between them are filled with layers of loose fibrous connective tissue, in which venous sinuses and hemocapillarie are registered. The wall of the venous sinuses is formed by flat endotheliocytes. In some places, it is intermittent due to sinusoidal hemocapillaries that open into the lumen of the venous sinuses (Figure 4C; Fathima and Lucy, 2014; Moghadam and Mohammadpour, 2017; Kot and Prokopenko, 2020).

According to Basha et al. (2009), adrenal cortical tissue in quails is formed from the peritoneal epithelium and interrenal mesonephros blastema on the third and fourth days of incubation. On the fourth day, cells of the medullary tissue of the adrenal gland migrate from the prevertebral nerve plexus of the ectoderm of the nerve crest to the cortical tissue. The location of cortical cell strands between sinusoids and cell differentiation begins

from the seventh to the tenth day of incubation. Invasion of medullary tissue cells into the cortical tissue is noted on day 18 of incubation.

In some studies, *Columba livia*, *Passer domesticus*, *Corvus splendens*, *Acridotheres tristis*, *Acridotheres ginginianus*, *Milvus migrans*, *Francolinus pondicerianus*, and *Bubulcus ibis* (Vyas, 1976) are used for cortical tissueand medullary tissue, interrenal tissue, and chromaffin tissue to describe the structure of the adrenal parenchyma in quails (El-Desoky and El-Zahraa, 2021), ducks (Fathima and Lucy, 2014), ostrich (Tang et al., 2009; Ye et al., 2017). The name of the first tissue corresponds to its origin, and the name of the second tissue is based on the ability of its cells to restore chromium, silver, and osmium oxi. The adrenal cortex of quail is represented by acidophilic cells. They are large, polyhedral, or columnar in shape, have acidophilic and vacuolated cytoplasm, and have a rounded or oval nucleus located eccentrically (El-Desoky and El-Zahraa, 2021). Regarding the adrenal gland of quails, the nuclei of acidophilic cells are large with nucleoli and coarse chromatin; the cytoplasm contains many mitochondria, ribosomes, lipid droplets, and endoplasmic reticulum (Basha et al., 2009). Depending on the number of lipid droplets and mitochondria, acidophilic cells are divided into two types. Cells of the first type contain numerous lipid droplets with several slightly larger globular mitochondria (Figure 5A, B; Moawad and Randa, 2017), while cells of the second type entail few lipid droplets (Figure 5C; Moawad and Randa, 2017).

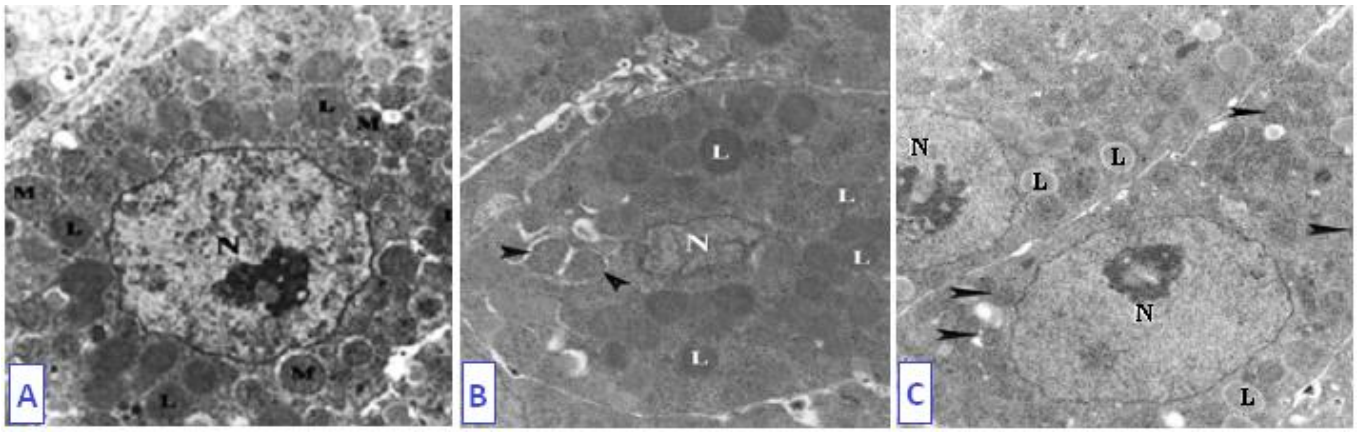


Figure 5. Electron micrographs of the adrenal cortex cells in a chicken. (**A**): N: Spherical basal nucleus, L: Numerous electron dense lipid droplets, M: Few mitochondria (Uranyle acetate and lead citrate stain, × 8000); (**B**): N: Irregular nucleus, L: Many electron dense lipid droplets, Black arrow heads: Few mitochondria (Uranyle acetate and lead citrate stain, × 8000); (**C**): N: Oval basally situated nuclei, L: Few lipid droplets, Black arrow heads: Numerous mitochondria (Uranyle acetate and lead citrate stain, × 8000, Moawad and Randa, 2017).

Basophilic cells are part of the chromaffin tissue of the adrenal glands of poultry. They have a polygonal or rounded shape, basophilic cytoplasm, and a large spherical nucleus located in the center and contains two or three nucleoli. In chickens, the cell height and diameter of their adrenal nuclei in males (8.72 ± 0.231 µm and 4.39 ± 0.359 µm, respectively) are higher than in females (8.67 ± 0.218 µm and 3.84 ± 0.326 µm, respectively, Sarkar et al., 2014). The cytoplasm of basophilic cells contains mitochondria with tubular crosses, ribosomes, endoplasmic reticulum, lipid droplets, and secretory granules (Figures 6A, B; El-Zoghby, 2010; Moawad and Randa, 2017). Depending on the shape of the secretory granules, basophilic cells are divided into two types. Epinephrine cells are composed of homogeneous polymorphic electron-dense secretory granules, and norepinephrine cells contain secretory granules with an electron-dense nucleus bounded by a light border (Figure 6C; Tang et al., 2009; El-Zoghby, 2010; Moawad and Randa, 2017). Prabhavathi et al. (2011) identified epinephrine, norepinephrine, and stellate-shaped satellite cells in the chromaffin tissue of the guinea fowl's adrenal glands. Differentiation of quail adrenal chromaffin cells into epinephrine and norepinephrine cells is completed by day 15 of incubation (Basha et al., 2009). In chickens, ganglion cells are registered among the cells of the medullary tissue of the adrenal glands (Moawad and Randa, 2017).

The placement and configuration of cell strands of cortical (interrenal) and medullary (chromaffin) tissues determine the formation of adrenal parenchyma zones in poultry (Ritchie and Pilny, 2008). Peripheral (subcapsular) and internal (central) zones are distinguished on the incision of the adrenal gland in a chicken (Moawad and Randa, 2017).

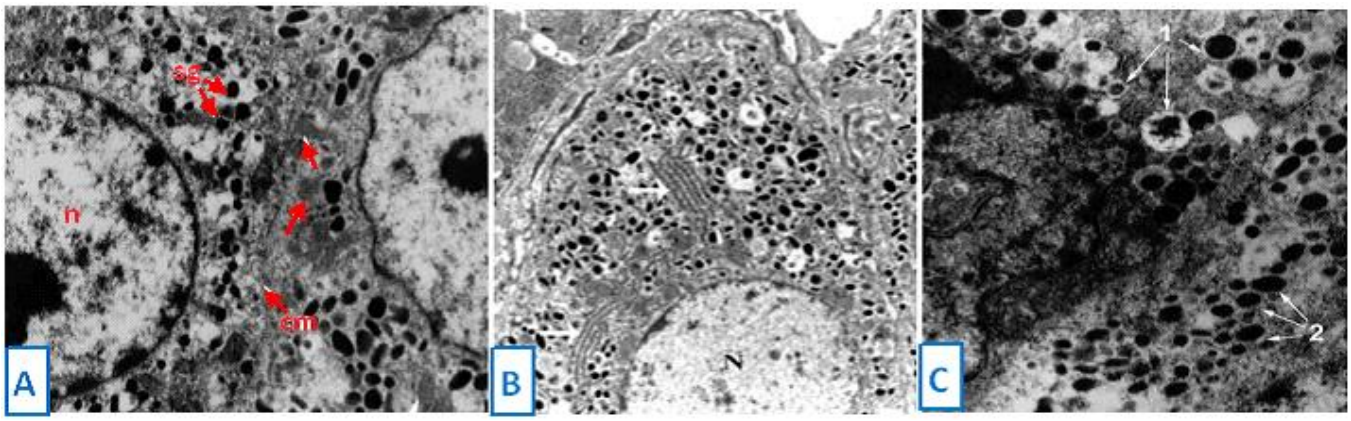


Figure 6. Electron micrographs of chromaffin cells of the adrenal gland in a goose. (**A**): n: nucleus, cm: Cell membrane, Arrows: Mitochondria, sg: Secretory granules (Uranyle acetate and lead citrate stain, × 1000, El-Zoghby, 2010), in chicken (**B**): n: Nucleus, Arrows: Strands of rough endoplasmic reticulum (Uranyle acetate and lead citrate stain, × 8000, Moawad and Randa, 2017), in geese (**C**): 1: Secretory granules of electron dense core surrounded by hollow electron lucent coat of chromaffin cells showing: norepinephrine, 2: Homogenous, polymorphic electron dense secretory granules of chromaffin cells showing: epinephrine (Uranyle acetate and lead citrate stain, × 1200, El-Zoghby, 2010).

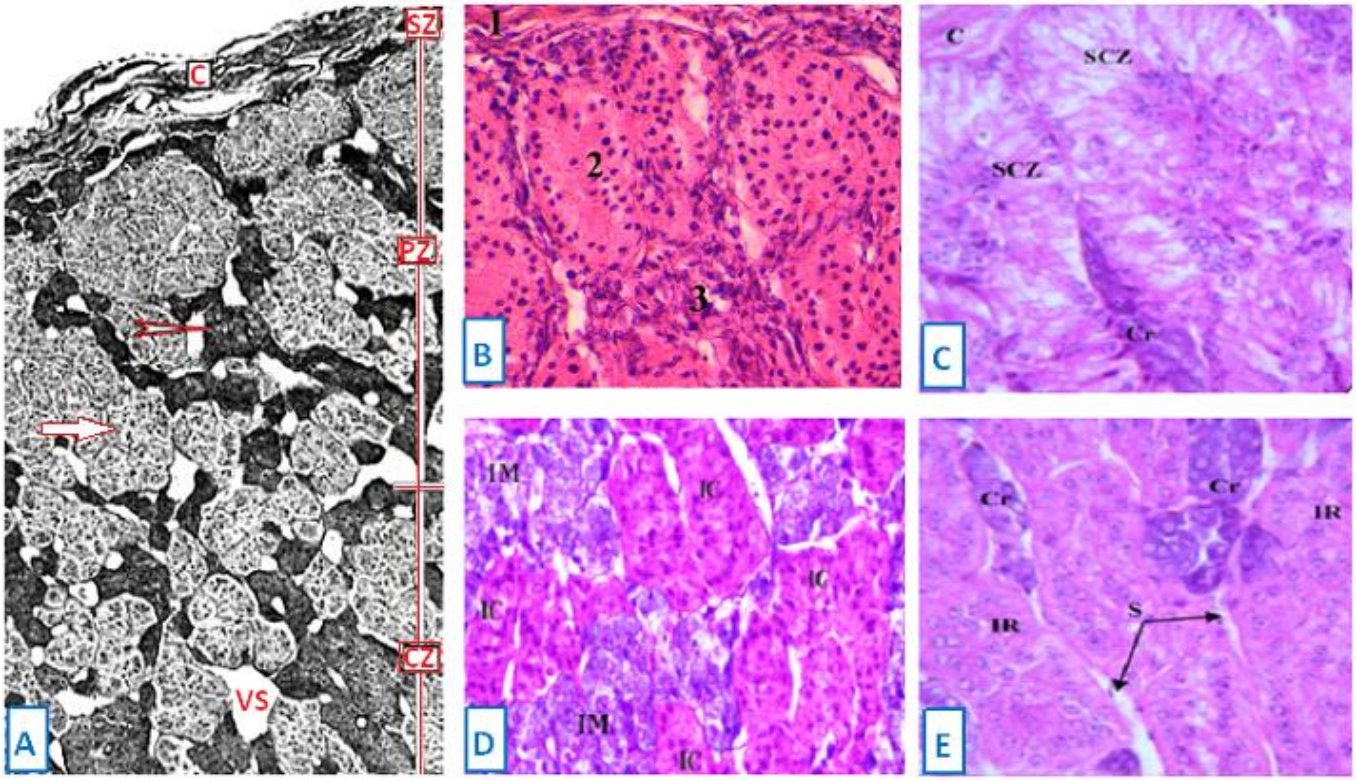


Figure 7. Photomicrographs of adrenal gland in chickens. (**A**): SZ: Subcapsular zone, PZ: Peripheral zone, CZ: Central zone, VS: Venous sinus, C: Capsule, Arrow: Cortical tissue, arrowhead: Medullare tissue (Azan, × 100, Humayun et al., 2012); in chicken (**B**): 1: Capsule, 2: Cortical tissue, 3: Medullare tissue (H&E, × 400, Kot and Prokopenko, 2020); in ostrich (**C**): 1: Capsule, 2: Subcapsular zone, Cr: Chromaffin tissue (H&E, × 400, Tang et al., 2009); in chicken (**D**): IC: Inner cortical cords, IM: Inner medullare cords (H&E, × 400, Moawad and Randa, 2017); in ostrich (**E**): IR: Interrenal tissue, Cr: Chromaffin tissue, S: Venous sinus (H&E, × 400, Tang et al., 2009).

Humayun et al. (2012) recorded a subcapsular layer, peripheral and central zones in the chicken adrenal gland (Figure 7A), which is consistent with the results of studies on guinea fowls (Moghadam and Mohammadpour, 2017) and quails (El-Desoky and El-Zahraa, 2021). The central and peripheral zones are distinguished for the adrenal gland of the ostrich. The latter zone consists of an external part (subcapsular zone) and an internal part (Tang et al.,

2009; Ye et al., 2017). In the subcapsular zone of the adrenal gland, cellular strands of cortical tissue have the appearance of loops in chickens (Figure 7B; Kot and Prokopenko, 2020) and quails (El-Desoky and El-Zahraa, 2021), and a type of bow in an ostrich (Figure 7C; Tang et al., 2009). Islands of medullary tissue are registered between them. In the guinea fowl adrenal gland, the subcapsular zone is represented only by medullary tissue (Moghadam and Mohammadpour, 2017). Cortical tissue cells form straight or arched cords in the central zone of the adrenal gland of chickens (Figure 7D; Moawad and Randa, 2017) and quails (El-Desoky and El-Zahraa, 2021). In the ostrich, the cords of the cortical tissue of the central zone are smooth and located on the right side of the periphery of the adrenal gland (Ye et al., 2017).

Micrometry

In the chicken adrenal gland, the proportion of medullary tissue in the central zone (49.7%) is twice as high as in the peripheral zone (24.8%), and the adrenal cortical-medullary ratio is 1.6:1 (Humayun et al., 2012). According to Sarkar et al. (2014), the adrenal cortical-medullary ratio of chicken in females (1.9:1) and males (1.43:1) is not the same. In duck ontogenesis, the adrenal cortical-medullary ratio changes from 1.15:1 (6 weeks of age) to 2:1 (18 weeks of age) due to an increase in the proportion of cortical tissue. At the beginning of egg laying, the layer hens experience stress, and the adrenal gland releases corticosterone and catecholamines into the blood, which help the adaptation to new conditions (Fathima and Lucy, 2014). Basha et al. (2009) recorded a decrease in the proportion of interrenal tissue and ganglion transformation of adrenal chromaffin tissue during molting in quails.

HISTOCHEMISTRY

Most histochemical reactions by which adrenal medulla hormones are released and detected are based on the property of these hormones to vigorously reduce chromium oxide and osmium silver (Moawad and Randa, 2017). Chromaffin granules of medullary cells of the chicken's adrenal gland acquire a greenish-yellow color in sections prefixed in Ortha's fluid and stained by Giemsa (Figure 8A; Moawad and Randa, 2017).

The adrenal histochemistry of the wild birds (*Columba livia*, *Passer domesticus*, *Corvus splendens*, *Acridotheres tristis*, *Acridotheres ginginianus*, *Milvus migrans*, *Francolinus pondicerianus*, and *Bubulcus ibis*) determined by their sexual activity. During the mating season of wild birds, the interrenal tissue of the central adrenal gland is characterized by the highest content of alkaline phosphatase, glycogen, acidic mucopoly-saccharides, and gross lipids. In the interrenal tissue of the subcapsular layer, moderate content of ascorbic acid is observed. The histochemical characteristics of chromaffin tissue remain unchanged throughout the year, except for acid phosphatase, the amount of which increases with the sexual activity of birds (Vyas., 1976).

In quails, the interrenal tissue is rich in glycogen, lipids, and cholesterol, and the chromaffin adrenal tissue is loaded with acidic mucopolysaccharides and ascorbic acid (Basha et al., 2009). Regarding the staining of histological sections of chicken adrenal glands with Sudan black B and Sudan III, the maximum amount of general and neutral lipids is recorded in cells of the interrenal tissue of the subcapsular zone, slightly less in such cells of the central zone (Figure 8B, C; Moawad and Randa, 2017).

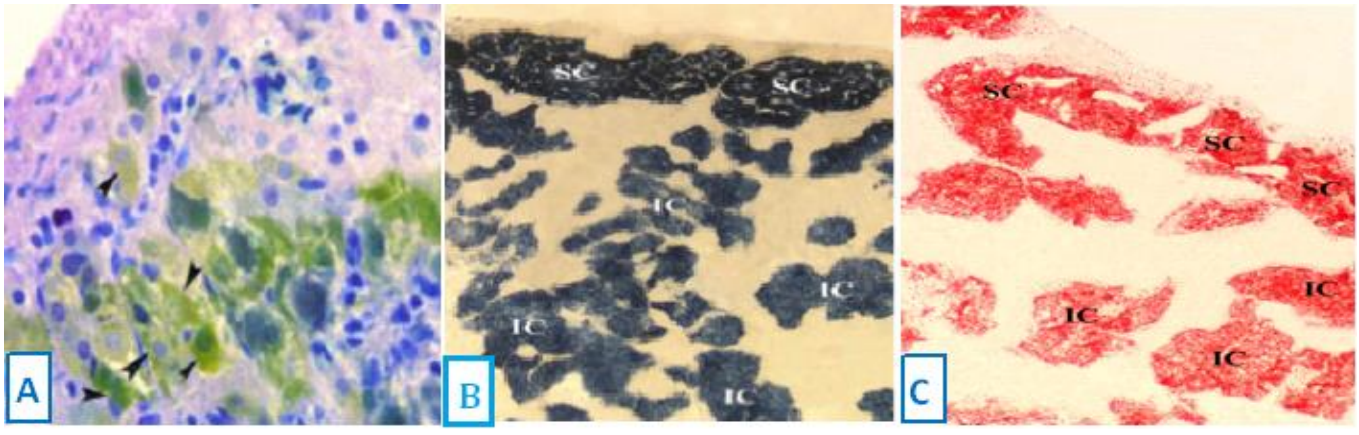


Figure 8. Photomicrographs of the adrenal gland of chickens. (**A**): Black arrow heads: Strong and moderate chromaffin reaction of greenish yellow color in medullary (Giemsa, × 1000), (**B**): SC: Intensive sudanophilic substances fill the subcapsular cortical cells, IC: Less intensive sudanophilic substances fill the inner cortical cells (Sudan black B, × 100), (**C**): SC: Strong positive reactions in subcapsular cortical cords, IC: Moderate reactions in inner cortical cords (Sudan III, × 200, Moawad and Randa, 2017).

CONCLUSION

This review indicated anatomical, histological, ultramicroscopic, histochemical, and morphometric methods of studying the adrenal gland of poultry are a matter of concern. The morphology of the adrenal gland in clinically healthy chickens, ducks, geese, and quails should be studied in depth. The obtained data can be used to assess differences in the morphofunctional state of the adrenal gland in poultry under the effect of various factors and pathological aspects.

DECLARATIONS

Acknowledgments

This research was self-funded and did not receive any financial assistance from any party.

Funding

There was no source of funds for this article.

Authors' contributions

Tatyana Kot contributed to data collection, database creation, and preparation of the manuscript. Svitlana Tkachuk was also involved in preparing the manuscript and data analysis. Svitlana Usenko, and Vladislav Prokopenko guided the research, photomicrographs and electron micrographs analysis, and manuscript preparation. 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 issue, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication, and/or submission, and redundancy.

REFERENCES

Afuwape O, Ladipo JK, Ogun O, Adeleye J, and Irabor D (2009). Pheochromocytoma in an accessory adrenal gland: A case report. Cases Journal, 2: 6271. DOI: https://www.doi.org/10.4076/1757-1626-2-6271

Basha S, Kannan T, and Ramesh G (2009). Age related changes of the adrenal gland in Japanese quail (*Coturnix coturnix japonica*). Tamilnadu Journal of Veterinary & Animal Sciences, 5(5): 198-202. Available at: https://ssrn.com/abstract=3524272

Batool F, Khan HA, and Saif-ur Rehman M (2020). Feeding ecology of blue rock pigeon (*Columba livia*) in the three districts of Punjab, Pakistan. Brazillian Journal of Biology, 80(4): 881-890. DOI: https://www.doi.org/10.1590/1519-6984.225451

Colcimen N and Cakmak G (2020). A stereological study of the renal and adrenal glandular structure of red-legged partridge (*Alectoris chukar*). Folia of Morphology, 80(1): 210-214. DOI: https://www.doi.org/10.5603/FM.a2020.0010

Di Lorenzo M, Barra T, Rosati L, Valiante S, Capaldo A, De Falco M, and Laforgia V (2020). Adrenal gland response to endocrine disrupting chemicals in fishes, amphibians and reptiles: A comparative overview. General and Comparative Endocrinology, 297: 113550. DOI: https://www.doi.org/10.1016/j.ygcen.2020.113550

Domyan ET and Shapiro MD (2017). Pigeonetics takes fight: Evolution, development, and genetics of intraspecific variation. Developmental Biology, 427(2): 241-250. DOI: https://www.doi.org/10.1016/j.ydbio.2016.11.008

El-Desoky SMM and El-Zahraa FAM (2021). Morphological and histological studies of the adrenal gland in the Japanese quail (*Coturnix japonica*). Microscopy Research and Technique, 84(10): 2361-2371. Available at: https://pubmed.ncbi.nlm.nih.gov/33908124/

El-Zoghby IM (2010). Light and electron microscope studies of the adrenal glands of the Egyptian geese (*Alopochen aegyptiacus*). Lucrări ştiinţifice-Medicină Veterinară, 53(12): 195-203. Available at: https://www.cabdirect.org/cabdirect/abstract/20103293050

Erdem E, Ozbaser F, Gurcan E, and Soysal M (2021). The morphological and morphometric characteristics of Alabadem pigeons. Turkish Journal of Veterinary & Animal Sciences, 45(2): 21. DOI: https://www.doi.org/10.3906/vet-2005-58

Fathima R and Lucy KM (2014). Morphological studies on the adrenal gland of kuttanad ducks (*Anas platyrhynchos domesticus*) during post hatch period. Journal of Agriculture and Veterinary Science, 7(6): 58-62. Available at: http://www.iosrjournals.org/iosr-javs/papers/vol7-issue6/Version-3/J07635862.pdf

Gaber W and Abdel-maksoud FM (2019). Interrenal tissue, chromaffin cells and corpuscles of Stannius of Nile tilapia (*Oreochromis niloticus*). Microscopy, 68(3): 195-206. DOI: https://www.doi.org/10.1093/jmicro/dfy146

Gjuen J and Jensen P (2022). Selection for reduced fear of humans changes intra-specific social behavior in red junglefowl-implications for chicken domestication. Genes, 13(1): 43. DOI: https://www.doi.org/10.3390/genes13010043

Hafez MH and Attia YA (2020). Challenges to the poultry industry: Current perspectives and strategic future after the COVID-19 outbreak. Frontiers in Veterinary Science, 7: 516. DOI: https://www.doi.org/10.3389/fvets.2020.00516

Kober A, Aoyama M, and Sugita S (2012). Morphological and histological studies on the adrenal gland of the Chicken (*Gallus domesticus*). The Journal of Poultry Science, 49(1): 39-45. DOI: https://www.doi.org/10.2141/jpsa.011038

Humayun KA, Aoyama M, and Sugita S (2012). Morphological and histological studies on the adrenal gland of the Chicken (*Gallus domesticus*). Journal of Poultry Science, 49(1): 39-45. DOI: https://doi.org/10.2141/jpsa.011038

Kot T and Prokopenko V (2020). Peculiarities of the morphology of the adrenal gland of chickens. Scientific Horizons, 5(90): 82-88. Available at: https://sciencehorizon.com.ua/web/uploads/pdf/419-%D0%A2%D0%B5%D0%BA%D1%81%D1%82%20%D1%81%D1%82%D0%B0%D1%82%D1%82%D1%96-608-1-10-20200722.pdf

Kot TF, Rudik SK, Guralska SV, Zaika SS, and Khomenko ZV (2021). Study of adrenal morphology from antiquity to the present day. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences, 23(101): 75-81. DOI: https://www.doi.org/10.32718/nvlvet10113

Lotfi CFP, Kremer JL, Passaia BS, and Cavalcante IP (2018). The human adrenal cortex: Growth control and disorders. Clinics, 73(1): e473s. DOI: https://www.doi.org/10.6061%2Fclinics%2F2018%2Fe473s

Lotveld P, Fallahshahroudi A, Bektic L, Altimiras J, and Jensen P (2017). Chicken domestication changes expression of stress-related genes in brain, pituitary and adrenals. Neurobiology of Stress, 7: 113-121. DOI: https://www.doi.org/10.1016/j.ynstr.2017.08.002

Manju Madhavan C and Varghese S (2016). Morphology and involution of human adrenal gland during foetal life. Journal of Evolution of Medical and Dental Sciences, 5(80): 5989-5994. DOI: https://www.doi.org/10.14260/jemds/2016/1352

Moawad U and Randa MH (2017). Histocytological and histochemical features of the adrenal gland of adult Egyptian native breeds of chicken (*Gallus Gallus domesticus*). Beni-Suef University Journal of Basic and Applied Sciences, 6(2): 199-208. DOI: https://www.doi.org/10.1016/j.bjbas.2017.04.001

Moghadam DP and Mohammadpour AA (2017). Histomorphological and stereological study on the adrenal glands of adult female guinea fowl (*Numida meleagris*). Comparative Clinical Pathology, 26: 1227-1231. DOI: https://www.doi.org/10.1007/s00580-017-2514-3

Muller BR, Medeiros HAS, de Sousa RS, and Molento CFM (2015). Chronic welfare restrictions and adrenal gland morphology in broiler chickens. Poultry Science, 94(4): 574-578. DOI: https://www.doi.org/10.3382/ps/pev026

Organization for economic cooperation and development/ Food and agriculture organization (OECD/FAO) (2016). OECD/FAO agricultural outlook 2016-2025. OECD Publishing., Paris, pp. 107-116. DOI: https://www.doi.org/10.1787/agr_outlook-2016-en

Organization for economic cooperation and development/ Food and agriculture organization (OECD/FAO) (2019). OECD/FAO Agricultural Outlook 2019-2028. OECD Publishing., Paris, pp. 166-167. DOI: https://www.doi.org/10.1787/agr_outlook-2019-en

Peng S and Broom D (2021). The sustainability of keeping birds as pets: Should any be kept?. Animals, 11(2): 582. DOI: https://www.doi.org/10.3390/ani11020582

Plakhotniuk E, Skobelskaya T, Lemeshchenk V, Kuevda E, and Saenko N (2021). Adaptive morphogenesis of the adrenal glands of ducks in conditions of hyperthermia. IOP Conference Series: Earth and Environmental Science, 937: 022014. Available at: https://iopscience.iop.org/article/10.1088/1755-1315/937/2/022014/pdf

Prabhavathi M, Basha SM, Sundaram V, Venkatasubramanian L, Leela V, and Ramesh G (2011). Electron microscopical study of adrenal gland in guinea foul. Indian Journal of Animal Researchi, 45(3): 215-218. Available at: https://arccjournals.com/journal/indian-journal-of-animal-research/ARCC810

Prokopenko VS (2022). Morphology of the adrenal gland blue rock pigeon (Columba livia L.). Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences, 24(105): 67-72. DOI: https://www.doi.org/10.32718/nvlvet10510

Prokopenko VS and Kot TF (2021a). Macroscopic characteristics of the avian adrenal gland. Bulletin of Sumy National Agrarian University. The Series: Veterinary Medicine, 4(55): 17-23. DOI: https://www.doi.org/10.32845/bsnau.vet.2021.4.3

Prokopenko VS and Kot TF (2021b). Features of the microscopic structure of the adrenal gland of geese. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences, 23(103): 10-14. DOI: https://www.doi.org/10.32718/nvlvet10302

Qureshi S, Khan MN, Shafi S, Mir MS, Adil S, and Khan A (2020). A study on histomorphology of adrenal gland in broiler chickens subjected to cold stress and its ameliorating remedies. International Journal of Current Microbiology and Applied Sciences, 9(4): 1160-1168. DOI: https://www.doi.org/10.20546/ijcmas.2020.904.137

Ritchie M and Pilny A (2008). The anatomy and physiology of the avian endocrine system. Veterinary Clinics of North America: Exotic Animal Practice, 11(1): 1-14. DOI: https://www.doi.org/10.1016/j.cvex.2007.09.009

Rudik O, Kot T, Guralska S, Dovhiy Y, and Zhytova O (2021). Micropathology of the internal organs of Japanese quails naturally infected with *Eimeria tenella*. Journal of World's Poultry Research, 11(3): 322-331. DOI: https://www.doi.org/10.36380/jwpr.2021.38

Kassaby SS, Velilla RE, and Shurbaji MS (2017). Adrenal cortical heterotopia in an inguinal hernia sac of an adult: A casereport and literature review. Human Pathology: Case Reports, 8: 13-15. DOI: https://www.doi.org/10.1016/j.ehpc.2016.08.007

Sarkar S, Islam MN, Adhikary NG, and Paul B (2014). Morphological and histological studies on the adrenal gland in male and female chicken (*Gallus domesticus*). International Journal of Biological and Pharmaceutical Research, 5(9): 715-718.

Scanes CG (2015). Avian endocrine system. Sturkie's avian physiology, Chapter 22, pp. 489-496. DOI: https://www.doi.org/10.1016/B978-0-12-407160-5.00022-1

Scanes CG (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poultry Science, 95(9): 2208-2215. DOI: https://www.doi.org/10.3382/ps/pew137

Scanes CG (2020). Avian physiology: Are birds simply feathered mammals?. Frontiers in Physiology, 11(9): 542466. DOI: https://www.doi.org/10.3389/fphys.2020.542466

Tang L, Peng KM, Wang JX, Luo HQ, Cheng JY, Zhang GY, Sun YF, Liu HZ, and Song H (2009). The morphological study on the adrenal gland of African ostrich chicks. Tissue and Cell, 41(4): 231-238. DOI: https://www.doi.org/10.1016/j.tice.2008.11.003

Tobias J, Sheard C, Pigot A, Devenish A, Yang J, Sayol F, Neate-Clegg M, Alioravainen N, Weeks T, Barber R et al. (2022). Avonet: morphological, ecological and geographical data for all birds. Ecology Letters, 25(3): 571-707. Available at: https://onlinelibrary.wiley.com/doi/epdf/10.1111/ele.13898

Vyas DK and Jacob D (1976). Seasonal study of the adrenal gland of some Indian avian species. Acta Anatomica, 95: 518-528. DOI: https://www.doi.org/10.1159/000144639

Ye LX, Wang JX, Li P, and Zhang XT (2017). Distribution and morphology of ghrelin immunostained cells in the adrenal gland of the African ostrich. Biotechnic & Histochemistry, 93(1): 1-7. DOI: https://www.doi.org/10.1080/10520295.2017.1372631

Zakrevska M and Tybinka A (2019). Histological characteristics of accessory adrenal glands of rabbits with different types of autonomous tonus. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, Series: Veterinary Sciences, 21(93): 125-130. DOI: https://www.doi.org/10.32718/nvlvet9322

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication
J. World Poult. Res. 13(1): 29-40, March 25, 2023

Research Paper, PII: S2322455X2300003-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.3

Thermal Manipulation During Incubation: Effects on Embryo Development, Production Performance, Meat Quality, and Thermal Tolerance of Broiler Chickens

Hèzouwè Tchilabalo Meteyake*, Abidi Bilalissi, Yaah Aimee Emmanuelle Kouame, Ombortime N'nanle, and Kokou Tona

Laboratoire des Techniques de Production Avicoles-Centre d'Excellence Régional sur les Sciences Aviaires-University of Lomé, Togo

* Corresponding authors Email**:** hezouwem0123@gmail.com

Received: 18 November 2022
Accepted: 10 January 2023

ABSTRACT

Thermal manipulations during the embryonic period have positive effects on thermotolerance and the productive performance of broilers subjected to acute heat stress. This study aimed to investigate the potential effects of Thermal manipulation during incubation (TMI) on productive performances and thermotolerance of broiler chickens growing in tropical climates. A total of 900 Cobb 500 broiler chicken eggs from a 35-week-old breeder flock were incubated in standard incubation conditions (37.8°C, 60% relative humidity) until day 7, when they were divided into 3 groups (300 eggs per group). The control group (C) was incubated at standard incubation conditions while T6 and T12 groups were subjected to, respectively, 6 hours/day and 12 hours/day of TMI (T° = 39.5°C, relative humidity = 65%, Embryonic day = 7-16). The relative embryo and albumen weight were determined from 10 to 18 days of incubation. The hatching event was checked between 450 and 504 hours of incubation, and egg hatchability, chick quality, and cloacal temperature were also determined. One hundred and twenty-five chicks from each incubation group were transferred to the farm and raised for 6 weeks. During this period, their post-hatch performances were determined. At week 6, blood samples were collected to measure T3, T4, and corticosterone hormone levels. Then, the 6-week-old broilers were slaughtered to determine meat yield and quality. Results showed that the chick's rectal temperature was significantly reduced in T6 and T12 groups compared to the C group, while hatchability and one-day-old chick weight were not affected. Final body weight and feed conversion ratio were significantly improved in the T12 group, compared to other groups. Thermal manipulation during incubation for 6 and 12 hours significantly reduced mortality rate and pectoralis major muscle drip loss while it increased muscle pH at 24 hours post-mortem (pH24). Corticosterone, T3, and T4 plasma hormone levels at week 6 were also significantly reduced by TMI. Therefore, exposing hatching eggs to 39.5°C and 65% of relative humidity from days 7 to 16 of incubation for 12 hours/day is recommended for the poultry industry in tropical climates.

Keywords: Chronic heat stress, Fast-growing broilers, Hatching and post-hatch performances, Thermal manipulation, Thermotolerance, Meat quality

INTRODUCTION

The poultry industry has been considered in recent years as a promising and emerging sector to satisfy the growing demand for animal protein in developing countries (Ayssiwede et al., 2009). However, this sector is confronted by many constraints, including heat stress. According to Lin et al. (2006), heat stress is the main limiting factor for the poultry industry in tropical and subtropical regions. Temperatures ranging from 35 to 45°C in some parts of the year are common in some tropical areas, causing morbidity and mortality in poultry (Akbarian et al., 2013). The situation is exacerbated by the globally averaged surface temperature projected to increase by 1.4 to 5.8°C over the period 1990 to 2100 (IPCC, 2001). It is known that the optimal temperature

range for broiler and layer production is 18-22°C (Charles, 2002; Aengwanich and Simaraks, 2004), with slight variations on both sides due to differences in age, sex, and line/strain. However, in the tropics and subtropics, chickens are generally kept in open houses and are therefore exposed daily to high environmental temperatures. Therefore they are susceptible to heat stress.

The ambient heat is one of the major constraints in poultry farming because of the enormous economic losses it causes in terms of mortality and reduction of productivity (Tesseraud and Temim, 1999). Heat stress causes a disturbance in the physiological status of chickens, including a reduction in certain hormones (Mujahid et al., 2007). Low T3 and high T4 hormone levels were observed in 42-day-old broilers under cyclic heat stress (Bueno et al. 2017). Sohail et al. (2010) also found an increase in plasma corticosterone levels in broilers reared under heat stress for 8 hours per day from 22 to 42 days.

The efficiency of certain vital functions, such as thermoregulation, is often impaired in broilers because the genetic selection of broiler strains is mainly aimed at increasing the growth rate of the animals (Havenstein et al., 2003). The cardiovascular and pulmonary systems, which play an important role in poultry thermoregulation, are less developed, therefore, cannot cope with the broiler chickens' growth rate (Havenstein et al., 2003). This makes them more sensitive to ambient heat and vulnerable to metabolic problems such as ascites, sudden death syndrome, and leg problems (De Smit et al., 2005). This is compounded by the fact that these commercial broilers (Cobb and Ross), usually raised in tropical and subtropical climates, are not mainly selected for these climates.

Various approaches, including genetic, technical, or feeding strategies, can be implemented to improve the tolerance of chickens to variations in thermal conditions (Oke et al., 2017; Oke, 2018, Meteyake et al., 2020). Heat acclimation is a strategy that could increase the thermotolerance of fast-growing broilers (Meteyake et al., 2020). The embryonic and early postnatal periods are the best times to improve the heat tolerance of chickens through thermal manipulation (Yahav 2009). When broilers are exposed to thermal stress during these periods, they acquire thermotolerance more easily in continued life (Al-Zghoul et al., 2013; Loyau et al., 2016). It was reported that adaptation to environmental conditions could be manipulated by exploiting the immaturity of the thermoregulatory mechanism system of chickens at the perinatal stage (Yahav and Mcmurtry 2001). In order to improve the thermal tolerance and welfare of poultry without altering growth and, thus, the economic viability of poultry industries, numerous studies on thermal manipulations in the perinatal and neonatal period have been conducted (Piestun et al., 2008; Nideou et al., 2019; Meteyake et al., 2020). These techniques induce rapid physiological and metabolic changes that persist for the life of the animal (Collin et al., 2011). A study by Meteyake et al. (2020) indicated positive effects of embryonic and neonatal acclimation techniques when applied individually, and in combination on Ross 308 broilers raised in a tropical climate; however, some deleterious effects, such as low hatchability, were observed. It became necessary to conduct further studies to establish optimal techniques. The adverse effect observed could be due to the duration of thermal manipulation applied to hatching eggs (Meteyake et al., 2020). Additionally, despite the plethora of reports on the thermotolerance of chickens, there is a scarcity of information on the effect of thermal manipulation duration during incubation on the productive performances of broilers reared in a tropical climate and in real uncontrolled environmental conditions (temperature and humidity). Thus, the present study aimed to investigate the effects of thermal manipulation during incubation (TMI) on, first, embryo development and production performances, second, thermo-tolerance, and third, meat quality of broiler chickens reared under a chronic hot environment.

MATERIAL AND METHODS

Ethical approval

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Experimental Animals (008/2021/BC-BPA/FDS-UL) of the University of Lome, Togo.

Study design

A total of 900 Cobb 500 broiler chicken hatching eggs from 35-week-old broiler breeders, provided by Incubel Hoogstraten, Belgium, were used for this study. Before incubation, these eggs were weighed, identified, and incubated in the same incubator (Petersime® Vision, Zulte, Belgium) at standard conditions (37.8°C and 60% of relative humidity [RH]) until day 7. Eggs were then divided randomly into three incubation treatment groups of 300 eggs each, including the control group (C group) and two thermal manipulation groups (T6 and T12 groups, Figure 1). The control group (C) was maintained at standard conditions while T6 and T12 groups were

subjected to a temperature of 39.5°C and 65% of RH for 6 and 12 hours daily, respectively, during the embryonic day (ED) 7-16. On day 18 of incubation, all the eggs were candled, and those with evidence of living embryos were weighed and transferred from the turning trays to hatching baskets, where they were subjected to standard conditions until ED 21.

At ED 21, 125 chicks per incubation group, selected randomly, were transferred to the farm and raised for 6 weeks. After 10 days of brooding, the chickens were raised at a natural ambient temperature, humidity, and ventilation in an open-sided poultry house (25 broilers with 5 replicates per group) and were randomly assigned to floor pens). They were reared on a floor litter with a stocking density of 10 broilers per m^2 (2 x 1.25 x 1.8 m for each pen) from 10 days of age onward and were subjected to the same prophylactic and light program (23 hours of light and 1 hour of darkness). All the chicks were vaccinated against the infectious bursal disease (BUR-706, France) vaccine via drinking water at the age of 5 and 19 days. Newcastle disease (Avinew® NeO, Boehringer Ingelheim, Lyon, France) and Avian infectious bronchitis disease (Bioral, Boehringer Ingelheim, Lyon, France) vaccines were also orally administrated at the age of 7 and 21 days. All the broilers were fed *ad libitum* and received the same non-pelleted feed (Table 1). At 6 weeks of age, 15 chickens per group were slaughtered for meat yield, and quality measurement, and blood samples were collected to determine levels of T3, T4, and corticosterone hormones.

Table 1. Calculated composition of experimental feed during the starter (0-10 days of age) and grower (11-42 days of age)

Ingredient (%)	Starter (1-10 days)	Grower (10-42 days)
White maize	53.5	62
Wheat bran	4	3
Fish meal[1]	8	8
Roasted soybeans	21.5	19.5
Brewery by product	5	2
Oyster shell	2	2.5
Broiler concentrate	5	2
Dl-methionine	0.5	0.5
L-lysine	0.5	0.5
Total	100	100
Chemical nutritional characteristics		
Metabolizable energy (kcal/kg)	3000	3065
Crude protein (%)	21.30	19.59
Crude fiber (%)	4.9	4.68
Lysine	1.45	1.33
Methionine ((%)	0.98	0.88
Methionine + cysteine (%)	1.20	1.08
Calcium	1.55	1.34
Total phosphorus	0.56	0.61

[1]A commercial fish meal containing 40% crude protein produced in Senegal and used by West African breeders.

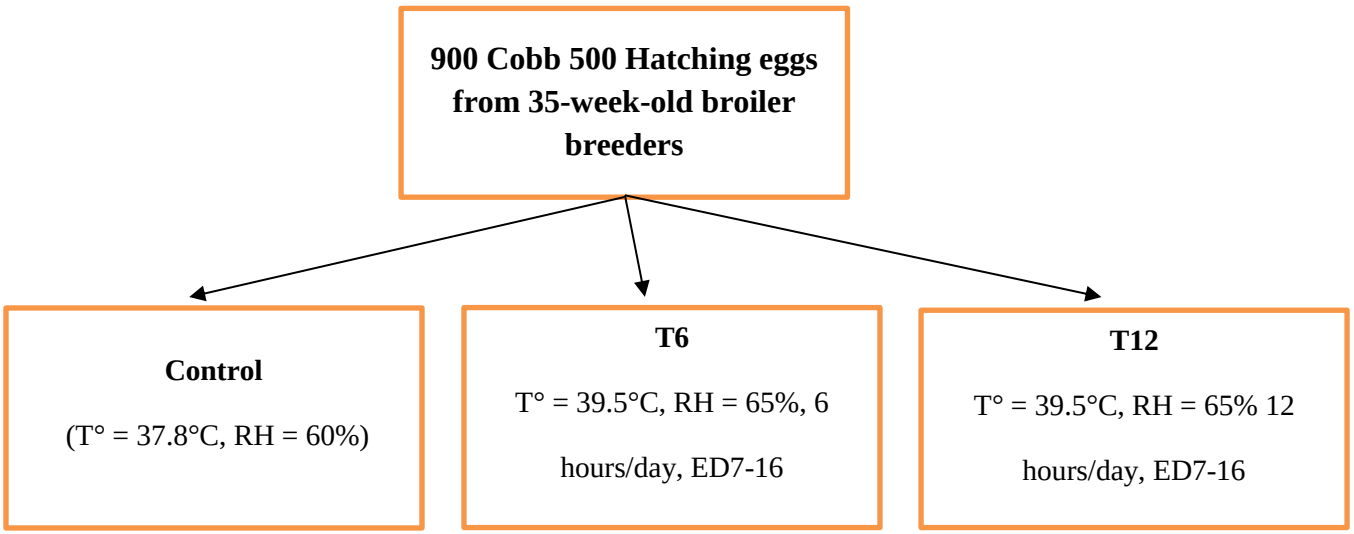


Figure 1. Study design. RH: Relative humidity; T°: Temperature; ED: Embryonic day

Data collection

Embryo and albumen weight, hatching event, hatchability, and chick quality

At embryo days 10, 12, 14, 16, and 18, a total sample of 10 eggs per group was broken, and the embryo and albumen weight were measured. These data were used to determine the Relative embryo weight (REW) and Relative albumen weight (RAW) using Formula 1:

$$\text{REW or RAW (\%)} = \frac{\text{Absolute Embryo or Albumen weight}}{\text{Egg weight}} \text{ X } 100 \qquad \text{(Formula 1)}$$

From 450 to 504 hours of incubation, the time of external pipping (EP) and hatching for individual eggs were recorded every three hours. The hatched chicks were recorded. These data were used to determine the external pipping time (time between setting and external pipping), external pipping duration (duration between external pipping and hatching), and the total incubation duration (time between setting and hatching). Fertile hatchability and total embryo mortality (TEM) were also determined using formulas 2 and 3.

$$\text{Fertile hatchability (\%)} = \frac{\text{Number of hatched chicks at the end of incubation}}{\text{Number of fertile eggs transferred to the hatching basket}} \text{ X } 100 \quad \text{(Formula 2)}$$

$$\text{Total embryo mortality (\%)} = \frac{\text{Number of unhatched but fertile eggs}}{\text{Number of fertile eggs transferred to the hatching basket}} \text{ X } 100 \quad \text{(Formula 3)}$$

One-day-old chick body weights and quality

At 21 days of incubation, the hatched chicks were weighed, and the chick quality was determined according to the Tona scoring method (Tona et al., 2003). According to this method, physical parameters were scored, including reflex, down and appearance, eyes, the conformation of legs, navel area, yolk sac, remaining membranes, and yolk. The chick quality score was defined as the sum of the scores assigned to each quality parameter.

Meteorological data during the rearing phase

Temperatures and RH in the poultry house were recorded daily at 07:00 a.m., 10:00 a.m., 01:00 p.m., and 04:00 p.m. using thermo-hygrometers (HTC-1, China). These data were used to calculate the temperature-humidity index (THI) using Formula 4 used by Bueno et al. (2020):

$$\text{THI} = 0.8\text{T} + \left(\frac{\text{RH}(\text{T}-14.3)}{100}\right) + 46.3 \qquad \text{(Formula 4)}$$

THI: Temperature-humidity index, T: Ambient temperature, RH: Relative humidity

Post-hatch growth performance and mortality rate

At the end of the hatch, chicks were transferred to the farm and weighed to determine their initial body weight. During the experimental period, amount of feed consumption, body weight, and mortalities were recorded weekly. Feed intake was determined as the difference between the amount of feed given and the remaining feed. The body weight gain was calculated as the difference between initial and final body weight. These data were used to determine the feed conversion ratio by dividing feed intake by body weight gain.

Blood sample collection and hormonal analysis

At an internal pipping stage, at hatch, and 6 weeks post-hatch, the blood samples (n = 15 per group) were collected (from the jugular vein at IP stage and brachial veins at hatch and at 6 weeks post-hatch) in heparinized tubes and centrifuged at 3,000 rpm for 15 minutes. The samples of plasma obtained were stored in a freezer at -20°C. A volume of 100 µL of plasma was used for tri-iodothyronine (T3), thyroxin (T4), and corticosterone concentrations determination by using the automated VIDAS systems, which is an enzyme-linked fluorescent assay (ELFA) technique. Antibody anti-T3 of mutton, marked by phosphatase alkaline and sodium azide, antibody anti-T4, marked by phosphatase alkaline and methylisothiazolone, and a derivative of cortisol, marked by phosphatase alkaline and sodium azide, provided by VIDAS were used, for the determination of the concentrations of T3, T4, and corticosterone, respectively. All the samples were run in a certain assay for each hormone to avoid inter-assay variability. Corticosterone concentration was determined just at 6 weeks of age.

Body and surface temperature

Neck and breast temperature were measured on 30-day-old chicks per group using an infrared thermometer. The rectal temperature was also measured using an electronic thermometer.

Meat yield and quality

At week 6, 15 broilers per group were slaughtered. Their carcass, breast, and tigh were weighed to calculate their yields in relation to body weight. The ultimate pH (pHu or pH24) of the *pectoralis major* muscle was measured at 24 hours postmortem using a pH meter (HI9125 portable waterproof pH/ORP meter, HANNA Instrument, Italy) by inserting the electrode in the left pectoralis major muscle (Zhang et al., 2017). The drip loss of *pectoralis major* muscle was also determined, as described by Zhang et al. (2017). Meat samples with a size of 3 cm (length) × 2 cm (width) × 1 cm (thickness), were weighed (W1) and suspended parallel to the longitudinal axis of the myofibers in netting and vacuumed bags and stored at 4°C. Samples were weighed after 48 hours (W2) hanging. The drip loss was calculated using the following formula:

$$\text{Drip loss at 48 hours (\%)} = \frac{W1 - W2}{W1} \text{ X } 100 \quad \text{(Formula 5)}$$

Where, W1 is weight at slaughter time and W2 denotes weight 48 hours after slaughter.

Statistical analysis

Graph Pad Prism 8.0 statistical analysis software was used for data analysis. ANOVA one-way test was used for statistical analysis. Shapiro-Wilk's test was used to check the normal distribution of data, and Levene's test to prove the homogeneity of variance. Percentage data were transformed into Arcsin values and then re-transformed into the original values after the analysis. The comparison between the different groups after ANOVA was made using the Tukey test. The significant level was set at $p < 0.05$.

RESULTS

Embryo weight

The evolution of the absolute and relative weights of embryos according to the treatments has been shown in Figure 2 (A and B). The figures show the increasing trajectory of absolute and relative embryo weights with age. The embryos of the three groups had similar absolute weights from day 10 to 16 ($p > 0.05$). However, on day 18 of incubation, embryos from C and T12 groups had a higher absolute weight than those of the T6 groups ($p < 0.05$). Regarding relative weight, the significant effect of embryo acclimation was only observed on days 14 (C = T12 > T6; $p < 0.05$) and 16 (C = T6 > T12; $p < 0.05$) of incubation.

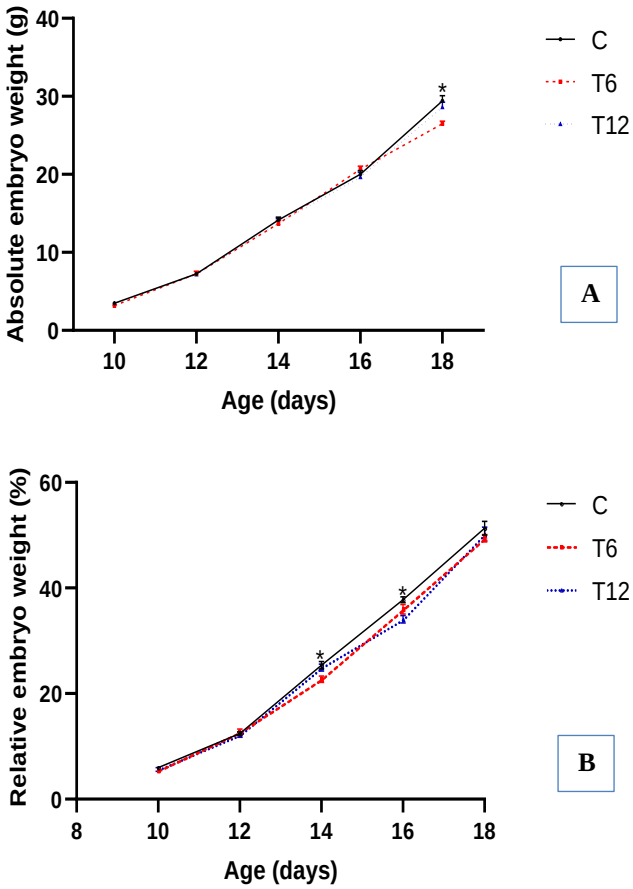


Figure 2. Absolute (A) and relative embryo weight (B) according to thermal manipulation and chicken embryo age. *: significant difference; $p < 0.05$; C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

Albumen weight

Absolute and relative albumen weights decreased with embryo age (figures 3A and 3B). Absolute and relative albumen weights were similar in all three groups except on day 16 of incubation, where the C group had a lower absolute weight ($p < 0.05$) and a lower relative weight ($p < 0.05$) than the other groups (T6 and T12).

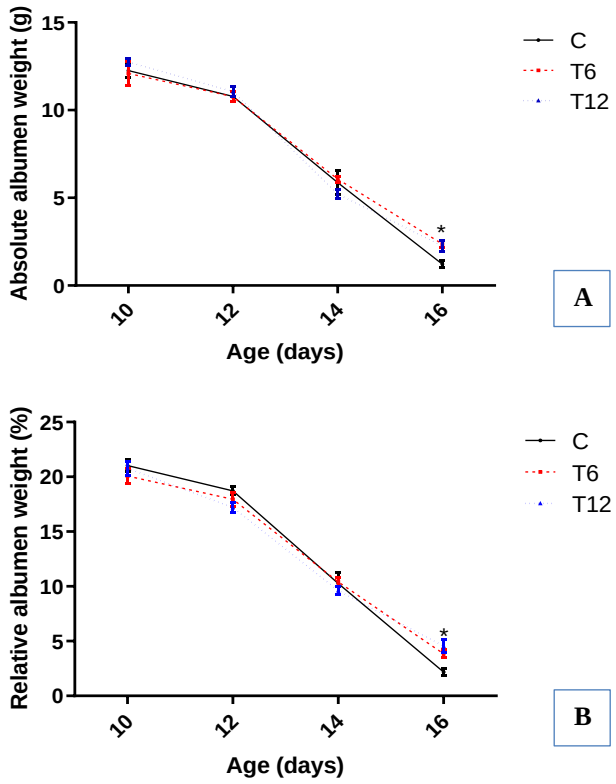


Figure 3. Absolute (A) and Relative albumen weight (B) according to thermal manipulation and chicken embryo age. *: significant difference; $p < 0.05$; C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

Egg weight loss at 18 days of incubation and hatching performance

The effect of thermal manipulation during incubation (TMI) on egg weight loss and hatching performance has been shown in Table 2. The egg weights before setting were 62.08 ± 0.17 g, 62.12 ± 0.17 g, and 62.25 ± 0.17 g, respectively in C, T6, and T12 groups. Egg weight loss at 18 days of incubation was higher in the control group compared to the T12 group ($p < 0.05$), however, there was no significant difference between T6 and other groups. Both duration of thermal manipulation significantly increased external pipping time ($p < 0.05$). The T6 group had external pipping about 4 hours and 2 hours after C and T12 groups, respectively. The T6 group had a significantly lower external pipping duration and a higher total incubation duration, compared to the other groups ($p < 0.05$). The T12 group had no significant external pipping duration and total incubation duration compared to the control. Hatchability and body weight of chicks of all groups at hatch were not significantly ($p > 0.05$) different among all the groups, while TMI during 12 hours decreased the chick's quality ($p < 0.05$).

Rectal, neck, and breast temperature

The effect of heat treatments on rectal, neck, and breast temperatures is shown in Table 3. The rectal temperature of chicks in the T12 group was significantly the lowest ($p < 0.05$). It was followed by the temperature of T6 group and C group, which had the highest rectal temperature. Regarding neck and breast temperature, it was significantly higher in the T6 group compared to the other groups ($p < 0.05$).

Meteorological data in the opened poultry house

The evolution of ambient temperature, RH, and THI in the poultry house is shown in Table 4. The ambient temperature increased to reach a peak at 01:00 pm and then decreased. The RH followed an opposite trend to that of ambient temperature. The highest THI was reached at 01.00 pm.

Table 2. Effect of thermal manipulation on hatching process, fertile hatchability, body weight, and quality of broiler chickens

Parameters	C	T6	T12	p value
Egg weight loss at ED 18 (%)	14.38 ± 0.15^{a}	13.7 ± 0.2^{ab}	13.41 ± 0.19^{b}	0.0007
EP time (hours)	466.1 ± 0.4394^{c}	470.8 ± 0.5321^{a}	468.6 ± 0.5287^{b}	<0.0001
EP duration (hours)	15.71 ± 0.508^{a}	13.57 ± 0.429^{b}	14.03 ± 0.556^{ab}	0.0059
Incubation time (hours)	481.8 ± 0.498^{b}	484.4 ± 0.465^{a}	482.6 ± 0.526^{b}	0.0005
Fertile hatchability (%)	84.68 ± 2.829	84.44 ± 2.750	83.59 ± 2.922	0.9602
Day old chick body weight (g)	44.89 ± 0.386	44.69 ± 0.451	44.27 ± 0.468	0.6121
Tona score (%)	85 ± 0.8460^{a}	82 ± 1.046^{a}	71 ± 1.369^{b}	<0,0001

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$) C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment, EP: External pipping, ED: Embryonic day

Table 3. Effect of thermal manipulation on rectal, neck, and side temperature of broiler chickens at hatch (at day 21 of incubation)

Parameters	C	T6	T12	p value
Rectal temperature (°C)	38.89 ± 0.07^{a}	38.59 ± 0.08^{b}	38.20 ± 0.0795^{c}	<0.0001
Neck temperature (°C)	36.34 ± 0.1635^{b}	37.12 ± 0.1680^{a}	36.51 ± 0.2294^{ab}	0.0121
Breast temperature (°C)	37.18 ± 0.1908^{b}	38.16 ± 0.1989^{a}	37.28 ± 0.2549^{b}	0.0041

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$) C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment, °C: Degree Celsius

Post-hatch performance

The post-hatch performance of the broiler chickens according to treatment is shown in Table 5. Daily feed intake (DFI) was reduced significantly by the thermal treatment ($p < 0.05$). The body weight at 6 weeks of age and the average daily weight gain were increased significantly by a TMI of 12 hours ($p < 0.05$). The feed conversion ratio was reduced significantly in the T12 group compared to the C group ($p < 0.05$). The FCR of the T6 group was not significantly different from the C group and T12 group. The mortality rate (MR) was significantly reduced by TMI ($p < 0.05$). The lowest MR was recorded in the T12 group.

T3, T4 and corticosterone hormone

The effect of TMI on thyroid hormone levels at the internal pipping stage, hatch, and at 6 weeks post-hatch is shown in Table 6. Regardless of the stage (at the internal pipping stage, at hatch, and at 6 weeks post-hatch), the level of T3 was significantly lower in TMI groups, compared to the control ($p < 0.05$). The level of T4 at the internal pipping and 6 weeks post-hatch was significantly lower in the T6 and T12 groups compared to the control group ($p < 0.05$). Plasma corticosterone concentration at 6 weeks of age was significantly lower in T6 and T12 groups compared to the control group ($p < 0.05$, Figure 4).

Meats yields, ultimate pH, and drip loss

The effect of thermal treatment on meat yields is shown in Table 7. The carcass yield of the control group was significantly lower than the other groups, which had similar carcass yields ($p < 0.05$). The thigh yield was not affected by thermal manipulation, while breast yield was increased significantly with 12 hours of TMI compared to the control group ($p < 0.05$). The pHu of meat was significantly higher in T6 group compared to the control group ($p < 0.05$, Figure 5), while drip loss was reduced significantly by thermal manipulation irrespective of TMI duration ($p < 0.05$, Figure 6).

Table 4. Temperature-humidity index, mean temperature, and relative humidity values in the open poultry house during the rearing phase

Schedules of the day / Parameters	7.00 am	10.00 am	01.00 pm	04.00 pm
Temperature (°C)	25.91 ± 0.25	30.5 ± 0.29	33.5 ± 0.2	29.77 ± 0.4
Relative Humidity (%)	46.665	44.835	35.815	39.335
THI	72.44	77.96	79.97	76.20

°C: Degree Celsius, THI: Temperature- humidity index

Table 5. Effect of thermal manipulation on post-hatch performance of broiler chickens

Parameters	C	T6	T12	p value
DFI (g)	76.72 ± 0.46^{a}	70.28 ± 0.411^{b}	70.03 ± 0.551^{b}	< 0.0001
DWG (g)	37.78 ± 0.34^{b}	37.72 ± 0.22^{b}	39.22 ± 0.28^{a}	0.0073
FCR	2.03 ± 0.05^{a}	1.89 ± 0.06^{ab}	1.79 ± 0.09^{b}	0.0335
MR (%)	9.09 ± 0.52^{a}	6.58 ± 0.60^{b}	3.927 ± 0.672^{c}	0.0007
FBW (g)	1631.6 ± 15.5^{b}	1629 ± 12.2^{b}	1691 ± 16^{a}	0.0250

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$) C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment, DFI: Daily feed intake, DWG: Daily weight gain, FCR: Feed conversion ratio, MR: Mortality rate, FBW: Final body weight.

Table 6. Effect of thermal manipulation on thyroid hormones of broiler chickens

Stage	Parameters	C	T6	T12	p value
IP	T3 (nmol/l)	27.02 ± 0.646[a]	16.29 ± 0.962[b]	12.68 ± 0.114[c]	<0.0001
	T4 (nmol/l)	33.32 ± 0.356[a]	25.21 ± 0.778[b]	19.31 ± 0.009[c]	<0.0001
Hatch	T3 (nmol/l)	3.748 ± 0.386[a]	2.049 ± 0.170[b]	2.542 ± 0.153[b]	0.0012
	T4 (nmol/l)	24.63 ± 0.934[b]	36.09 ± 0.329[a]	37.91 ± 0.832[a]	<0.0001
Six weeks post-hatch	T3 (nmol/l)	2.763 ± 0.018[a]	2.11 ± 0.1091[c]	2.438 ± 0.0414[b]	<0.0001
	T4 (nmol/l)	16.29 ± 0.464[a]	6.025 ± 0.221[c]	9.728 ± 0.2425[b]	<0.0001

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$). C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment, nmol/l: nanomol per liter, IP: Internal pipping

Table 7. Effect of thermal manipulation on meat yields of broiler chickens

Parameters	C	T6	T12	p value
Carcass yield (%)	70.21 ± 0.2222[b]	72.43 ± 0.4498[a]	72.74 ± 0.3032[a]	<0.0001
Breast yield (%)	22.82 ± 0.3277[b]	23.50 ± 0.3673[ab]	24.46 ± 0.2934[a]	0.0044
Thigh yield (%)	22.14 ± 0.4907	21.95 ± 0.4907	20.89 ± 0.5618	0.1668

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$) C: control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

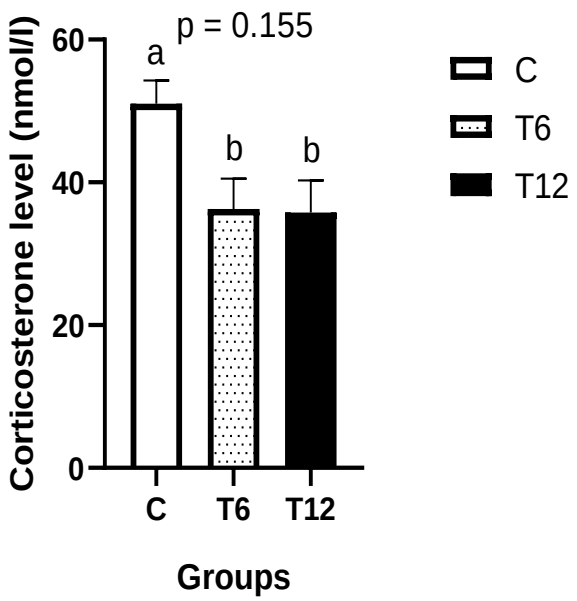


Figure 4. Effect of thermal manipulation during incubation on plasma corticosterone concentration of 6 weeks old broiler chickens. [a,b] Different letters indicate significant ($p < 0.05$) differences among treatments C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

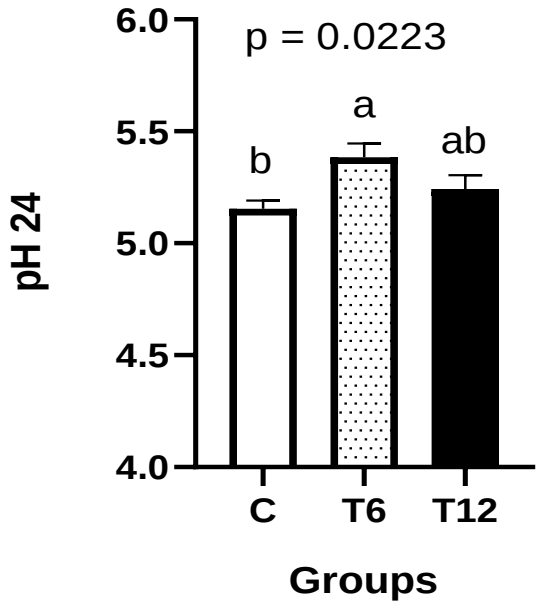


Figure 5. Effect of thermal manipulation on meat ultimate pH of broiler chickens. [a,b] Different letters indicate significant ($p < 0.05$) differences among treatments C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

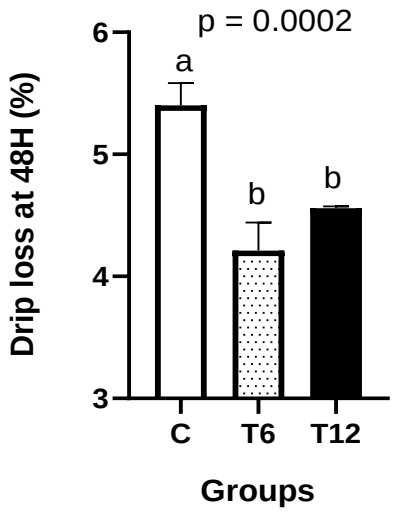


Figure 6. Effect of thermal manipulation on meat drip loss of broiler chickens at 48 hours post-slaughter. [a,b] Different letters indicate significant ($p < 0.05$) differences among treatments C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

DISCUSSION

This study clearly showed that the duration of thermal manipulation during incubation affected embryo development, hatching and post-hatching performance, meat quality, and thermotolerance of Cobb broilers. Regarding the effects of TMI on embryo development at certain embryonic ages, the present study indicated a significant effect of TMI on absolute and relative yolk sac, albumen, and embryo weights. These results are similar to those of Molenaar et al. (2011) and Maatjens et al. (2014), who observed that eggshell temperature influenced yolk absorption and embryo development during incubation.

Regarding hatching performance, similar to the findings of a study by Meteyake et al. (2020), indicating the 12-hour TMI reduced egg weight loss at 18 days of age. The increase in incubation temperature was combined with 65% RH to reduce water loss caused by the high temperature. Relative humidity of 65% appears too high for a 12 h TMI but not for 6 hours since egg weight loss was similar to controls in the group subjected to a 6 hours TMI.

Incubation time was longer in T6 group compared to T12 group, which had a similar incubation time to the control group in the present study. Thermal manipulation during incubation would have reduced the metabolism of the embryos. This could explain why the relative weight of embryos in the T6 group was lower compared to the other groups at certain ages. Moraes et al. (2004) observed a delay in the hatching processes of eggs incubated at 39.0°C for 2 hours per day from day 13 to day 17 of incubation.

Higher incubation temperature depresses hatchability depending upon the embryogenesis stage and temperature's duration and intensity (Halle and Tzschentke, 2011). Thermal manipulation during incubation did not significantly affect hatchability and chick weight in the current study. The present results are in contrast with those of Meteyake et al. (2020), who observed a reduction in hatchability and body weight of Ross 308 day-old chicks subjected to TMI (39.5°C and 65% for 12 hours). This discrepancy could be attributed to the genetic strain of the broiler (Cobb 500) used in the present study. Therefore, a TMI applied for 12 hours would not be enough to induce an augmentation of embryonic mortality in Cobb 500 strain. However, a decrease in the quality of chicks from the eggs subjected to TMI of 12 hours was observed. This result is similar to the studies by Piestun et al. (2015) and Meteyake et al. (2020). A duration of 12 hours of thermal manipulation was sufficient to elicit negative effects on the navel closure and then the chick's quality in this study.

Thermal manipulation during incubation reduced the rectal temperature of hatched chicks in the present study. Collin et al. (2007), Al-Zghoul et al. (2015), Al-Rukibat et al. (2017) observed a low rectal temperature in chicks hatched from the eggs subjected to TMI treatments. This reduction in the body temperature suggested a reduction in metabolism, and thus, better thermotolerance, as evidenced by T3 levels in day-old chicks subjected to TMI. Thermal manipulation during incubation of 6 hours increased the body temperature of the neck and breast in day-old chicks. These results are similar to those reported by Morita et al. (2016), who observed an increase in head, back, and neck temperatures in day-old chicks from eggs subjected to continuous heat treatment of 39°C from day 13 of incubation to hatching. This thermal treatment would have reduced the thickness of the skin and increased the density of blood vessels in the skin, thereby increasing the thermolysis capacity of the chicks. These results were not observed in chicks from the eggs subjected to 12 hours of TMI. This suggests that the duration of TMI would affect broilers' thermolysis and, thus, their thermotolerance.

During the post-hatch growth phase, the chickens were reared in an open-sided poultry house in a tropical temperature where the daily ambient temperature and RH were highest between 10:00 am and 01:00 pm. The ambient temperature during this period exceeded the

optimal rearing temperature (between 22 and 24°C) for adult chickens. It was observed that temperature and RH were inversely proportional. The cyclic variation of ambient temperature and RH in the current study is consistent with environmental conditions in tropical climates. Temperature-humidity index (THI) is an indicator which is used when the effects of heat stress are evaluated (Zulovich et al., 1990). According to Purswell et al. (2012), thermal comfort indices, such as the THI integrate the effects of temperature and humidity and may offer a means to predict the effects of thermal conditions on performance. The THI values were as high as 80 around 10:00 a.m. and even more at 1:00 p.m. This shows that the broilers were thermally stressed every day throughout the experiment, as it has been shown that THI values above 79.92 indicate that hens are exposed to heat stress (Yakubu et al., 2018).

In this study, the higher daily weight gain and final body weight of chickens in the T12 group are consistent with those of Al-Zghoul et al. (2019). It was reported that TMI improved broiler weights by stimulating muscle development and growth via myoblast proliferation (Piestun et al., 2015). This effect was not observed in the T6 group, suggesting that a TMI during 6 hours in the current study was insufficient to stimulate myoblast development and growth.

The feed intake of the chickens decreased as a function of TMI duration in this study. This result confirms that Meteyake et al. (2020) observed a decreased feed intake in acclimated chickens. The decrease in feed intake would be due to a decrease in metabolism and, thus, heat production, corresponding to the low plasma T3 and T4 levels in 6-week-old acclimated chickens. The levels of thyroid hormones are important indicators of metabolic activity (Todini, 2007). Jenkins et al. (2004) stated that thyroid hormones significantly influence the metabolism, development, and thermoregulatory mechanisms of the chicks in the post-hatch period and consequently, on metabolic heat production. The decrease in heat production in chickens subjected to TMI led to better thermotolerance, as shown by the decrease of corticosterone levels in the T6 and T12 groups. Blood corticosterone levels are commonly analyzed as a stress index in chickens (Mahmoud et al., 2004). The results of the current study are similar to those of Piestun et al. (2008), who observed a decrease in blood corticosterone levels in acclimatized chickens subjected to a thermal change at the end of rearing.

The decrease in feed conversion ratio in this study could be explained by the fact that despite a decrease in the feed intake of chickens in the T12 group, they had a higher weight gain than the other group. In agreement with this study result, Meteyake et al. (2020) observed an improvement in the feed conversion ratio in chickens from eggs subjected to TMI.

The increased carcass yield of the TMI chickens in the current study is probably due to the myoblast proliferation, particularly in the breast muscle, which had a higher yield. These results are similar to those of Piestun et al. (2015), indicating that TMI stimulates muscle development and growth via myoblast proliferation. The reduced meat drip loss in the present study is consistent with the observation of Yalçin et al. (2005), who found lower drip loss in breast meat of broilers exposed to 39.6°C from embryonic day 10 to 18. According to AL-Sagan et al. (2020), pH24 is one of the most important physical traits for the qualitative profile of meat and is commonly utilized to assess the sensory qualities of the technological properties of meat. Meat pH is related to the water-holding capacity (Jung et al., 2010), which is correlated positively with the texture, juiciness, and flavor of the meat. In the present study, TMI reduced drip loss, increased the ultimate pH of the meat, and then improved the processing ability of the meat, particularly in the T6 group. These results are similar to those of Yalçin et al. (2022), who reported that an increase in the incubation temperature induced an increase of pH24.

CONCLUSION

In conclusion, thermal manipulation during incubation influenced embryo development and hatching performance. Thermal manipulation during incubation for 12 hours reduced day-old chicks' quality without affecting hatchability and body weight. Thermal manipulation during incubation affected the body and rectal temperature. Thermal manipulation during incubation decreases chicks' rectal temperature. A TMI during 6 hours increases chicks' ability to dissipate heat. Moreover, post-hatch performance and meat quality were improved by TMI. Generally, exposing hatching eggs to 39.5°C and 65% of relative humidity from day 7 to day 16 of incubation during 12 hours/day is recommended for the poultry industry in the tropical climate.

DECLARATION

Acknowledgments

This study was financially supported by the Regional Excellence Center on Poultry Sciences (CERSA) of the

university of Lomé, Togo. The authors express warm gratitude to World Bank IDA 5424, the main sponsor of CERSA.

Authors' contributions

Meteyake Hèzouwè Tchilabalo contributed to the experimental design, data collection, data analysis, and manuscript drafting. Bilalissi Abidi, Kouame Yaah Aimee Emmanuelle and N'nanle Ombortime contributed to data collection and revising the manuscript. Tona Kokou contributed to the design and supervision of the experiment and to the revising of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Ethical consideration

The authors have made sure that the work 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.

Availability of data and materials

The necessary data will be provided by authors according to reasonable requests.

REFERENCES

Aengwanich W and Simaraks S (2004). Pathology of heart, lung, liver and kidney in broilers under chronic heat stress. Songklanakarin Journal of Science and Technology, 26(3): 418-424. Available at: http://rdo.psu.ac.th/sjst/journal/26-3/13chicken-stress.pdf

Akbarian A, Golian A, Kermanshahi H, Farhoosh R, Raji AR, De Smet S, and Michiels J (2013). Growth performance and gut health parameters of finishing broilers supplemented with plant extracts and exposed to daily increased temperature. Spanish Journal of Agricultural Research, 11(1): 109-119. DOI: https://www.doi.org/10.5424/sjar/2013111-3392

Al-Rukibat RK, Al-Zghoul MB, Hananeh WM, Al-Natour MQ, and Abu-Basha EA (2017). Thermal manipulation during late embryogenesis: Effect on body weight and temperature, thyroid hormones, and differential white blood cell counts in broiler chickens. Poultry Science, 96(1): 234-240. DOI: https://www.doi.org/10.3382/ps/pew298

Al-Sagan AA, Khalil S, Hussein EOS, and Attia YA (2020). Effects of fennel seed powder supplementation on growth performance, carcass characteristics, meat quality, and economic efficiency of broilers under thermoneutral and chronic heat stress conditions. Animals, 10(2): 206. DOI: https://www.doi.org/10.3390/ani10020206

Al-Zghoul MB, Ismail ZB, Abd Elhafeed SD, Al-Ramadan A, Althnaian TA, Al-Ramadan SY, Abdelhadi MA, Albokhadaim IF, Al Busadah KA, Eljarah A, et al. (2015). Hsp90, Hsp60 and HSF-1 genes expression in muscle, heart and brain of thermally manipulated broiler chicken. Research in Veterinary Science, 99: 105-111. https://www.doi.org/10.1016/j.rvsc.2014.12.014

Al-Zghoul MB, Dalab AE, Ababneh MM, Jawasreh KI, Al Busadah KA, and Ismail ZB (2013). Corrigendum to thermal manipulation during chicken embryogenesis results in enhanced Hsp70 gene expression and the acquisition of thermotolerance. Research in Veterinary Science, 95(2): 502-507. DOI: https://www.doi.org/10.1016/j.rvsc.2013.12.008

Al-Zghoul MB, Sukker H, and Ababneh MM (2019). Effet de la manipulation thermique des embryons de poulets de chair sur la réponse au stress oxydatif induit par la chaleur. [Effect of thermal manipulation of broilers embryos on the response to heat-induced oxidative stress]. Poultry Science, 98(2): 991-1001. DOI : https://www.doi.org/10.3382/ps/pey379

Ayssiwede SB, Azebaze SPA, and Missohou A (2009). Essais de substitution du maïs par le sorgho dans: Effets sur les performances zootechniques des poulets de chair. [Substitution trials of maize by sorghum in: Effects on the zootechnical performance of broiler chickens]. Revue Africaine de Santé et de Productions Animales, 7 : 25-32. Available at : https://b2n.ir/s21256

Bueno JPR, de Mattos Nascimento MRB, da Silva Martins JM, Marchini CFP, Gotardo LRM, de Sousa GM, Mundim AV, Guimarães EC, Rinaldi FP, and Rinaldi FP (2017). Influência da idade e do estresse cíclico de calor no perfil bioquímico sérico em frangos de corte. [Influence of age and cyclic heat stress on the serum biochemical profile of broiler chickens] Semina: Ciências Agrárias.. Available at: https://agris.fao.org/agris-search/search.do?recordID=DJ20210239915

Bueno JPR, Gotardo LRM, Dos Santos AM, Litz FH, Olivieri OCL, Alves RLOR, Moraes CA, and Nascimento MRBM (2020). Effect of cyclic heat stress on thyroidal hormones, thyroid histology, and performance of two broiler strains. International Journal of Biometeorology, 64(7): 1125-1132. DOI: https://www.doi.org/10.1007/s00484-020-01886-0

Charles DR and Walker AW (2002). Responses to the thermal environment. In: D.R. Charles and A.W. Walker (Editors), Poultry environment problems: A guide to solutions. Nottingham University Press., UK, pp. 1-16. Available at: https//www.cabdirect.org/cabdirect/abstract/20023096268

Collin A, Berri C, Tesseraud S, Requena Rodon FE, Rodon FR, Skiba-Cassy S, Crochet S, Duclos MJ, Rideau N, Tona K, et al. (2007). Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poultry Science, 86(5): 795-800. DOI: https://www.doi.org/10.1093/ps/86.5.795

Collin A, Bedrani L, Loyau T, Mignon-Grasteau S, Métayer-Coustard S, Praud C, De Basilio V, Requena Rodon F, Bastianelli D, Duclos M, et al. (2011). L'acclimatation embryonnaire: Une technique innovante pour limiter les mortalités liées au stress thermique chez le poulet. Productions Animales, 24(2) : 191-198. DOI: https://www.doi.org/10.20870/productions-animales.2011.24.2.3253

De Smit L, Tona K, Bruggeman V, Onagbesan O, Hassanzadeh M, Arckens L, and Decuypere E (2005). Comparison of three lines of broilers differing in ascites susceptibility or growth rate. 2. Egg weight loss, gas pressures, embryonic heat production, and physiological hormone levels. Poultry Science, 84(9): 1446-1452. DOI: https://www.doi.org/10.1093/ps/84.9.1446

Halle I and Tzschentke B (2011). Influence of temperature manipulation during the last 4 days of incubation on hatching results, post-hatching performance and adaptability to warm growing conditions in broiler chickens. The Journal of Poultry Science, 48(2): 97-105. DOI: https://www.doi.org/10.2141/jpsa.010056

Havenstein GB, Ferket PR, and Qureshi MA (2003). Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science, 82(10): 1509-1518. DOI: https://www.doi.org/10.1093/ps/82.10.1509

Intergovernmental panel on climate change (IPCC) (2001). Climate Change 2001: The scientific basis. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change. Available at: https://www.ipcc.ch/site/assets/uploads/2018/03/WGI_TAR_full_report.pdf

Jenkins SA and Porter TE (2004). Ontogeny of the hypothalamo–pituitary–adrenocortical axis in the chicken embryo: A review.

Domestic Animal Endocrinology, 26(4): 267-275. DOI: https://www.doi.org/10.1016/j.domaniend.2004.01.001

Jung S, Choe JH, Kim B, Yun H, Kruk ZA, and Jo C (2010). Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers. Meat Science, 86(2): 520-526. https://www.doi.org/10.1016/j.meatsci.2010.06.007

Lin H, Jiao HC, Buyse J, and Decuypere E (2006). Strategies for preventing heat stress in poultry. World's Poultry Science Journal, 62(1): 71-86. DOI: https://www.doi.org/10.1079/WPS200585

Loyau T, Hennequet-Antier C, Coustham V, Berri C, Leduc M, Crochet S, Sannier M, Duclos MJ, Mignon-Grasteau S, Tesseraud S et al. (2016). Thermal manipulation of the chicken embryo triggers differentialgene expression in response to a later heat challenge. BMC Genomics, 17(1): 329. DOI: https://www.doi.org/10.1186/s12864-016-2661-y

Maatjens CM, Reijrink IAM, Molenaar R, van der Pol CW, Kemp B, and van den Brand H (2014). Température and CO2 during the hatching phase. I. Effects on chicks' quality and organs development. Poultry Science, 93(3): 645-654. DOI: https://www.doi.org/10.3382/ps.2013-03490

Mahmoud UT, Abdel-Rahman MA, and Darwish MH (2014). Effects of propolis, ascorbic acid and vitamin E on thyroid and corticosterone hormones in heat stressed broilers. Journal of Advanced Veterinary Research, 4(1): 18-27. Availaible at: https://advetresearch.com/index.php/AVR/article/view/68

Mahmoud KZ, Edens FW, Eisen EJ, and Havenstein GB (2004). Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers (Gallus gallus domesticus) subjected to cyclic heat stress. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 137(1): 35-42. DOI: https://www.doi.org/10.1016/j.cbpc.2003.09.013

Meteyake HT, Bilalissi A, Oke OE, Voemesse K, and Tona K (2020). Effect of thermal manipulation during incubation and heat challenge during the early juvenile stage on production parameters of broilers reared under a tropical climate. European Poultry Science, 84: 1-16. DOI: https://www.doi.org/10.1399/eps.2020.318

Molenaar R, Hulet R, Meijerhof R, Maatjens CM, Kemp B, and van den Brand H (2011). High eggshell temperatures during incubation decrease growth performance and increase the incidence of ascites in broiler chickens. Poultry Science, 90(3): 624-632. DOI: https://www.doi.org/10.3382/ps.2010-00970

Moraes VMB, Malheiros RD, Bruggeman V, Collin A, Tona K, van As P, Onagbesan OM, Buyse J, Decuypere E, and Macari M (2004). The effect of timing of thermal conditioning during incubation on embryo physiological parameters and its relationship to thermotolerance in adult broiler chickens. Journal of Thermal Biology, 29(1): 55-61. DOI: https://www.doi.org/10.1016/j.jtherbio.2003.10.006

Morita VDS, Almeida VRD, Matos JB, Vicentini TI, van den Brand H, and Boleli IC (2016). Incubation temperature during fetal development influences morphophysiological characteristics and preferred ambient temperature of chicken hatchlings. PloS One, 11(5): e0154928. DOI: https://www.doi.org/10.1371/journal.pone.0154928

Mujahid A, Pumford NR, Bottje W, Nakagawa K, Miyazawa T, Akiba Y, and Toyomizu, M (2007). Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. The Journal of Poultry Science, 44(4): 439-445. DOI: https://doi.org/10.2141/jpsa.44.439

Nideou D, N'nanlé ON, Kouame YAE, Chrysostome C, Gbeassor M, Decuypere E, and Tona K (2019). Effect of high temperature during first and second halves of incubation on layer chicken embryo physiology. International Journal of Poultry Science, 18(12): 626-633. DOI: https://www.doi.org/10.3923/ijps.2019.626.633

Oke OE (2018). Evaluation of physiological response and performance by supplementation of *Curcuma longa* in broiler feed under hot humid tropical climate. Tropical Animal Health and Production, 50: 1071-1077. DOI: https://www.doi.org/10.1007/s11250-018-1532-8

Oke OE, Emeshili UK, Iyasere OS, Abioja MO, Daramola JO, Ladokun AO, Abiona JA, Williams TJ, Rahman SA, Rotimi SO et al. (2017). Physiological responses and performance of broiler chickens offered olive leaf extract under hot humid tropical climate. Journal of Applied Poultry Research, 26(3): 376-382. DOI: https://www.doi.org/10.3382/japr/pfx005

Piestun Y, Shinder D, Ruzal M, Halevy O, Brake J, and Yahav S (2008). Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermotolerance. Poultry science, 87(8): 1516-1525. DOI: https://www.doi.org/10.3382/ps.2008-00030

Piestun Y, Yahav S, and Halevy O (2015). Thermal manipulation during embryogenesis affects myoblast proliferation and skeletal muscle growth in meat-type chickens. Poultry Science, 94(10): 2528-2536. DOI: https://www.doi.org/10.3382/ps/pev245

Purswell JL, Dozier III WA, Olanrewaju HA, Davis JD, Xin H, and Gates RS (2012). Effect of temperature-humidity index on live performance in broiler chickens grown from 49 to 63 days of age. American Society of Agricultural and Biological Engineers. IX International Livestock Environment Symposium. p. 3. DOI: https://www.doi.org/10.13031/2013.41619

Sohail MU, Ijaz A, Yousaf MS, Ashraf K, Zaneb H, Aleem M, and Rehman H (2010). Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poultry Science, 89(9): 1934-1938. DOI: https://doi.org/10.3382/ps.2010-00751

Tesseraud S and Temim S (1999). Modifications métaboliques chez le poulet de chair en climat chaud: Conséquences nutritionnelles. INRAE Productions Animales, 12(5): 353-363. DOI: https://www.doi.org/10.20870/productions-animales.1999.12.5.3894

Todini L (2007). Thyroid hormones in small ruminants: Effects of endogenous, environmental and nutritional factors. Animal, 1(7): 997-1008. DOI: https://www.doi.org/10.1017/s1751731107000262

Tona K, Bamelis F, De Ketelaere B, Bruggeman V, Moraes VM, Buyse J, Onagbesan O, and Decuypere E (2003). Effects of egg storage time on spread of hatch, chick quality, and chick juvenile growth. Poultry Science, 82(5): 736-741. DOI: https://www.doi.org/10.1093/ps/82.5.736

Yahav S (2009). Alleviating heat stress in domestic fowl: Different strategies. World's Poultry Science Journal, 65(4): 719-732. DOI: https://www.doi.org/10.1017/s004393390900049x

Yahav S and McMurtry JP (2001). Thermotolerance acquisition in broiler chickens by temperature conditioning early in life—the effect of timing and ambient temperature. Poultry Science, 80(12): 1662-1666. DOI: https://www.doi.org/10.1093/ps/80.12.1662

Yakubu A, Epko EI, and Oluremi OI (2018). Physiological adaptation of sasso laying hens to the hot-dry tropical conditions. Agriculturae Conspectus Scientificus, 83(2): 187-193. Available at: https://hrcak.srce.hr/file/298892

Yalçin S, Özkan S, and Shah T (2022). Incubation temperature and lighting: Effect on embryonic development, post-hatch growth, and adaptive response. Frontiers in Physiology, 13: 899977. DOI: https://www.doi.org/10.3389/fphys.2022.899977

Zhang C, Zhao X, Wang L, Yang L, Chen X, and Geng Z (2017). Resveratrol beneficially affects meat quality of heat-stressed broilers which is associated with changes in muscle antioxidant status. Animal Science Journal, 88(10): 1569-1574. DOI: https://www.doi.org/10.1111/asj.12812

Zulovich JM and DeShazer JA (1990). Estimating egg production declines at high environmental temperatures and humidities. Paper-American Society of Agricultural Engineers, pp. 1-15. Available at https://www.cabdirect.org/cabdirect/abstract/19912449755

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication
J. World Poult. Res. 13(1): 41-47, March 25, 2023

Research Paper, PII: S2322455X2300004-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.4

Identification of Probiotic Bacteria Isolated from Domestic Chickens (*Gallus domesticus*) Using the 16S rRNA Gene Method

Dirayah Rauf Husain *, Riuh Wardhani, Fiqha Septia Ningsih, and Fuad Gani

Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia

*Corresponding author's Email: dirayahrh@unhas.ac.id

Received: 12 October 2022
Accepted: 30 November 2022

ABSTRACT

The intestines of domestic poultry (*Gallus domesticus*) are one of the potential sources of probiotic bacteria that can produce antibacterial agents. The objective of this study was to identify the types of probiotic bacteria obtained from the digestion of domestic poultry using the molecular analysis method of 16S rRNA gene sequencing. Observations were conducted on colony morphology, gram staining, biochemical tests, and antibacterial activity using the diffusion agar method. Molecular analysis of DNA extraction was carried out, followed by the amplification of samples using a 16S rRNA universal primer. Dielectrophoresis and sequencing were performed on the 16S rRNA gene. The identification of morphological observations, gram staining, and biochemical tests showed that probiotic bacteria isolates, including Gram-positive, rod-shaped, rounded colony form, flat elevation, entire nonmotile edge, and catalase-negative, could ferment all carbohydrate content in the TSIA medium. The antibacterial potential was also found in probiotic bacteria, as evidenced by the inhibition zone formed in the test. The results of the bacterial gene sequences of PaTa5 probiotic bacterial isolates had a similarity of 98.37% with *Lactobacillus plantarum.* These findings indicated the presence of some bacteria species that have antibacterial activity in the intestines of domestic chickens (*Gallus domesticus*).

Keywords: *Lactobacillus plantarum*, Native chicken, Probiotic, 16S rRNA

INTRODUCTION

Probiotics are live microorganisms that positively affect the health of organisms and can balance the bacteria in the digestive tract by suppressing the growth of harmful pathogenic bacteria (Anadón et al., 2016; Vahdatpour and Babazadeh, 2016). One of the potential sources of probiotic bacteria is found in domestic poultry (Husain et al., 2017; Husain et al., 2019). The habitat of domestic poultry in the open environment causes the composition of the population of microorganisms that live in the digestive tract to be suspected to be high. Microbiota in the digestive system can form colonies that protect the digestive tract by sticking to epithelial tissue on the enterocyte wall, thereby reducing colony formation from pathogenic bacteria (Shang et al., 2018).

The presence of bacteria in the digestive tract of chickens is partly due to the interaction of bacteria and the environment that contaminates the chicken's body through the feed. The difference in chickens' age will also influence the differences in the types of bacteria that exist (Talaiekhozani et al., 2015). The majority of bacteria found in adult chickens is dominated by the *Lactobacillus* genus, which is one of the variations in the composition of the bacterial population between 2-day-old chickens and adult chickens (Sugiharto et al., 2018).

In an effort to find potential bacterial strains as antibiotics, the identification of bacterial strains continues to develop from conventional physiological and biochemical characterizations. Identification by this method requires a long time, and identification results are less accurate. An efficient, highly sensitive, and specific

To cite this paper: Husain DR, Wardhani R, Ningsih FS, and Gani F (2023). Identification of Probiotic Bacteria Isolated from Domestic Chickens (*Gallus domesticus*) Using the 16S rRNA Gene Method. *J. World Poult. Res.*, 13 (1): 41-47. DOI: https://dx.doi.org/10.36380/jwpr.2023.4

molecular-based identification technique is currently being developed by analyzing 16S rRNA gene sequencing (16S ribosomal ribonucleic acid, S states Svedberg, the ribosome measurement unit) pioneered by Woese and Fox in 1977.

The objective of the current study was to identify potential types of probiotic bacteria originating from domestic chickens using the 16S rRNA method.

MATERIALS AND METHODS

Ethical approval

This research was conducted at the Microbiology Laboratory, Hasanuddin University, Indonesia. The procedure in this study followed the standards of the guidelines for the use of animals (Buchanan et al., 2012).

Sampling and isolation

Domestic chickens (*Gallus domesticus*) were obtained from Takalar, South Sulawesi, Indonesia (5°08.184'S,119°29.520'E). The inner walls of the chicken intestine were scraped and then inserted into a physiological sterile NaCl solution and diluted with graded dilution. A serial dilution was done at concentrations from 10^{-1} to 10^{-6}. 1 mL solution from the inoculated dilution in the De Mann–Rogosa–Sharpe agar (MRSA) medium (the pour plate method), then incubated at 37°C for 24-48 hours. The isolate obtained was coded PaTa5.

Morphological and biochemical characterization

Morphological observations were conducted by observing the characteristics revealed by the colonies formed using a stereo microscope. Gram staining technique was done by painting bacterial cells with a solution of violet crystals, lugol, alcohol-acetone, and safranin to determine the type of gram in bacteria. Biochemical characterization was done using several tests, including the Triple Sugar Iron Agar Test (TSIA), Motility Test, and catalase test (Husain et al., 2022).

Inhibition test

The test was carried out *in vitro* by the agar diffusion method using a 10 mm blank disk. The test media was incubated at 37 for 1×24 hours, and the inhibition area was measured using calipers. Incubation was continued for up to 2×24 hours to determine the properties of the active compounds in the culture (Husain and Wardhani, 2021).

DNA extraction

The Geneaid PrestoTM Mini gDNA Bacteria Kit's DNA (Geneid, Taiwan) extraction procedure was used to extract genomic DNA (Geneaid, 2017).

16S rRNA gene amplification

This technique was performed on isolated DNA samples using a previously prepared PCR reaction mixture consisting of 16 l 2 Mytaq HS Red Mix, 2 µl of each of the 16S rRNA Primary Forward gene 63F and the Primary Reversal gene 387R, and 4 μl of H2O. Tubes corresponding to amplifications of samples were filled with 22 µl PCR reaction mixture, then 5 μl of nuclease water and 5 µl of DNA extract were added. Amplification was carried out for 30 cycles, and each cycle consisted of denaturation at 95°C for 5 minutes, at 95°C for 1 m, annealing at 57°C for 1 minute, extending at 72°C for 1 m, and post-extending at 72°C for 10 m (Sune et al., 2020).

PCR products by electrophoresis detection

The amplified DNA was separated with 1% agarose gel electrophoresis. The ethidium bromide (UltraPure™, United States) dye was used to observe the results, and the UV-transilluminator was used to detect them. The results of the detection were recorded according to the method described by Marzuki et al. (2015).

DNA sequencing

The basic local alignment search tool (BLAST) program was used to compare the partial DNA sequences of 16S rRNA with the public database (NCBI) to find homologies. Query cover and E-value values could be seen in the blast analysis results. Query cover indicates the percentage of sample used in the BLAST analysis, and the E-value shows the level of probability statistically of an item. A low E value indicates a high level of homology (Madden et al., 2013). Clustal was used to perform multiple alignments, and a phylogenetic tree was constructed using MEGA version 6.0 utilizing the neighbor-joining technique and bootstrap values derived from 1,000 replications (Sune et al., 2020).

RESULTS AND DISCUSSION

Morphological and biochemical characterization

Based on the results of colony morphological tests, the characteristics of a colony were cream-colored, smooth surface of the colony, the entire edge, and the shape of flat elevation (Table 1). Morphological characterization was used to observe the morphology of bacterial colonies. Microorganisms grown on various media show different macroscopic appearances on growth (Wafula et al., 2015).

The results of gram staining on probiotic bacteria showed that the shape of the bacterial cell was in the form of a stem (bacilli). Probiotic bacterial isolates were also

classified as Gram-positive bacteria, where the results of staining indicated a purple color (Figure 1). Based on the gram staining results, the nature of bacterial cell walls against crystal violet dye stain (primary color) and safranin (opponent color) could be seen (Aisha et al., 2017).

Table 1. Morphological characteristics of *Lactobacillus plantarum*

Color	Colony Surface	Shape	Edge	Elevation
Cream	Smooth	Circle	Entire	Flat

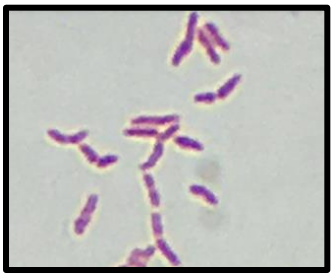


Figure 1. Gram staining of *Lactobacillus plantarum*

The morphological character of probiotic bacterial cells was obtained following the characteristics of probiotic bacteria classified as *Lactobacillus* gram-positive bacteria (Mannan et al., 2017). Gram-positive bacteria have a thick cell wall and a cell membrane layer so that when the bacteria become dehydrated by giving alcohol, 96% of the pores will shrink, which causes the main color (crystal violet) not to get out (Peristiawati et al., 2019).

The results obtained from the TSIA Test revealed that the PaTa5 probiotic isolate was not formed by gas and black sediment (H_2S), and the yellow Slant and Butt parts indicated that the PaTa5 isolate was acidic. Therefore, it could ferment the three types of sugar, namely glucose, lactose, and sucrose. TSIA media contain sugar, glucose, and lactose/sucrose (Dalyn, 2014). Probiotic bacteria obtain energy, while it only depends on fermentative metabolism. There are types of lactic acid bacteria that can ferment the three types of sugar found in the TSIA medium, namely glucose, lactose, and sucrose. It is marked in yellow on the slant (slanted agar) and yellow on the Butt (Murugan, 2017).

Motility test observation results showed that the isolate of PaTa5 probiotic bacteria was nonmotile since there was no propagation around the inoculation area. The PaTa5 isolate did not have a movement tool (flagella). Probiotic bacteria were nonmotile due to their minimal capacity for biosynthetic activity. Energy acquisition is totally dependent on the fermentative metabolism that takes place in its place (Wu et al., 2017).

The catalase test results were negative, indicating that air bubbles (O_2) did not form when the probiotic bacteria isolate was dripped with H_2O_2 solution. Isolates showed negative results on the catalase test. It can be concluded that the isolate of Lactic acid bacteria was homofermentative (Mannan et al., 2017).

Inhibition test

The inhibition test on pathogenic bacteria aims to determine the ability of isolates of probiotic bacteria obtained from domestic poultry *Gallus domesticus* to inhibit pathogenic bacteria, especially on the test bacteria used, namely *Escherichia coli* (*E. coli*, negative gram) as enteric bacteria and *Staphylococcus aureus* (*S. aureus*, positive gram) as pathogenic bacteria (Rouger et al., 2017). *E. coli* and *S. aureus* are bacteria often found in the digestive tract of small poultry intestines and are pathogenic.

The PaTa5 probiotic bacterial isolate had a clear zone diameter in the *E. coli* test bacteria of 17.5 mm, an incubation period of 1×24 hours, and 18 mm during the incubation period of 2×24 hours (Figure 2). Meanwhile, the *S. aureus* test bacteria obtained a diameter of 12.5 mm during the incubation period of 1×24 hours and 13 mm during the incubation period of 2×24 hours (Li et al., 2016). The inhibition zone of 10-15 mm was classified as weak, 15-20 as moderate, and strong if> 20 mm. Therefore, the isolate of the PaTa5 probiotic bacteria had a moderate inhibitory effect on *E. coli*, while it was classified as having a weak inhibitory effect on the *S. aureus* bacteria. Probiotic bacteria are a type of bacteria that occupy the gastrointestinal tract. They can inhibit the growth of some bacteria by producing various antibacterial components (organic acids, hydrogen peroxide, and bacteriocin) that can suppress the growth of pathogenic bacteria (Husain et al., 2019; Weerapong et al., 2016).

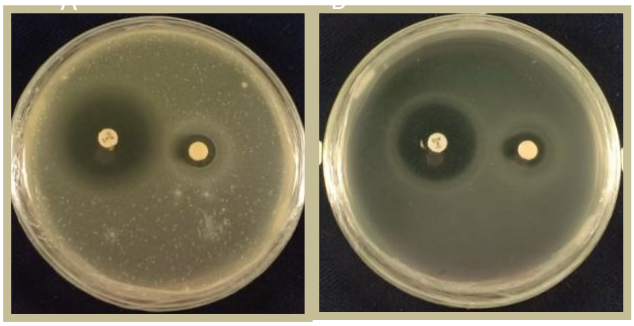


Figure 2. Inhibition test results for *Escherichia coli* (A) and *Staphylococcus aureus* (B)

16S rRNA gene amplification

Genomic DNA isolated and extracted from probiotic bacterial isolates was then analyzed by PCR to amplify the 16S rRNA gene using universal primers for 16S rRNA genes, namely forward primer 63F and reserve primer 1387R.

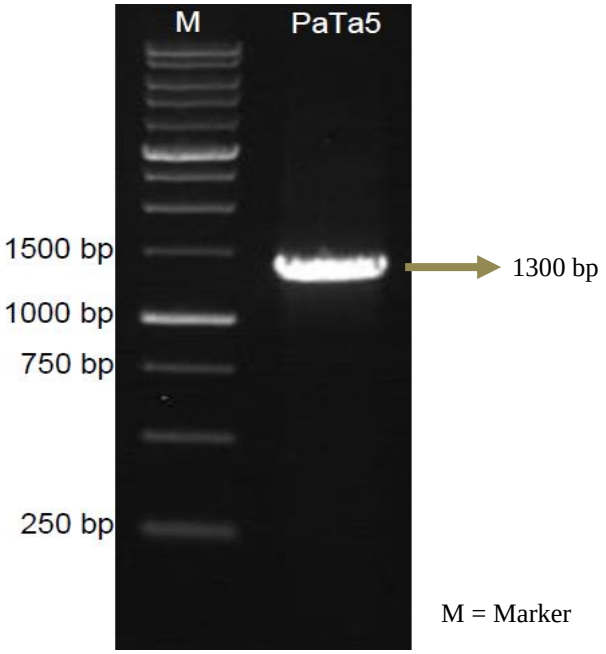


Figure 3. Results of 16S rRNA gene amplification with 63F and 1387R primers

The pairing of the 63F and 1387R PCR primary designs for 16S rRNA gene amplification of bacteria were systematically evaluated and tested in terms of the specificity and efficiency of various types of bacteria and samples in the environment. Primer pairs are more useful for 16S rRNA gene amplification in ecology and systematic studies. This primer could identify organism coryneform and genus *Micrococcus* (Gram-positive, high guanine (G) and cytosine (C), *Eubacterium*, and *Proteobacteria*. In addition, 63F was found to have a greater hybridization potential, compared to other primers (27F and 1392R), where the primer cannot amplify the conserved area optimally compared to the primary pair 63F and 1387R (Marchesi et al., 1998). The amplification results were electrophoretic with agarose 1.5% and visualized by UV light.

Based on the results of electrophoresis visualization, the primer used could be amplified well in PaTa5 bacteria probiotic isolates and is at a size of about 1300 bp (Figure 3). As can be seen in Figure 3, the pattern of DNA bands resulting from PCR amplification was formed using primers in the form of a single band. The DNA bands of PaTa5 probiotic bacteria isolates appear thick. This shows the DNA of the amplified PaTa 5 sample had a high concentration, indicating that the PaTa5 sample was well amplified using 63F and 1387R primers.

The suitability of the primers greatly affected the identification results. Misuse of primers could lead to the amplification of other regions in the genome that are not targeted or otherwise. No genomic regions were amplified. Optimization of annealing temperature was also a factor that affected the success of identification (Rychlik et al., 1990; Guillen et al., 2016).

The PaTa5 sample was amplified using a universal primer pair 63F and 1387R, which showed a clear and thick DNA band, then proceeded to the next stage of DNA sequencing. The intensity of the fragment during visualization was feasible to be used at a later stage with a clearly visible band, and no smears were found. The difference in band thickness indicated different DNA concentrations (Kuhn et al., 2018).

16S rRNA fragments by sequencing analysis

DNA sequencing is the process of sequencing a DNA base. This process uses the principle of enzymatically polymerizing DNA reactions (Kchouk et al., 2017). In order to determine the identity of the bacteria, BLAST first determines the base type.

Several parameters, including Query cover, E-value, and ident, can be seen in Table 2. The query cover indicated the percentage of samples used in the BLAST analysis, and the E-value showed the level of probability statistically of an item. Of the four parameters, the most accurate was the E-value. A high percentage of homology was indicated by a smaller e-value (Madden et al., 2013).

Identification results were 98% since differences in several pairs of nucleotide bases aligned. This can be caused due to mutation. Mutations are defined as changes in the DNA base. Mutations can occur spontaneously, where one of the bases is lost from the nucleotides through hydrolysis (Beyene et al., 2010).

Analysis of the 16S rRNA gene sequence shows that the isolate of the PaTa5 probiotic bacteria obtained from the intestine of domestic poultry *Gallus domesticus* had the closest resemblance to *Lactobacillus plantarum* (*L. plantarum*). The *L. plantarum* bacteria can be classified into the domain of Bacteria, phylum of *Firmicutes*, class of *Bacilli*, order of *Lactobacillales*, family of *Lactobacillaceae*, genus of *Lactobacillus*, and plantarum species of *Lactobacillus* (Zheng et al., 2020).

Table 2. Sequencing analysis result of PaTa5 isolates

Homologous Lactic acid bacteria species	Query coverage (%)	E Value	Identities
Lactobacillus plantarum strain CAU:227	99 %	0.0	98.37%
Lactobacillus plantarum strain CAU:222	99 %	0.0	98.37%
Lactobacillus plantarum subsp. strain Ni997	99 %	0.0	98.37%
Lactobacillus plantarum strain MMB03	99 %	0.0	98.36%
Lactobacillus plantarum strain KLDS 1.0344	99 %	0.0	98.36%
Lactobacillus plantarum strain YLL-03	99 %	0.0	98.36%

CONCLUSION

Morphological characterization shows that PaTa5 isolate is a gram-positive with bacillus (rods) form. PaTa5 isolates could ferment all carbohydrate content in the TSIA medium, nonmotile in the SIM medium, and negative results at the catalase test. The inhibition test for pathogen bacteria shows a clear zone (inhibition zone) around the PaTa5 isolate. Metabolite was produced by this isolate from the inhibition zone in the *E. coli* test bacteria of 17.5 mm during the incubation period of 1×24 hours, and 18 mm during the incubation period of 2×24 hours. Meanwhile, the *S. aureus* test bacteria obtained a diameter of 12.5 mm during the incubation period of 1×24 hours and 13 mm during the incubation period of 2×24 hours. The results of the bacterial gene sequences of PaTa5 probiotic bacterial isolates have a similarity of 98.37% with *L. plantarum*. Probiotic bacteria are often used as a food fermentation agent since they can improve food flavor. Considering the obtained results of the current study, probiotic bacteria have biological activity, such as antibacterial, making them suitable as fermentation agents for the production of functional foods and beverages.

DECLARATIONS

Acknowledgments

The authors acknowledge the facilities, and scientific and technical support from the Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia. The authors received no financial support for the research, authorship, and/or publication of this article.

Authors' contribution

Dirayah Rauf Husain and Riuh Wardhani conceptualized. Dirayah Rauf Husain, Riuh Wardhani, Fiqha Septia Ningsih, and Fuad Gani implemented the research. Dirayah Rauf Husain, Riuh Wardhani, and Fiqha Septia Ningsih wrote the manuscript. Riuh Wardhani and Fuad Gani commented on research improvement. All authors have read and agreed to the last version of the manuscript.

Competing interests

There is no competing interest exists in this research.

Ethical consideration

All authors have checked statistical analysis as well as 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 the article will be provided by the corresponding author according to reasonable requests.

REFERENCES

Aisha A, AlMahadin S, Eltarabily K, Shah PN, and Ayyash M (2017). Characterization of potential probiotic lactic acid bacteria isolated from camel milk. LWT - Food Science and Technology, 79: 316-325. DOI: https://www.doi.org/10.1016/j.lwt.2017.01.041

Anadón A, Larrañaga MRM, Ares I, and Martínez M (2016). Probiotics: Safety and toxicity considerations. Nutraceuticals, chapter 65, pp. 1081-1105. DOI: https://www.doi.org/10.1016/B978-0-12-821038-3.00065-3

Beyene D, Baguma Y, Mukasa SB, Sun C, and Jansson C (2010). Characterization and role of isoamylase1 (meisa1) gene in Cassava. African Crop Science Journal, 18(1): 1-8. DOI: https://www.doi.org/10.4314/acsj.v18i1.54187

Buchanan KL, Carere C, Perera TB, and Jennings DJ (2012). Guidelines for the treatment of animals in behavioral research and teaching. Animal Behavior, 83(1): 301-309.

Dalynn Biologicals (2014). Triple sugar iron agar. pp. 1-2. Available at: http://www.dalynn.com/dyn/ck_assets/files/tech/TT95.pdf

Geneaid (2017). Instruction manual. Presto™ mini gDNA bacteria kit. pp. 1-8. Available at: https://www.geneaid.com/data/files/1605607121726389188.pdf

Guillen IA, Camacho H, Tuero D, Bacardi D, Palenzuela O, Aguilera A, Silva JA, Estrada R, Gell O, Su´arez J et al. (2016). PCR conditions for 16S primers for analysis of microbes in the colon of rats. Journal of Biomolecular

Techniques, 27(3): 105-112. DOI: https://www.doi.org/10.7171/jbt.16-2703-002

Husain DR, Dwyana Z, Ambeng, Anggareni A, and Sulfahri (2017). Evaluation of bacteria from gallus domesticus as a potential probiotic in boiler chicks: Effect on growth performance and feed conversion ratio. International Journal of Poultry Science, 16(2): 43-49. DOI: https://www.doi.org/10.3923/ijps.2017.43.49

Husain DR and Wardhani R (2021). Antibacterial activity of endosymbiotic bacterial compound from Pheretima sp. earthworms inhibit the growth of Salmonella Typhi and *Staphylococcus aureus*: *In vitro* and in silico approach. Iranian Journal of Microbiology, 13(4): 537-543. DOI: https://www.doi.org/10.18502/ijm.v13i4.6981

Husain DR, Wardhani R, and Erviani AE (2022). Antibacterial activity of bacteria isolated from earthworm (Pheretima sp.) gut against Salmonella typhi and *Staphylococcus aureus*: *In vitro* experiments supported by computational docking. Biodiversitas, 23(2): 1125-1131. DOI: https://www.doi.org/10.13057/biodiv/d230257

Husain DR, Gunawan S, and Sulfahri (2019). Antimicrobial potential of lactic acid bacteria from domestic chickens gallus domesticus from south Celebes, Indonesia, in different growth phases: *In vitro* experiments supported by computational docking. Iranian Journal of Microbiology, 12(1): 62-69. DOI: https://www.doi.org/10.18502/ijm.v12i1.2519

Kuhn R, Böllmann J, Krahl K, Bryant IM, and Martienssen M (2018). Data on DNA gel sample load, gel electrophoresis, PCR and cost analysis. Data in Brief, 16: 732-751. DOI: https://www.doi.org/10.1016/j.dib.2017.11.082

Kchouk M, Gibrat JF, and Elloumi M (2017). Generations of sequencing technologies: From first to next generation. Biology and Medicine, 9(3): 1000395. Available at: https://www.walshmedicalmedia.com/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

Li WR, Li HL, Shi QS, Sun TL, Xie XB, Song B, and Huang XM (2016). The Dynamics and mechanism of the antimicrobial activity of tea tree oil against bacteria and fungi. Applied Microbial and Biotechnology, 100(20): 8865-8875. DOI: https://www.doi.org/10.1007/s00253-016-7692-4

Madden T (2013). The blast sequence analysis tool. The NCBI handbook. Chapter 16. Available at: https://www.unmc.edu/bsbc/docs/NCBI_blast.pdf

Mannan SJ, Rezwan R, Rahman MS, and Begum K (2017). Isolation and biochemical characterization of *lactobacillus* species from yogurt and cheese samples in Dhaka Metropolitan Area. Bangladesh Pharmaceutical Journal, 20(1): 27-33. DOI: https://www.doi.org/10.3329/bpj.v20i1.32090

Marchesi JR, Sato T, Weightman AJ, Martin TA, John C, Fry JH, Hiom SJ, and Wade WG (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Applied and Environmental Microbiology, 64(2): 795-799. DOI: https://www.doi.org/10.1128/aem.64.2.795-799.1998

Murugan K (2017). Bacteriological analysis in the gut of cirrhinus cirrhosus and screening microbiology the effect of bacteriocin against common Gram-negative bacterial pathogens. World Journal of Pharmaceutical Research, 4(7): 930-975. Available at: https://www.wjpr.net/abstract_file/3142

Peristiawati, Sanjaya Y, and Trinaya C (2019). Isolation and identification of potential culturable probiotics bacteria from intestine of Anguilla bicolor. Journal of Physics: Conference Series, 1280(2): 022005. Available at: https://iopscience.iop.org/article/10.1088/1742-6596/1280/2/022005

Rychlik W, Spencer WJ, Rhoads RE (1990). Optimization of the annealing temperature for DNA amplification *in vitro*. Nucleic Acids Research, 18(21): 6409-6412. DOI: https://www.doi.org/10.1093/nar/18.21.6409

Rouger A, Tresse O, and Zagorec M (2017). Bacterial contaminants of poultry meat: Sources, species, and dynamics. Microorganism, 5(3): 50. DOI: https://www.doi.org/10.3390/microorganisms5030050

Shang YS, Kumar S, Oakley B, and Woo KK (2018). Chicken gut microbiota: Importance and detection technology. Frontiers in Veterinary Science, 5: 254. DOI: https://www.doi.org/10.3389/fvets.2018.00254

Sune D, Rydberg A, Augustisson, Serrander L, and Jungestrom MB (2020). Optimization of 16S rRNA gene analysis for use in the diagnostic clinical microbiology service. Journal of Microbiological Methods, 170: 105854. https://www.doi.org/10.1016/j.mimet.2020.105854

Sugiharto, Yudiarti T, Isroli, Widiastuti E, and Wahyuni HI (2018). Hematological parameters and selected intestinal microbiota populations in the Indonesian indigenous crossbred chickens fed basal diet supplemented with multi-strain probiotic preparation in combination with vitamins and minerals. Veterinary World, 11(6): 874-882. DOI: https://www.doi.org/10.14202/vetworld.2018.874-882

Talaiekhozani A, Alaee S, and Ponraj M (2015). Guidelines for quick application of biochemical tests to identify unknown bacteria. Accounts of Biotechnology Research, 2(2): 1-26. Available at: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4101035

Vahdatpour T and Babazadeh D (2016). The effects of Kefir rich in probiotic administration on serum enzymes and performance in male Japanese quails. Journal of Animal & Plant Sciences, 26(1): 34-39. Available at: http://thejaps.org.pk/docs/v-26-01/05.pdf

Wafula EN, Kinyua J, Kariuki D, Muigai A, Mwirichia R, and Kibet T (2015). Morphological characterization of soil bacteria in Ngere tea catchment area of Murang'a County, Kenya. International Journal of Life Sciences Research, 3(1): 121-134. Available at: https://www.researchpublish.com/papers/morphological-characterization-of-soil-bacteria-in-ngere-tea-catchment-area-of-muranga-county-kenya

Weerapong W, Malila Y, Sorapukdee S, Swetwiwathana A, Benjakul S, and Vissessangguan W (2016). Bacteriocins from lactic acid bacteria and their applications in meat and meat product. Meat Science, 120: 118-132. DOI: https://www.doi.org/10.1016/j.meatsci.2016.04.004

Wu Z, Wu J, Cao P, Jin Y, Pan D, Zeng X, and Guo Y (2017). Characterization of probiotic bacteria involved in fermented milk processing enriched with folic acid. Journal of Dairy Science, 100(6): 4223-4229. Available at: https://www.journalofdairyscience.org/article/S0022-0302(17)30342-9/pdf

Zheng J, Wittouck S, Salvetti E, Franz CM, Harris H M, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J et al. (2020). A taxonomic note on the genus *lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *lactobacillaceae* and *leuconostocaceae*. International Journal of Systematic and Evolutionary Microbiology, 70(4): 2782-2858. https://www.doi.org/10.1099/ijsem.0.004107

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication
J. World Poult. Res. 13(1): 48-60, March 25, 2023

Research Paper, PII: S2322455X2300005-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.5

Impact of Prebiotic Supplementation on Productive Performance, Carcass Traits, and Physiological Parameters of Broiler Chickens under High Stocking Density Condition

Essam Mohamed Hassan Karar[1]*, Abdel-Rahman Mohamed Mohamed Atta[2], Hassan Bayoumi Ali Gharib[2], and Mohamed Abdel-Rahman Abdel-Hamed El-Menawey[2]

[1]*Miser Arabian poultry company, Cairo, Egypt*
[2]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt*

Corresponding author's E-mail: Essamkarar@outlook.com

Received: 09 January 2023
Accepted: 23 February 2023

ABSTRACT

The present study was performed to investigate the effect of increasing stocking density, prebiotic supplementation, and the interactions on broiler chicken performance and some physiological parameters. A total of 912 one-day-old chickens were used in this study, and they were randomly divided into six groups with 4 replicates each. The experiment included three levels of stocking densities (10, 13, and 15 broiler chicken/m^2) in 6 groups. Groups 1, 3, and 5 were maintained without prebiotic supplementation, while groups 2, 4, and 6 received a diet supplemented with prebiotics in water (1cm/liter). Reducing stocking densities and adding prebiotics improved body weight, feed consumption, feed conversion ratio, hemoglobin, packed cell volume, oxidative stress parameters (total antioxidant capacity), and European production efficiency factor, while decreasing malondialdehyde levels. On the other hand, stocking density and prebiotic supplementation did not affect dressing percentage, the relative weight of giblet parts, hind part, front part, and lymphoid organs (thymus and bursa of Fabricius). In conclusion, adding prebiotics at 1 cm/liter (Mannan-oligo saccharide and B-Glucan) can partially mitigate the negative effects of high stocking density on production performance, physiological and oxidative stress parameters, and European production efficiency factor.

Keywords: Antioxidant biomarkers, Broiler chicken, β-glucan, Mannan oligosaccharide, Oxidant, Prebiotic, Stocking density

INTRODUCTION

Poultry has become an important industry in the economies of countries, and it plays an important role in providing animal protein at reasonable prices compared to meat and fish. The poultry industry has recently produced food with high progress with a decrease in production cost Nasr et al. (2021). Poultry has a fast production cycle and high feed conversion ratio compared to different other types of farm animals except for fish. Broiler chickens should be supplied with the best environmental conditions to fulfill their genetic potential for growth because any flaw in optimal conditions can reduce performance (Feddes et al. 2002). The stocking density is one of the important factors in the poultry industry. Stocking density is an expression of live weight or housed birds per square meter of floor space (Meluzzi and Sirri, 2009). Increasing stocking density had benefits that included increasing income, achieving full use of the available area, and improving productivity Gholami et al. (2020). High environmental temperature is one of the most critical factors that affect broiler chickens' performance. High stocking density may be led to reduced dissipation of body heat to the air due to the reduction of airflow at the level of the bird. Due to high stocking densities, some factors that may decrease performance include poor air quality because of increased ammonia, difficulty access to feed and water, and unsuitable air exchange. Decreasing floor

space for broiler chickens may reduce feed efficiency, carcass quality, and growth rate and increase mortality (Feddes et al., 2002; Škrbić et al., 2011; Mahrose et al., 2019).

Prebiotics are natural feed supplements that cause many economic advantages by improving broiler chickens' feed efficiency, decreasing mortality rates, and increasing growth rates (Yaqoob et al. 2021). Stress from high stocking densities had a negative effect on microbial population, growth performance, and gut morphology, while the supplementation of prebiotics can reduce the deleterious effect of stress and microbial dysbiosis in the gut of broiler chickens under the condition of high stocking densities (Kridtayopas et al., 2019). A study by Nikpiran et al. (2013b) was conducted to investigate the influence of prebiotics on the performance, blood enzymes, and organ weight of Japanese Quails. They found a diet containing 1 g/kg prebiotic. The pax (yeast cells of *Saccharomyces cerevisiae*) has positive effects on performance. Dietary prebiotics is supposed to be probable important replacements for antibiotic growth promoters in poultry production due to their improvement in productive performance and health status (Froebel et al., 2019). Recently, there was great attention to the use of prebiotics instead the use of antibiotics in the zootechnical sector (Prentza et al. 2022). Using Fermacto® as a prebiotic at a level of 1.6 g/kg in quail's diet improved its growth performance, and it may be due to enhancing digestion, improving intestinal lumen health, and absorption of nutrients by different enzymes (Nikpiran et al. 2014). Different types of oligo and polysaccharides, including mannan-oligosaccharide (MOS), fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), pyrodextrins, isomalto-oligosaccharides (IMO), lactulose and beta-glucan are generally regarded as prebiotics (Alloui et al., 2013). Adding prebiotics (Aspergillus meal) at a level of 1.6 g/kg to a quail diet has beneficial effects on performance parameters (Babazadeh et al. 2011). This study aimed to determine the effects of different stocking densities and prebiotic supplementation on productive performance, oxidative stress, and physiological parameters.

MATERIAL AND METHODS

Ethical approval

The present study was done and approved by Institutional Animal Care and Use Committee (CU-IACUC) at Cairo University, Cairo, Egypt, under the approval code CU/II/F/42/22.

Study design

A total of 912 unsexed One-day-old Cobb 500 broiler chickens with an average weight of 48 g were used in this study. The trial period lasted from one day of age to slaughter (35 days). The broiler chicks were divided randomly into 6 groups, each group repeated 4 times. The experiment included 3 levels of stocking density (10, 13, and 15 broiler chicken/m^2). The stocking density of groups 1 and 2 was 10 broiler chicken/m^2, groups 3 and 4 were 13 broiler chicken/$m^{2,}$ while groups 5 and 6 were 15 broiler chicken/m^2. Groups 1, 3, and 5 were kept without prebiotic supplementation, while groups 2, 4, and 6 were supplemented with prebiotics in their water 1 cm/liter, Table 1). Feed and water were offered *ad libitum* during the experiment period (35 days). The vaccination program is shown in Table 2. Broiler chickens received starter (1-10 days), grower (11-24 days), and finisher (25-35 days) diets. The compositions of diets are indicated in Table 3.

Broiler chickens were exposed to 23 hours of light, and one hour of dark during the first 3 days, followed by20 hours of light and 4 hours of dark till the end of the experiment, the light intensity was 25 lux for the first 7 days, and then 10 lux after 7 days of age. The brooding temperature was set at 32^{o}C on the first day, and gradually reduced to 24^o C by the end of the third week, then maintained at 24^{o}C until the end of the experiment.

Table 1. Experimental design of the study

Group	Dietary treatment
T1	10 bird/m^2+0 prebiotic
T2	10 bird/m^2+1 cm prebiotic/liter water
T3	13 bird/m^2+0 prebiotic
T4	13 bird/m^2+1 cm prebiotic/liter water
T5	15 bird/m^2+0 prebiotic
T6	15 bird/m^2+1 cm prebiotic/liter water

T: Treatment

Table 2. Vaccination program of broiler chickens (Cobb 500) in the present study

Age (days)	Type	Method	Dose
6	Infectious Bronchities (IB primer) +Newcastle disease (ND-Hitchener B1)	Eye drops	1 dose
10	Newcastle disease (ND- Hitchener B1) + Avian Influenza (H5n3)	Injection under the skin neck	0.5 ml dose
12	Infectious Bursal disease (IBD 78)	Eye drops	1 dose
16	Infectious Bronchities (IBird)	Eye drops	1 dose
18	Newcastle disease (ND-Lasota)	Eye drops	1 dose

Corporation and country made of vaccines: CEVA company, France

Table 3. Composition and calculated analysis of starter, grower, and finisher diets of broiler chickens

Diets	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-35 days)
Ingredients (%)			
Yellow corn	51.2	56.0	61.5
Soya bean meal (46%)	41.3	36.0	30.5
Vegetable oil	3.0	3.5	3.7
Dicalcium phosphate	2.2	2.1	2.1
Limestone	0.8	0.9	0.9
NaCl (salt)	0.4	0.4	0.4
DL-Methionine	0.3	0.3	0.3
Permix*	0.4	0.4	0.3
L-lysine-Hcl	0.2	0.2	0.2
Threonine	0.1	0.2	0.1
Antitoxin	0.1	0	0
Anti-clostridium	0	0	0
Antibiotic	0	0	0
Total	100	100	100
Calculated values			
Crude protein (%)	23.0	21.0	19.0
Crude fat (%)	5.4	6.0	6.4
ME (Kcal/Kg)	2972.7	3056.2	3127.7
Crude fiber (%)	4.1	3.9	3.6
Calcium (%)	0.9	1.0	0.9
Total P (%)	0.8	0.8	0.7
Available P (%)	0.5	0.5	0.5
Lysine (%)	1.4	1.3	1.1
Methionine (%)	0.7	0.6	0.6
Methionine + Cystine (%)	1.0	1.0	0.9
Threonine (%)	1.0	0.9	0.8
Ash (%)	6.3	6.0	5.7

*Each gram premix contained: vitamin A (transretinyl acetate) 9,000 IU; vitamin D3 (cholecalciferol) 2,600 IU; vitamin E (dl-α-tocopheryl acetate) 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride) 300 mg; nicotinic acid 30 mg; pantothenic acid (d-calcium pantothenate) 10 mg; folic acid 0.6 mg; biotin 0.07 mg; manganese (MnO) 70 mg; zinc (ZnO) 60 mg; iron (FeSO4 H2O) 40 mg; copper (CuSO4 5H2O) 7 mg; iodine (Ca(IO3)2) 0.7 mg; selenium (Na2SeO3) 0.3 mg.

Prebiotic

Each liter of prebiotic contains 62.5 g of Mannan-oligo saccharide and 62.5 gm of B-Glucan. Prebiotics were added to the water for 8 hours per day from day 8 until the end of the experiment.

Data collection

Productive performance

The body weight (BW) of the chickens and feed consumption of each group were recorded weekly, and then the Feed Conversation Ratio (FCR) was estimated. European Production Efficiency Factor (EPEF) was calculated at the end of the experiment according to Equation 1:

European Production Efficiency Factor = [Livability % × BW (kg) / Age (d) x FCR] ×100

Carcass characteristics

After 35 days of age, 10 chickens were randomly selected from each batch, weighed, slaughtered, blood filtered, feathered, and then eviscerate. Dressing, front part, hind part, liver, gizzard, heart, spleen, bursa of fabricius, and thymus were weighed, and the relative weight was calculated.

Hematological and oxidative stress parameters

Blood samples were randomly collected at 35 days of age from 10 chickens from each group. The volume of samples was two ml and they were collected from the wing vein, without anticoagulant into a clean centrifuge tube. Three ml of heparinized blood samples were centrifuged at 2500 rpm for 15 minutes (Dacie and Lewis, 1991). Individual plasma samples were stored in a deep freezer at -20^{o}C until the biochemical analysis.

Hemoglobin value and packed cell volume

The hemoglobin concentration (g /100 ml blood) was determined by the spectrophotometer (Jenway, United Kingdom). Packed cell volume was determined by using microhematocrit tubes, blood centrifuged at 4000 rpm/ minute for 10 minutes, and the mean of the reading obtained was recorded (Dacie and Lewis, 1991).

Total antioxidant capacity and malondialdehyde

Plasma samples were assayed for Total antioxidant

capacity (T-AOC), and Malondialdehyde (MDA) was determined with a spectrophotometer by Colorimetric Method using commercial detection kits (Diamond Biodiagnostic, Egypt)

Statistical analysis

Enumeration data of the mortality and relative organ weight were tested by the Arcsine transformation method (Roger, 2013). A two-way analysis of variance was used to analyze the data by the least squares procedure of the general linear model (GLM) of SAS program (SAS, 2014). Sources of variation were stocking density and prebiotic supplementation The separation of means was done using Duncan's multiple range test (Duncan, 1955) for comparisons among the significant means ($p < 0.05$).

RESULTS AND DISCUSSION

Productive performance

Live body weight

The results of the present study in Table 4 showed that there was a negative relationship between stocking density and BW throughout the experimental period. The low density had a significantly ($p < 0.05$) higher BW than those of medium and high density. Significant differences ($p < 0.05$) in BW were observed between medium and high density at one, four, and five weeks of age, while at two and three weeks of age, no significant differences were observed ($p < 0.05$). Kridtayopas et al. (2019) consider high stocking density as a stress factor which in turn leads to reduced growth performance, gut bacteria, and intestinal morphology. It was reported that high stocking density could decrease final body weight at 42 days (Sekeroglu et al., 2009). In the same line, Tong et al. (2012) found that increasing stocking density decreased body weight and weight gain. Likewise, Ali (2013) obtained that the highest body weight and daily body weight gain were recorded in the lowest density.

The current results also explain that prebiotic supplementation significantly improved body weight throughout the experimental period ($p < 0.05$). This result agrees with Chae et al. (2006), who reported that weight gain improved due to adding β-glucan supplementation to the broiler chicken diet. In addition, Abdel-Hafeez et al. (2017) reported that birds fed a diet supplemented with prebiotics had a greater body weight. Xu et al. (2003) indicated that feeding broilers diet containing 0.4% fracto-oligosaccharieds (FOS) increased the average daily gain. In another study, Shendare et al. (2008) recorded that the BW of broiler chickens fed a diet with 0.01% mannan-oligosaccharide (MOS) was higher as compared to the control group. Likewise, El-Sheikh et al. (2009) reported that adding 0.2% MOS to the diet of Mandarah hens increased BW and BWG. Tavaniello et al. (2018) found that prebiotics increased broiler's BW irrespective of 3 different routes of delivery (*in ovo*, in water, and *in ovo* + in water) as compared to the control group. In addition, Nikpiran et al. (2014) reported that using Fermacto® as a prebiotic at a level of 1.6 g/kg in quail's diet had increased body weight as compared to the control group. Nikpiran et al. (2013b) found that body weight was higher than the control group when they added 1 g/kg Thepax (prebiotic) to the Japanese Quails diet. Babazadeh et al. (2011) reported that the body weight of quail that fed a diet containing 1.6 g/kg (*Aspergillus* meal) as a prebiotic at a level of was higher than the control group.

Feed consumption

The results of the current study showed that stocking density and the interaction with prebiotics had a significantly higher ($p < 0.05$) weekly and total feed consumption during the experiment period, while prebiotics improved weekly feed consumption for the second, third weeks, and the total feed consumption (Table 5). The Lower stocking density resulted in a significant increase ($p < 0.05$) in weekly feed consumption at one and five weeks of age, while at three and four weeks of age, medium groups consumed the highest amount of feed, on the other hand, during the second week the high-density group consumed significantly more feed as compared with other groups ($p < 0.05$). The interaction effect showed that adding 1 cm β-glucan+MOS /liter drinking water had a contradicting effect on daily feed consumption.

These results agree with previous studies by Dozier et al. (2005); Dozier et al. (2006) reported that feed intake was negatively affected by increasing stocking density (Dozier et al., 2005; Dozier et al., 2006). In the same line, Tong et al. (2012) reported that daily feed intake reduced significantly as density increased. Likewise, Cengiz et al. (2015) indicated that feed intake was significantly decreased at high stocking density (20 birds/m^2) as compared with low stocking density (10 birds/m^2). Heidari et al. (2018) found that feed intake had reduced due to increasing stocking density from 12 to 18 birds/m^2. Recently, Miao et al. (2021) demonstrated that high stocking density (20 birds/m^2) significantly decreased feed intake as compared with low stocking density 14 birds/m^2.

The current results indicated that prebiotic

supplementation did not affect feed consumption. These results are in agreement with Benites et al. (2008), who found that the addition of Mannan Oligosaccharide from Bio-Mos (1.0 kg/ton or 0.5 kg/ton) and SAF-Mannanto (0.5 kg/ton) in broiler's diets had no significant effect on feed consumption. Also, Sohail et al. (2013) reported that 0.5% MOS had no significant effect on feed consumption when the broilers were reared under heat stress. Güçlü (2011) fed a Japanese quail diet for 12 weeks with different levels of MOS 0.5 and 1 kg/ton, and they found that prebiotics had no significant effect on feed consumption. Likewise, Iqbal et al. (2017) fed Japanese quail diets supplemented with 0.25%, 0.50%, and 1.0% MOS for 15 weeks, and they found no significant effect on feed intake. And disagree with Piray et al. (2007), who showed that prebiotics increased feed intake during 0-42 days of age. Likewise, it was reported that dietary prebiotic supplementation of 0.5 g mannan oligosaccharide/kg from the first day of age until 42 days of age improved feed consumption (Bozkurt et al., 2009). Abdel-Raheem et al. (2011) reported that cumulative feed intake had increased for birds fed a diet with MOS 2 g/kg in the starter phase and 0.5 g/kg in the grower phase compared to control groups. Also, Rehman et al. (2020) reported that using MOS as a prebiotic in broiler chickens' diet with different levels (0, 1, and 1.5 g/kg) had improved feed intake.

Feed conversion ratio

The current results explained that increasing density resulted in a negative effect on the commutative feed conversion ratio, the best value was recorded when stocking density was 10 birds/m^2 at four and five weeks of age (Table 6). The interaction showed that the low-density group supplemented with prebiotics had the best FCR compared with other groups. The current results agree with the finding of Guardia et al. (2011), who reported that increasing stocking density from 12 to 17 birds/m^2 had a negative effect on FCR and reduced growth performance. Palizdar et al. (2017) found that increasing stocking density had increased FCR, which was the highest value at 21.3 birds/m^2. Abo-Ghanima et al. (2021) indicated that total FCR increased due to increasing stocking density from 25 to 40 kg/m^2.

The current results also explained that cumulative FCR was improved when birds were supplemented with prebiotics. Bozkurt et al. (2009) reported that improving feed conversion was due to stimulated growth of the beneficial microflora in the GIT induced by prebiotic 0.5 g mannan oligosaccharide/kg. Nikpiran et al. (2013a) observed an improvement in broiler performances and FCR due to adding prebiotic Turbo Tox® (1 g/kg) to the diet. In the same line, Waqas et al. (2019) observed an improvement in FCR due to adding MOS 0.2, 0.4, and 0.6 g/Kg. Likewise, Abd-Elsamee et al. (2021) reported that FCR significantly improved when broilers fed diets supplemented with a combination of β-glucan + MOS from yeast or mushroom at rates of 0.01%, 0.02% and 0.03% as compared to control. Nikpiran et al. (2014) reported that FCR had improved due to using Fermacto® as a prebiotic at a level of 1.6 g/kg in the quail's diet as compared to the control group. Nikpiran et al. (2013b) found that FCR had improved due to adding 1 g/kg Thepax to the Japanese Quails diet. Adding prebiotics (*Aspergillus* meal) at a 1.6 g/kg level to a quail diet improved FCR (Babazadeh et al. 2011).

Table 4. Least-square means ± SE of broiler chickens' body weight affected by density, prebiotic, and their interaction at day 35 of age

Items	BW0 (g)	BW1 (g)	BW2 (g)	BW3 (g)	BW4 (g)	BW5 (g)
Density						
10 birds/m^2	49.92 ± 0.27	218.61 ± 1.54^{a}	518.47 ± 4.44^{a}	1022.29 ± 8.87^{a}	1578.21 ± 12.64^{a}	2190.98 ± 21.69^{a}
13 birds/m^2	48.80 ± 0.23	209.06 ± 1.24^{b}	504.94 ± 3.00^{b}	1004.16 ± 6.09^{b}	1530.59 ± 9.08^{b}	2011.90 ± 17.66^{b}
15 birds/m^2	49.62 ± 0.21	203.02 ± 0.94^{c}	510.48 ± 2.36^{b}	1007.85 ± 4.46^{b}	1483.01 ± 6.22^{c}	1842.39 ± 13.19^{c}
Prebiotic						
0 Prebiotic	49.37 ± 0.19	208.36 ± 1.03^{b}	506.84 ± 2.91^{b}	1008.60 ± 5.37^{b}	1514.94 ± 6.21^{b}	1943.32 ± 14.34^{b}
1cm/liter water Prebiotic	49.48 ± 0.19	210.02 ± 1.02^{a}	514.64 ± 2.18^{a}	1012.29 ± 4.78^{a}	1533.63 ± 8.62^{a}	2041.18 ± 15.96^{a}
Interaction						
10 birds/m^2*0 Prebiotic	49.97 ± 0.36	217.58 ± 2.22^{a}	511.03 ± 7.70^{b}	1004.33 ± 14.18^{c}	1543.46 ± 12.47^{b}	2188.75 ± 28.28^{b}
10 birds/m^2*1cm/liter water	49.88 ± 0.40	219.65 ± 2.15^{a}	525.91 ± 4.36^{a}	1040.25 ± 10.46^{a}	1634.96 ± 20.80^{a}	2203.32 ± 33.00^{a}
13 birds/m^2*0 Prebiotic	48.70 ± 0.35	206.97 ± 2.00^{c}	501.36 ± 4.59^{c}	1005.30 ± 8.48^{c}	1537.43 ± 11.66^{c}	1887.24 ± 21.67^{d}
13 birds/m^2*1cm/liter water	48.91 ± 0.32	211.15 ± 1.47^{b}	508.51 ± 3.87^{b}	1003.03 ± 8.77^{c}	1523.75 ± 13.94^{d}	2137.38 ± 23.97^{c}
15 birds/m^2*0 Prebiotic	49.55 ± 0.28	203.38 ± 1.10^{d}	508.66 ± 3.46^{b}	1014.22 ± 6.46^{b}	1491.61 ± 8.52^{f}	1832.50 ± 17.85^{f}
15 birds/m^2*1cm/liter water	49.70 ± 0.31	202.66 ± 1.54^{d}	512.30 ± 3.21^{b}	1001.47 ± 6.14^{c}	1474.42 ± 9.04^{e}	1852.23 ± 19.43^{e}

$^{a-f}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$)

Table 5. Least-square means ± SE of broiler chickens' weekly feed intake affected by stocking density, prebiotic, and their interaction at day 35 of age

Items	FW1 (g)	FW2 (g)	FW3 (g)	FW4 (g)	FW5 (g)	TF (g)
Density						
10 birds/m^2	196.37 ± 1.37^{a}	407.82 ± 4.71^{b}	738.37 ± 7.95^{b}	952.63 ± 11.38^{b}	995.81 ± 27.37^{a}	3094.68 ± 42.46^{a}
13 birds/m^2	179.87 ± 1.04^{b}	408.62 ± 1.64^{b}	753.08 ± 3.88^{a}	968.96 ± 11.62^{a}	949.90 ± 32.14^{b}	3080.56 ± 41.74^{a}
15 birds/m^2	177.12 ± 0.39^{b}	431.35 ± 2.86^{a}	697.37 ± 7.47^{c}	931.77 ± 15.24^{c}	911.05 ± 34.60^{c}	2971.55 ± 50.60^{b}
Prebiotic						
0 Prebiotic	184.58 ± 2.90	412.44 ± 5.04^{b}	726.77 ± 10.67^{b}	952.19 ± 10.37	946.28 ± 29.02	3037.71 ± 40.81^{b}
1cm/liter water Prebiotic	184.33 ± 2.45	419.42 ± 2.82^{a}	732.45 ± 6.52^{a}	950.05 ± 12.05	958.22 ± 25.17	3060.15 ± 38.10^{a}
Interaction						
10 birds/m^2*0 Prebiotic	197.50 ± 2.32^{a}	399.62 ± 6.93^{e}	737.70 ± 13.19^{c}	944.77 ± 18.69^{c}	1002.25 ± 41.64^{a}	3084.38 ± 62.52^{b}
10 birds/m^2*1cm/liter water	195.25 ± 1.60^{a}	416.02 ± 3.28^{b}	739.05 ± 11.01^{c}	960.50 ± 14.65^{b}	989.37 ± 41.66^{b}	3104.98 ± 66.59^{a}
13 birds/m^2*0 Prebiotic	178.75 ± 1.93^{c}	406.02 ± 2.15^{d}	757.72 ± 1.08^{a}	971.60 ± 22.25^{a}	946.67 ± 50.88^{c}	3082.03 ± 72.51^{b}
13 birds/m^2*1cm/liter water	181.00 ± 0.70^{b}	411.22 ± 1.85^{c}	748.45 ± 7.41^{b}	966.32 ± 11.45^{a}	953.12 ± 47.17^{c}	3079.10 ± 53.58^{b}
15 birds/m^2*0 Prebiotic	177.50 ± 0.28^{c}	431.67 ± 5.97^{a}	684.90 ± 11.65^{e}	940.20 ± 12.13^{c}	889.92 ± 53.27^{e}	2946.73 ± 70.83^{d}
15 birds/m^2*1cm/liter water	176.75 ± 0.75^{d}	431.02 ± 1.62^{a}	709.85 ± 4.62^{d}	923.35 ± 29.84^{d}	932.17 ± 49.52^{d}	2996.38 ± 80.75^{c}

$^{a-e}$Mean, within a column, with different superscripts are significantly different ($p < 0.05$). FW: Weekly feed intake, TF: Total feed intake.

Table 6. Least-square means ± SE of broiler chickens' weekly cumulative feed conversion ratio affected by stocking density, prebiotic, and their interaction at day 35 day of age

Items	FCR1	FCR2	FCR3	FCR4	FCR5
Density					
10 birds/m^2	0.90 ± 0.01^{a}	1.15 ± 0.01^{b}	1.31 ± 0.01^{a}	1.44 ± 0.02^{b}	1.50 ± 0.02^{c}
13 birds/m^2	0.86 ± 0.01^{b}	1.16 ± 0.01^{b}	1.33 ± 0.01^{a}	1.51 ± 0.01^{a}	1.62 ± 0.04^{b}
15 birds/m^2	0.87 ± 0.01^{b}	1.19 ± 0.01^{a}	1.29 ± 0.01^{b}	1.50 ± 0.01^{a}	1.71 ± 0.01^{a}
Prebiotic					
0 Prebiotic	0.88 ± 0.01	1.17 ± 0.01	1.31 ± 0.01	1.49 ± 0.01	1.64 ± 0.03^{a}
1cm/liter water Prebiotic	0.87 ± 0.01	1.17 ± 0.01	1.31 ± 0.01	1.48 ± 0.01	1.58 ± 0.03^{b}
Interaction					
10 birds/m^2*0 Prebiotic	0.91 ± 0.01^{a}	1.14 ± 0.01^{c}	1.32 ± 0.02^{b}	1.48 ± 0.03^{b}	1.50 ± 0.03^{c}
10 birds/m^2*1cm/liter water	0.89 ± 0.01^{b}	1.16 ± 0.01^{b}	1.30 ± 0.01^{b}	1.41 ± 0.01^{c}	1.50 ± 0.03^{c}
13 birds/m^2*0 Prebiotic	0.86 ± 0.01^{d}	1.16 ± 0.01^{b}	1.34 ± 0.01^{a}	1.50 ± 0.01^{b}	1.73 ± 0.03^{a}
13 birds/m^2*1cm/liter water	0.85 ± 0.01^{d}	1.16 ± 0.01^{b}	1.33 ± 0.01^{a}	1.51 ± 0.01^{a}	1.52 ± 0.01^{c}
15 birds/m^2*0 Prebiotic	0.87 ± 0.01^{c}	1.20 ± 0.03^{a}	1.27 ± 0.01^{c}	1.49 ± 0.01^{b}	1.70 ± 0.01^{b}
15 birds/m^2*1cm/liter water	0.87 ± 0.01^{c}	1.18 ± 0.01^{a}	1.31 ± 0.01^{b}	1.52 ± 0.02^{a}	1.71 ± 0.04^{a}

$^{a-d}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$). FCR: feed conversion ratio

Table 7. Least-square means ± SE of broiler chickens' carcass traits affected by stocking density, prebiotic, and their interaction at 35 day of age

Items	Dressing W (%)	Front part (%)	Hind part (%)	Liver W (%)	Heart W (%)	Gizzard W (%)
Density						
10 birds/m^2	71.24 ± 0.57	41.58 ± 0.78	29.70 ± 0.76	2.40 ± 0.07	0.46 ± 0.02	1.99 ± 0.06
13 birds/m^2	70.84 ± 0.53	41.11 ± 0.87	29.79 ± 0.61	2.62 ± 0.07	0.5 ± 0.02	2.01 ± 0.07
15 birds/m^2	71.44 ± 0.61	40.85 ± 0.63	30.59 ± 0.33	2.47 ± 0.11	0.48 ± 0.02	2.15 ± 0.12
Prebiotic						
0 Prebiotic	71.37 ± 0.46	41.31 ± 0.56	29.94 ± 0.52	2.43 ± 0.05	0.48 ± 0.01	2.04 ± 0.05
1cm/liter water Prebiotic	70.98 ± 0.47	41.05 ± 0.67	30.11 ± 0.45	2.57 ± 0.08	0.48 ± 0.01	2.07 ± 0.09
Interaction						
10 birds/m^2*0 Prebiotic	72.14 ± 0.85	42.40 ± 1.35	29.18 ± 1.41	2.43 ± 0.12	0.47 ± 0.04	2.01 ± 0.07
10 birds/m^2*1cm/liter water	70.34 ± 0.70	40.75 ± 0.77	30.22 ± 0.64	2.36 ± 0.07	0.45 ± 0.01	1.98 ± 0.11
13 birds/m^2*0 Prebiotic	70.38 ± 0.52	40.48 ± 0.63	30.16 ± 0.65	2.57 ± 0.07	0.49 ± 0.01	2.14 ± 0.09
13 birds/m^2*1cm/liter water	71.30 ± 0.94	41.75 ± 1.64	29.42 ± 1.07	2.68 ± 0.14	0.51 ± 0.04	1.89 ± 0.10
15 birds/m^2*0 Prebiotic	71.58 ± 0.92	41.05 ± 0.83	30.47 ± 0.37	2.29 ± 0.08	0.49 ± 0.02	1.96 ± 0.12
15 birds/m^2*1cm/liter water	71.29 ± 0.85	40.64 ± 0.99	30.70 ± 0.57	2.66 ± 0.20	0.48 ± 0.03	2.34 ± 0.20

$^{a-b}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$). W: Weight

Table 8. Least-square means ± SE of broiler chickens' lymphoid organs affected by density, prebiotic, and their interaction at day 35 of age

Items	Spleen W (%)	Thymus W (%)	Bursa of fabricius W (%)
Density			
10 birds/m^2	0.11 ± 0.007	0.79 ± 0.02^{c}	0.08 ± 0.007
13 birds/m^2	0.12 ± 0.008	0.93 ± 0.02^{b}	0.09 ± 0.005
15 birds/m^2	0.12 ± 0.007	0.99 ± 0.01^{a}	0.11 ± 0.002
Prebiotic			
0 Prebiotic	0.12 ± 0.005	0.90 ± 0.02	0.09 ± 0.004
1cm/liter water Prebiotic	0.12 ± 0.006	0.91 ± 0.02	0.10 ± 0.005
Interaction			
10 birds/m^2*0 Prebiotic	0.12 ± 0.001	0.78 ± 0.03	0.08 ± 0.001
10 birds/m^2*1cm/liter water	0.11 ± 0.006	0.80 ± 0.03	0.08 ± 0.009
13 birds/m^2*0 Prebiotic	0.12 ± 0.001	0.93 ± 0.03	0.09 ± 0.005
13 birds/m^2*1cm/liter water	0.11 ± 0.001	0.92 ± 0.02	0.10 ± 0.001
15 birds/m^2*0 Prebiotic	0.11 ± 0.004	0.98 ± 0.02	0.10 ± 0.002
15 birds/m^2*1cm/liter water	0.13 ± 0.001	1.01 ± 0.02	0.11 ± 0.004

[a-b] Mean, within a column, with different superscripts are significantly different ($p < 0.05$). W: Weight.

Carcass traits

The results of carcass traits in Table 7 showed that neither stocking density nor prebiotic supplementation affect the relative weight of all carcass trait parameters in the current experiment. The current results are in agreement with those of Thomas et al. (2004), who reported that stocking density did not affect the carcass traits significantly. In addition, Sekeroglu et al. (2011) indicated that stocking density had no significant effect on the percentage of carcass yield. On the other hand, Dozier et al. (*2006)*; Onbasilar et al. (2008) reported that high stocking densities had a negative effect on final body weight. On the other hand, Yalçınkaya et al. (2012) found that adding 1 g/kg MOS to the broiler diet did not affect the percentage of carcass yield. However, Tavaniello et al. (2018) reported that prebiotics positively affected breast muscle weight and yield and can positively affect carcass traits and meat quality. Piray et al. (2007) revealed a positive effect on carcass quality when broiler chickens were fed prebiotic fermacto at the level of 1.5 and 3 g/kg, compared to the control group. Ahiwe et al. (2019) reported that adding yeast mannan (YM) at 0.15 or 0.20 g/kg to broiler chickens' diet significantly improved the dressing percentage as compared to the control group. The results of Simitzis et al. (2012) demonstrated that the stocking density of 13 birds/m^2 had a lower liver weight than that of 6 birds/m^2. Sekeroglu et al. (2011) reported that stocking density did not significantly affect liver or heart weights. Waqas et al. (2019) reported that the weight of the liver, gizzard, and heart was higher in birds receiving 0.6 g/Kg of MOS than those of the control group. Waqas et al. (2019) found that gizzard, heart, and liver percentages were increased in birds fed 0.6 g/Kg of MOS compared to those fed a control diet. Also, Rehman et al. (2020) found that using three levels of MOS in a broiler's diet had significant effects on liver, heart, and gizzard weights.

Lymphoid organs

The results of lymphoid organs in Table 8 demonstrated that neither stocking density nor prebiotic supplementation affected the relative weight of the spleen and bursa of Fabricius. However, the relative weight of the thymus was significantly increased with the increase in stocking density ($p < 0.05$). Houshmand et al. (2012) reported that the spleen and bursa of Fabricius weights did not significantly affect by stocking density. Likewise, Azzam and El-Gogary (2015) reported that stocking density had no significant effect on the bursa of Fabricius or spleen weights. Thymus weight did not affect by increasing stocking density, and the highest weight was for the medium density 20.35 g, while low and high density was 19.20 and 19.85 g, respectively. The results agree with Tong et al. (2012), who reported that different stocking densities had no significant effect on thymus weight. Thymus weight did not affect by increasing stocking density, and the highest weight was for the medium density 20.35 g, while low and high density was 19.20 and 19.85 g, respectively. Qaid et al. (2016)

reported that the relative weights of the thymus did not affect by stocking density. In addition, Houshmand et al. (2012) reported that adding prebiotics (Bio-Mos) to the starter and finisher diets at 2 and 1 g/kg, respectively, had no significant effect on the spleen or bursa of Fabricius weights. Guo et al. (2003) reported that adding β-Glucan to the diet had increased the relative weights of the spleen, thymus, and bursa as compared to the control. Usama et al. (2018) added β-Glucan + MOS to the diet, had increased the percentage of lymphoid organs. Chand et al. (2019) obtained that the relative weight of the thymus, spleen, and bursa of Fabricius was significantly increased due to adding 100 g/kg MOS to the broiler diet as compared to the control group. On the other hand, Simitzis et al. (2012) reported that increasing stocking density from 6 to 13 birds/m^2 decreased spleen weight and high density had lower weights of spleen and bursa of Fabricius than those in low density. In addition, Ali (2013) reported that high stocking density had reduced the bursa of Fabricius weight and its percentage at day 42 of age.

Hematological parameters

Hemoglobin and packed cell volume

The hemoglobin concentration of chickens at medium density was significantly higher than those of the other densities ($p < 0.05$, Table 9). On the other hand, birds at low density had significantly the lowest value ($p < 0.05$). These results disagreed with Sekeroglu et al. (2011), who found that stocking density had no significant effect on hemoglobin.

The current study showed that supplementation significantly increased the Hg value from 7.05 g/dl to 6.24 g/dl, compared to the control group. The interaction between the density and prebiotic showed that all densities had been affected by adding the prebiotic supplement and had a higher value than those un-supplemented group. The results agreed with those of Mohammed et al. (2016) and Oni et al. (2020) reported that prebiotic supplementation improved hemoglobin value compared to the control group.

There was a significant effect due to stocking density and their interaction on PCV, while prebiotics had no significant effect as compared to low-density and un-supplemented groups. Broiler chickens at high and low stocking density almost had the same PCV value, and it was higher than the medium density value ($p < 0.05$). In the present study, the interaction showed that adding prebiotics to low and high-density birds improved PCV values. AL-Kassie et al. (2008) reported that when the prebiotic was added to the broiler chickens' diet at 10 g/kg increased the percentage of PCV at day 42 of age. Muhammad et al. (2020) and Tarabees et al. (2021) found that adding Isomalto-oligosaccharides 0.5 g/kg to broiler chickens' diet had no significant effect on hemoglobin or PCV.

Oxidative stress parameters

Total antioxidant capacity

Stocking density, prebiotic, and their interaction had a significant effect on TAOC in chickens reared in low density which had the significantly highest level of TOAC followed by those at high and then medium density ($p < 0.05$, Table 9). This result is in agreement with those reported by Zhao et al. (2009) and Cai et al. (2019), that stress caused by high stocking density influenced the antioxidant status of broiler chickens. The results of Wang et al. (2021) demonstrated that adding 200 mg/Kg apple pectic oligosaccharide to breeder chickens' diet increased TAOC values.

Malondialdehyde

The results showed that MDA raised due to the increase in stocking density. Chickens in low density had significantly the lowest value, while MDA in those in medium and high density had almost high concentrations ($p < 0.05$). These results agreed with Simsek et al. (2009), who reported that stocking density of up to 22 birds/m^2 may lead to oxidative stress and MDA increased when the stocking density increased.

On the other hand, the results demonstrated that prebiotic supplementation had no effect on MDA ($p < 0.05$). The interaction between density and prebiotic refers to contradicted results. The results were in agreement with those of Tarabees et al. (2021), who found that prebiotic Isomalto-oligosaccharides had no significant effect on MDA. However, Zhou et al. (2019) noted a decrease in MDA content when they added MOS at levels of 0.5, 1, and 1.5 g/kg to the diet.

European production efficiency factor

The best significant value of EPEF was found at the low density of (415.83) flowed by those in 13 bird/m^2 and then in 15 bird/m^2 ($p < 0.05$, Table 10). Gholami et al. (2020) conducted a study to evaluate the effect of stocking density on broiler chicken performance and economic profit. They found that the stocking density significantly affected economic income when the study included four densities of 10, 15, 17, and 20 chicks/m2,

and the highest earnings were a density of 20 chicks/m2. Nasr et al. (2021) studied the impact of stocking density on growth performance to recommend a better density with low production cost and high quality. They found that the medium density of 18 birds/m^2 revealed better performance and the best from the economic point of view than the high density of 20 birds/m^2. Adding prebiotics to the broiler chicken diet positively affected the European production efficiency factor and increased the value to 372.25 compared with the control 341.24. Lowry et al. (2005) reported that using β-glucan as a prebiotic in the diet for broilers challenged with *Salmonella* had decreased mortality rate and economic loss for broiler chickens. Waqas et al. (2019) added different levels of MOS 0.2, 0.4, and 0.6 g/Kg to broiler chicken's diet. They found that the broilers diet with 0.6 g/kg MOS led to the best profit percentage per 1 Kg of meat as compared with the control. In addition, Ibrahim et al. (2021) reported that adding prebiotic AGRIMOS (a high source of mannan oligosaccharides and β glucans) at a level of 0.1% to broiler chicken diets low in protein (95, 90, and 85% of NRC) has a useful effect on economic value.

Table 9. Least-square means ± SE of hematological and oxidative stress parameters affected by broiler chickens' density, prebiotic, and their interaction at day 35 of age

Items	Hg (g/dL)	PCV (%)	TAOC (mM / L)	MDA (nmol/ml)
Density				
10 birds/m^2	5.53 ± 0.24^{c}	28.05 ± 1.40^{a}	0.32 ± 0.02^{a}	16.81 ± 1.81^{b}
13 birds/m^2	7.47 ± 0.37^{a}	26.80 ± 0.97^{b}	0.12 ± 0.01^{c}	18.51 ± 0.75^{a}
15 birds/m^2	6.92 ± 0.27^{b}	28.45 ± 0.84^{a}	0.14 ± 0.01^{b}	18.06 ± 1.40^{a}
Prebiotic				
0 Prebiotic	6.24 ± 0.25^{b}	27.36 ± 0.96	0.16 ± 0.02^{b}	17.80 ± 1.22
1cm/liter water Prebiotic	7.05 ± 0.30^{a}	28.16 ± 0.82	0.23 ± 0.02^{a}	17.78 ± 1.04
Interaction				
10 birds/m^2*0 Prebiotic	5.01 ± 0.26^{c}	25.50 ± 2.45^{d}	0.27 ± 0.02^{b}	14.57 ± 2.33^{c}
10 birds/m^2*1cm/liter water	6.06 ± 0.35^{b}	30.60 ± 0.90^{a}	0.38 ± 0.03^{a}	19.05 ± 2.69^{a}
13 birds/m^2*0 Prebiotic	7.17 ± 0.39^{a}	27.30 ± 1.21^{c}	0.09 ± 0.01^{d}	19.12 ± 0.89^{a}
13 birds/m^2*1cm/liter water	7.78 ± 0.63^{a}	26.30 ± 1.57^{c}	0.15 ± 0.02^{c}	17.89 ± 1.25^{b}
15 birds/m^2*0 Prebiotic	6.54 ± 0.35^{b}	29.30 ± 0.84^{b}	0.14 ± 0.02^{c}	19.71 ± 2.49^{a}
15 birds/m^2*1cm/liter water	7.31 ± 0.41^{a}	27.60 ± 1.45^{c}	0.14 ± 0.01^{c}	16.40 ± 1.19^{b}

$^{a-b}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$). TAOC: Total antioxidant capacity, MDA: Malondialdehyde, Hg: Hemoglobin, PCV: Packed cell volume

Table 10. Least-square means ± SE of European production efficiency factor affected by broiler chickens' density, prebiotic, and their interaction at 35th day of age

Items	EPEF (%)
Density	
10 birds/m^2	415.83 ± 15.44^{a}
13 birds/m^2	351.09 ± 19.30^{b}
15 birds/m^2	303.32 ± 10.51^{c}
Significant level	<.0001***
Prebiotic	
0 Prebiotic	341.24 ± 17.46^{b}
1cm/liter water Prebiotic	372.25 ± 18.54^{a}
Significant level	0.0565*
Interaction	
10 birds/m^2*0 Prebiotic	414.45 ± 19.06^{a}
10 birds/m^2*1cm/liter water	417.22 ± 27.36^{a}
13 birds/m^2*0 Prebiotic	307.23 ± 16.37^{c}
13 birds/m^2*1cm/liter water	394.96 ± 13.71^{b}
15 birds/m^2*0 Prebiotic	302.04 ± 6.39^{c}
15 birds/m^2*1cm/liter water	304.59 ± 21.77^{c}
Significant level	0.0530*

$^{a-c}$ Mean, within a column, with different superscripts are significantly different ($p < 0.05$). EPEF: European Production Efficiency Factor

CONCLUSION

It can conclude that adding prebiotics (Mannan-oligo saccharide and B-Glucan) can partially ameliorate the adverse effect of high stocking density on productive performance, physiological and oxidative stress parameters, and European production efficiency factor.

DECLARATION

Acknowledgments

All authors are thankful to the Professor of poultry physiology" Prof. Dr. Abdel-Rahman Mohamed Mohamed Atta" for the study idea and his effort in facilitating this trial. Sincere thanks to Miser Al-Rabiea for the poultry Company and Miser Arabian poultry company for funding the authors with chicks and Vaccines. Moreover, Ismailia Miser for the poultry company for funding the authors with the Basal diet.

Authors' contribution

Essam Mohamed Hassan Karar collected the data, performed the data analysis, and wrote the manuscript draft. Abdel-Rahman Mohamed Mohamed Atta for the study idea, designed the study and revised the manuscript. Mohamed Abdel-Rahman Abdel-Hamed El-Menawey was responsible for the scientific material collection used in the experiment and revised the manuscript. Hassan Bayoumi Ali Gharib was responsible for the laboratory analysis. All authors have read and approved the final data and manuscript.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Competing interests

All research authors agree to publish this research and do not have any conflict of interest.

Ethical consideration

This research was truthful and did not plagiarize or pattern any other papers or ideas. Any fabrication or falsification did not find in this research. This article or any scientific results did not submit to any journals.

REFERENCES

Abdel-Hafeez HM, Saleh ESE, Tawfeek SS, Youssef IMI, and Abdel-Daim ASA (2017). Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. Asian-Australasian Journal of Animal Sciences, 30(5): 672-682. DOI: https://www.doi.org/10.5713/ajas.16.0535

Abdel-Raheem SM and Abd-Allah SMS (2011). The Effect of single or combined dietary supplementation of mannan oligosaccharide and probiotics on performance and slaughter characteristics of broilers. International Journal of Poultry Science, 10(11): 854-862. DOI: https://www.doi.org/10.3923/ijps.2011.854.862

Abd-Elsamee MO, Abd-Elhakim AES, Elsharkawy RR, and Elsherif HMR (2021). Impact of using different sources and levels of β-glucan and mannan oligosaccharide on performance traits of broiler chicks. Advances in Animal and Veterinary Sciences, 9(11): 1851-1862. DOI: https://www.doi.org/10.17582/journal.aavs/2021/9.11.1851.1862

Abo-Ghanima MM, Swelum AA, Shukry M, Ibrahim SA, Abd El-Hack ME, Khafaga AF, Alhimaidi AR, Ammari AA, El-Tarabily KA, and Younis MEM (2021). Impacts of tea tree or lemongrass essential oils supplementation on growth, immunity, carcass traits, and blood biochemical parameters of broilers reared under different stocking densities. Poultry Science, 100(11): 101443. DOI: https://www.doi.org/10.1016/j.psj.2021.101443

Ahiwe EU, Omede AA, Abdallh ME, Chang'a EP, Al-Qahtani M, Gausi H, and Graham H, and Iji PA (2019). Response of broiler chickens to dietary supplementation of enzymatically hydrolyzed glucan or mannan yeast products. Journal of Applied Poultry Research, 28(4): 892-901. DOI: https://www.doi.org/10.3382/japr/pfz047

Ali AHH (2013). Productive performance and immune response as affected by broiler stocking density. Egyptian Poultry Science Journal, 33(4): 795-804.

Al-Kassie GAM, Al-Jumaa YMF, and Jameel YJ (2008). Effect of probiotic (Aspergillus niger) and prebiotic (Taraxacum officinale) on blood picture and biochemical properties of broiler chicks. International Journal of Poultry Science, 7(12): 1182-1184. DOI: http://www.doi.org/10.3923/ijps.2008.1182.1184

Alloui MN, Szczurek W, and Świątkiewicz S (2013). The usefulness of prebiotics and probiotics in modern poultry nutrition: A review. Annals of Animal Science, 13(1): 17-32. DOI: https://www.doi.org/10.2478/v10220-012-0055-x

Azzam MMM and El-Gogary MR (2015). Effects of dietary threonine levels and stocking density on the performance, metabolic status and immunity of broiler chickens. Asian Journal of Animal and Veterinary Advances, 10(5): 215-225. DOI: https://www.doi.org/10.3923/ajava.2015.215.225

Babazadeh D, Vahdatpour T, Nikpiran H, Jafargholipour MA, and Vahdatpour S (2011). Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (Coturnix japonica). Indian Journal of Animal Sciences, 81(8): 870-874. Available at: https://www.cabdirect.org/cabdirect/abstract/20113289755

Benites V, Gilharry R, Gernat AG, and Murillo JG (2008). Effect of dietary mannan oligosaccharide from bio-mos or SAF-mannan on live performance of broiler chickens. Journal of Applied Poultry Research, 17(4): 471-475. DOI: https://www.doi.org/10.3382/japr.2008-00023

Bozkurt M, Küçükyılmaz K, Çatlı AU, and Çınar M (2009). The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. South African Journal of Animal Science, 39(3): 197-205. DOI: https://www.doi.org/10.4314/sajas.v39i3.49152

Cai CH, Zhao RX, Wang P, Wang JS, Li KX, Zhan XA, and Wang KY (2019). Effects of different stocking densities on growth performance, antioxidant ability, and immunity of finishing broilers. Animal Science Journal, 90(4): 583-588. DOI: https://www.doi.org/10.1111/asj.13148

Cengiz Ö, Köksal BH, Tatll O, Sevim Ö, Ahsan U, Üner AG, Beyaz D, Büyükyörük S, Yakan A, and Önol AG (2015). Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. Poultry Science, 94(10): 2395-2403. DOI:

https://www.doi.org/10.3382/ps/pev194

Chae BJ, Lohakare JD, Moon WK, Lee SL, Park YH, and Hahn TW (2006). Effects of supplementation of β-glucan on the growth performance and immunity in broilers. Research in Veterinary Science, 80(3): 291-298. DOI: https://www.doi.org/10.1016/j.rvsc.2005.07.008

Chand N, Shamsullah, Rafiullah, Khan RU, Mobashar M, Naz S, Rowghani E, and Khan MA (2019). Mannanoligosaccharide (MOS) in broiler ration during the starter phase: 1. Growth performance and intestinal histomorpholgy. Pakistan Journal of Zoology, 51(1): 173-176. DOI: https://www.doi.org/10.17582/journal.pjz/2019.51.1.173.176

Dacie JV and Lewis SM (1991). Practical haematology, 7th Edition. ChurchhillL Livingstone., London. pp. 1-73.

Dozier WA, Thaxton JP, Branton SL, Morgan GW, Miles DM, Roush WB, Lott BD, and Vizzier-Thaxton Y (2005). Stocking density effects on growth performance and processing yields of heavy broilers. Poultry Science, 84(8): 1332-1338. DOI: https://www.doi.org/10.1093/ps/84.8.1332

Dozier WA, Thaxton JP, Purswell JL, Olanrewaju HA, Branton SL, and Roush WB (2006). Stocking density effects on male broilers grown to 1.8 kilograms of body weight. Poultry Science, 85(2): 344-351. DOI: https://www.doi.org/10.1093/ps/85.2.344

Duncan DB (1955). The multiple ranges and multiple F tests. Biometrics, 11(1): 1-42. DOI: https://www.doi.org/10.2307/3001478

El-Sheikh AMH, Abdalla EA, and Maysa MH (2009). Study on productive performance, hematological and immunological parameters in a local strain of chicken as affected by mannan oligosaccharide under hot climate conditions. Egyptian Poultry Science Journal, 29(1): 287-305. Available at: https://www.cabdirect.org/cabdirect/abstract/20113110343

Feddes JJ, Emmanuel EJ, and Zuidhoft MJ (2002). Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. Poultry Science, 81(6): 774-779. DOI: http://www.doi.org/10.1093/ps/81.6.774

Froebel LK, Jalukar S, Lavergne TA, Lee JT, and Duong T (2019). Administration of dietary prebiotics improves growth performance and reduces pathogen colonization in broiler chickens. Poultry Science, 98(12): 6668-6676. DOI: https://www.doi.org/10.3382/ps/pez537

Gholami M, Chamani M, Seidavi A, Sadeghi AA, and Aminafschar M (2020). Effects of stocking density and environmental conditions on performance, immunity, carcase characteristics, blood constitutes, and economical parameters of cobb 500 strain broiler chickens. Italian Journal of Animal Science, 19(1): 524-535. DOI: https://www.doi.org/10.1080/1828051X.2020.1757522

Guardia S, Konsak B, Combes S, Levenez F, Cauquil L, Guillot JF, Moreau-Vauzelle C, Lessire M, Juin H, and Gabriel I (2011). Effects of stocking density on the growth performance and digestive microbiota of broiler chickens. Poultry Science, 90(9): 1878-1889. DOI: https://www.doi.org/10.3382/ps.2010-01311

Güçlü BK (2011). Effects of probiotic and prebiotic (mannanoligosaccharide) supplementation on performance, egg quality and hatchability in quail breeders. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 58(1): 27-32. DOI: https://www.doi.org/10.1501/vetfak_0000002445

Guo Y, Ali RA, and Qureshi MA (2003). The influence of β-glucan on immune responses in broiler chicks. Immunopharmacology and Immunotoxicology, 25(3): 461-472. DOI: https://www.doi.org/10.1081/IPH-120024513

Heidari S and Toghyani M (2018). Effect of stocking density and methionine levels on growth performance and immunity of broiler chicks. Iranian Journal of Applied Animal Science, 8(3): 483-489. Available at: https://ijas.rasht.iau.ir/article_542688.html

Houshmand M, Azhar K, Zulkifli I, Bejo MH, and Kamyab A (2012). Effects of prebiotic, protein level, and stocking density on performance, immunity, and stress indicators of broilers. Poultry Science, 91(2): 393-401. DOI: https://www.doi.org/10.3382/ps.2010-01050

Ibrahim NSK, Ahmed ABNS, and Abdel-Raheem GSE (2021). Impact of dietary supplementation of prebiotics on the growth performance and immunity in broilers fed low protein diets. Assiut Veterinary Medical Journal, 67(171): 103-119. DOI: http://www.doi.org/10.21608/avmj.2021.205249

Iqbal MA, Hussain A, Roohi N, Arshad MI, and Khan O (2017). Effects of mannan-oligosaccharides-supplemented diets on production performance of four close-bred flocks of Japanese quail breeders. South African Journal of Animal Sciences, 47(3): 290-297. DOI: https://www.doi.org/10.4314/sajas.v47i3.5

Kridtayopas C, Rakangtong C, Bunchasak C, and Loongyai W (2019). Effect of prebiotic and synbiotic supplementation in diet on growth performance, small intestinal morphology, stress, and bacterial population under high stocking density condition of broiler chickens. Poultry Science, 98(10): 4595-4605. DOI: https://www.doi.org/10.3382/ps/pez152

Lowry VK, Farnell MB, Ferro PJ, Swaggerty CL, Bahl A, and Kogut MH (2005). Purified β-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against Salmonella enterica serovar Enteritidis. International Journal of Food Microbiology, 98(3): 309-318. Available at: https://pubag.nal.usda.gov/download/39276/pdf

Mahmoud UT, Mahmoud MA, Abdel–Mohsein HS, and Amen OA (2018). AGRIMOS® prebiotics: Effect on behavior, performance, cecal microbial population and humeral immunity in broiler chickens. Journal of Advanced Veterinary Research, 8(3): 49-59. Available at: https://www.advetresearch.com/index.php/AVR/article/view/303

Mahrose KM, Alagawany M, Abd El-Hack ME, Mahgoub SA, and Attia FAM (2019). Influences of stocking density and dietary probiotic supplementation on growing Japanese quail performance. Anais da Academia Brasileira de Ciencias, 91(2): e20180616. DOI: https://www.doi.org/10.1590/0001-3765201920180616

Meluzzi A and Sirri F (2009). Welfare of broiler chickens. Italian Journal of Animal Science, 8(S1): 161-173. DOI: https://www.doi.org/10.4081/ijas.2009.s1.161

Miao ZQ, Dong YY, Qin X, Yuan JM, Han MM, Zhang KK, Shi SR, Song XY, Zhang JZ, and Li JH (2021). Dietary supplementation of methionine mitigates oxidative stress in broilers under high stocking density. Poultry Science, 100(8): 101231. DOI: https://www.doi.org/10.1016/j.psj.2021.101231

Mohammed LS, Kamel ER, Abo-Salem M, ST A, and RM ES (2016). Effect of probiotics, prebiotics, synbiotics, organic acids and enzymes supplementation on broiler chicks' immunity in relation to the economic performance. Benha Veterinary Medical Journal, 30(2): 34-44. DOI: https://doi.org/10.21608/bvmj.2016.31327

Nasr MAF, Alkhedaide AQ, Ramadan AAI, Hafez AESE, and Hussein MA (2021). Potential impact of stocking density on growth, carcass traits, indicators of biochemical and oxidative stress and meat quality of different broiler breeds. Poultry Science, 100(11): 101442. DOI: https://www.doi.org/10.1016/j.psj.2021.101442

Nikpiran H, Taghavi M, Khodadadi A, and Athari SS (2013a). Influence of probiotic and prebiotic on broiler chickens performance and immune status. Journal of Novel Applied Sciences, 2(8): 256-259. Available at: https://jnasci.org/wp-content/uploads/2013/08/256-259.pdf

Nikpiran H, Vahdatpour T, Babazadeh D, and Vahdatpour S (2013b). Effects of *Saccharomyces cerevisiae*, Thepax and their combination on blood enzymes and performance of Japanese quails (Coturnix japonica). Journal of Animal and Plant Sciences, 23(2): 369-375.

Nikpiran H, Vahdatpour T, Daryoush B, Tabatabaei SM, and Vahdatpour S (2014). Effects of functional feed additives on growth influenced

hormones and performance of Japanese quails (Coturnix japonica). Greener Journal of Biological Sciences, 4(2): 039-044. DOI: https://www.doi.org/10.15580/gjbs.2014.2.021014096

Onbasilar EE, Poyraz Ö, Erdem E, and Ozturk H (2008). Influence of lighting periods and stocking densities on performance, carcass characteristics and some stress parameters in broilers. Archiv Fur Geflugelkunde, 72(5): 193-200. Available at: https://hdl.handle.net/20.500.12587/4068

Oni OO, Alabi JO, and Adebayo RM (2020). Comparative efficacy of lactose and mannan-oligosaccharides on performance, nutrient digestibility and health status of broiler chicks. Indian Journal of Poultry Science, 55(2): 121-128. DOI: https://www.doi.org/10.5958/0974-8180.2020.00017.3

Palizdar MH, Daylami MK, and Pourelmi MR (2017). Effects of high stocking density on growth performance, blood metabolites and immune response of broilers (ROSS 308). Journal of Livestock Science, 8: 196-200. Available at: http://livestockscience.in/wp-content/uploads/stocking-densities.pdf

Piray AH, Kermanshahi H, Tahmasbi AM, and Bahrampour J (2007). Effects of cecal cultures and aspergillus meal prebiotic (Fermacto) on growth performance and organ weights of broiler chickens. International Journal of Poultry Science, 6(5): 340-344. DOI: http://www.doi.org/10.3923/ijps.2007.340.344

Prentza P, Castellone F, Legnardi M, Antlinger B, Segura-Wang M, Kefalas G, Fortomaris P, Argyriadou A, Papaioannou N, Stylianaki I et al. (2022). Effects of a multi-genus synbiotic (PoultryStar® sol) on gut health and performance of broiler breeders. Journal of World's Poultry Research. 12(4): 212-229. DOI: https://www.doi.org/10.36380/jwpr.2022.24

Qaid M, Albatshan H, Shafey T, Hussein E, and Abudabos AM (2016). Effect of stocking density on the performance and immunity of 1-to 14-d- old broiler chicks. Revista Brasileira de Ciencia Avicola, 18(4): 683-692. DOI: https://www.doi.org/10.1590/1806-9061-2016-0289

Rehman A, Arif M, Sajjad N, Al-Ghadi MQ, Alagawany M, Abd El-Hack ME, Alhimaidi AR, Elnesr SS, Almutairi BO, Amran RA et al. (2020). Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. Poultry Science, 99(12): 6946-6953. DOI: https://www.doi.org/10.1016/j.psj.2020.09.043

Roger EK (2013). Experimental design procedures for the behavioral sciences, 4th Edition. Sage., Los Angeles. p. 107.

Sekeroglu A, Demir E, Sarica M, and Ulutas Z (2009). Effects of housing systems on growth performance, blood plasma constituents and meat fatty acids in broiler chickens. Pakistan Journal of Biological Sciences, 12(8): 631-636. DOI: http://www.doi.org/10.3923/pjbs.2009.631.636

Sekeroglu A, Sarica MUSA, Gulay MS, and Duman M (2011). Effect of stocking density on chick performance, internal organ weights and blood parameters in broilers. Journal of Animal and Veterinary Advances, 10(2): 246-250. Available at: https://www.cabdirect.org/cabdirect/abstract/20113067439

Shendare RC, Gongle MA, Rajput AB, Wanjari BV, and Mandlekar SM (2008). Effect of supplementation of Manno-Oligosaccharide and b-glucans on maize based meal on commercial broilers. Veterinary World, 1(1): 13-15. Available at: http://www.veterinaryworld.org/2008/January/Supplementation.pdf

Simitzis PE, Kalogeraki E, Goliomytis M, Charismiadou MA, Triantaphyllopoulos K, Ayoutanti A, Niforou K, Hager-Theodorides AL, and Deligeorgis SG (2012). Impact of stocking density on broiler growth performance, meat characteristics, behavioural components and indicators of physiological and oxidative stress. British Poultry Science, 53(6): 721-730. DOI: https://www.doi.org/10.1080/00071668.2012.745930

Simsek UG, Dalkilic B, Ciftci M, and Yuce A (2009). The influences of different stocking densities on some welfare indicators, lipid peroxidation (MDA) and antioxidant enzyme activities (GSH, GSH-Px, CAT) in broiler chickens. Journal of Animal and Veterinary Advances, 8(8): 1568-1572. Available at: https://medwelljournals.com/fulltext/?doi=javaa.2009.1568.1572

Skrbic Z, Pavlovski Z, Lukic M, Petricevic V, Djukic-Stojcic M, and Zikic D (2011). The effect of stocking density on individual broiler welfare parameters: 2. Different broiler stocking densities. Biotechnology in Animal Husbandry, 27(1): 17-24. DOI: http://www.doi.org/10.2298/BAH1101017S

Sohail MU, Ijaz A, Younus M, Shabbir MZ, Kamran Z, Ahmad S, Anwar H, Yousaf MS, Ashraf K, Shahzad AH et al. (2013). Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. Journal of Applied Poultry Research, 22(3): 485-491. DOI: https://www.doi.org/10.3382/japr.2012-00682

Tarabees R, Hafez HM, Shehata AA, Allam TS, Setta A, and Elsayed MSA (2021). Effects of probiotic and/or prebiotic supplementations on immune response, haematology, oxidant-antioxidant biomarkers, and cytokine mRNA expression levels in the caeca of broilers infected with salmonella. Poultry Science Journal, 9(1): 41-52. DOI: https://www.doi.org/10.22069/psj.2021.18438.1637

Tavaniello S, Maiorano G, Stadnicka K, Mucci R, Bogucka J, and Bednarczyk M (2018). Prebiotics offered to broiler chicken exert positive effect on meat quality traits irrespective of delivery route. Poultry Science, 97(8): 2979-2987. DOI: https://www.doi.org/10.3382/ps/pey149

Thomas DG, Ravindran V, Thomas DV, Camden BJ, Cottam YH, Morel PCH, and Cook CJ (2004). Influence of stocking density on the performance, carcass characteristics and selected welfare indicators of broiler chickens. New Zealand Veterinary Journal, 52(2): 76-81. DOI: https://www.doi.org/10.1080/00480169.2004.36408

Tong HB, Lu J, Zou JM, Wang Q, and Shi SR (2012). Effects of stocking density on growth performance, carcass yield, and immune status of a local chicken breed. Poultry Science, 91(3): 667-673. DOI: https://www.doi.org/10.3382/ps.2011-01597

Usama TM, Manal AM, Hosnia SA, and Omar AA (2018). AGRIMOS® Prebiotics: Effect on Behavior, Performance, Cecal Microbial Population and Humeral Immunity in Broiler Chickens. Journal of Advanced Veterinary Research, 8(3):49-59. Available at: https://advetresearch.com/index.php/AVR/article/view/303

Wang J, Zhang C, Zhao S, Ding X, Bai S, Zeng Q, Zhang K, Zhuo Y, Xu S, Mao X et al. (2021). Dietary apple pectic oligosaccharide improves reproductive performance, antioxidant capacity, and ovary function of broiler breeders. Poultry Science, 100(4): 100976. DOI: https://www.doi.org/10.1016/j.psj.2020.12.073

Waqas M, Mehmood S, Mahmud A, Saima, Hussain J, Ahmad S, Tahir KM, Rehman A, Zia MW, and Shaheen MS (2019). Effect of yeast based mannan oligosaccharide (Actigen™) supplementation on growth, carcass characteristics and physiological response in broiler chickens. Indian Journal of Animal Research, 53(11): 1475-1479. DOI: https://www.doi.org/10.18805/ijar.B-923

Xu ZR, Hu CH, Xia MS, Zhan XA, and Wang MQ (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Science, 82(6): 1030-1036. DOI: http://www.doi.org/10.1093/ps/82.6.1030

Yalçinkaya I, Çinar M, Yildirim E, Erat S, Başalan M, and Güngör T (2012). The effect of prebiotic and organic zinc alone and in combination in broiler diets on the performance and some blood parameters. Italian Journal of Animal Science, 11(3): e55. DOI: https://www.doi.org/10.4081/ijas.2012.e55

Yaqoob MU, El-Hack MEA, Hassan F, El-Saadony MT, Khafaga AF, Batiha GE, Yehia N, Elnesr SS, Alagawany M, El-Tarabily KA et al. (2021). The potential mechanistic insights and future implications for the effect of prebiotics on poultry performance, gut microbiome, and intestinal morphology. Poultry Science, 100(7):

101143. DOI: https://www.doi.org/10.1016/j.psj.2021.101143

Zhao JP, Chen JL, Zhao GP, Zheng MQ, Jiang RR, and Wen J (2009). Live performance, carcass composition, and blood metabolite responses to dietary nutrient density in two distinct broiler breeds of male chickens. Poultry Science, 88(12): 2575-2584. DOI: https://www.doi.org/10.3382/ps.2009-00245

Zhou M, Tao Y, Lai C, Huang C, Zhou Y, and Yong Q (2019). Effects of mannanoligosaccharide supplementation on the growth performance, immunity, and oxidative status of partridge shank chickens. Animals, 9(10): 817. DOI: https://www.doi.org/10.3390/ani9100817

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 61-70, March 25, 2023

Research Paper, PII: S2322455X2300006-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.6

Effects of Black Pepper, Turmeric, and Fennel on Essential and Non-essential Chemical Contents of Egg

Lopamudra Samantaray[1] * and Yashaswi Nayak[2]

[1]*Department of Zoology, School of Applied Sciences, Jatni, India*
[2]*Centurion University of Technology and Management, Jatni-752050, Odisha, India*

Corresponding author's E-mail: lopamudraray88@gmail.com

Received: 19 January 2023
Accepted: 08 March 2023

ABSTRACT

The use of essential oils (EOs) in animal feeding has gained attention as a potential antibiotic growth promoter replacement over the past two decades. The current study aimed to determine the impact of three feed additives, namely black pepper, turmeric, and fennel, on the productivity of laying hens, the chemical composition, and macro- and microelement content in layer eggs. A total of 280 chicks aged 75 days were randomly divided into 7 treatments (5 replicates of 8 chicks). One treatment group was provided as the unsupplemented control. The other six treatment groups, namely D0 (basal diet [BD] control), D1 (BD + 1% of black pepper), D2 (BD + 1% of turmeric), D3 (BD + 1% of fennel), D4 (BD + 0.5% of black pepper + 0.5% of turmeric), D5 (BD + 0.5% of black pepper + 0.5% of fennel), D6 (BD + 0.5% of turmeric + 0.5% of fennel) were supplemented with varying levels of phytobiotics. The result of the study indicated that the egg weight, Hen-day-production (HDP), egg quality, and haugh unit significantly improved with a combined supplementation of phytobiotics (D4, D5, and D6 diets) when compared with the control. However, there were no significant differences in the chemical composition of eggs. The X-ray fluorescence spectrometer analysis of eggs revealed the presence of 17 significant elements, including phosphorous, sulfur, chlorine, potassium, calcium, manganese, iron, copper, zinc, and bromine. The study findings showed that the combined supplementation of phytobiotics lowered K and Cl, whereas Zn, Ca, S, and Cu contents positively increased in hen eggs by including phytobiotic in the diet. In conclusion, the EOs of phytobiotics as dietary supplementation at 1% and 0.5% could improve the HDP, egg weight, and egg mass, including nutrient elements in the egg.

Keywords: Egg, Essential oil, Hen, Mineral, Phytobiotic

INTRODUCTION

Poultry eggs are a worthy source of essential nutrients, proteins, vitamins, and minerals for human beings. However, the use of conventional antibiotic feed additives or antibiotic growth promoters in poultry feed has caused numerous complications in public health, such as antibiotic residue in poultry products and the development of antibiotic resistance in consumers (Saeed et al., 2018). Consumer anxiety about food safety could stimulate the animal feed production revolution and enhance the use of phytobiotic feed additives (Abd El-Ghany, 2020). Herbs belong to phytobiotic feed additives and contain secondary metabolites or bioactive compounds such as alkaloids, carotenoids, phenols, and flavonoids (Mohanraj et al., 2018; Ivanisova et al., 2020). These phytogenic attributes play a key role in producing healthy and safe poultry eggs, ultimately contributing to the maintenance of consumer health (Samantaray and Nayak, 2022).

The biological, physical, and chemical properties of poultry eggs are well known as they are a rich source of protein, fat, vitamins, and minerals. However, the investigations on the effect of phytobiotic feed additives and the possibility of positive impact on poultry eggs are scanty (Mirzaei et al., 2022). The objective of the present study was to evaluate the effect of three herbs, namely black pepper, turmeric, and fennel, on poultry eggs. These herbs are widely distributed and commonly used in Indian households as spices due to their considerable importance for human consumption in ayurvedic science and also rich in secondary metabolites like phytopolyphenols, polypropanoids, and flavonoids (Kumar et al., 2017). As

previous studies noted, phytochemicals, such as capsaicin (Liu et al., 2021), curcumin (Yadav et al., 2020), caffeoylquinic acid, and rutin (Castaldo et al., 2021) add a positive impact on the quality of egg and health of layer chickens.

Eggs are a good source of minerals that are macro elements, microelements, and trace elements. Elements, such as phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), and calcium (Ca) belong to macro elements (Welch and Graham, 2004). Ions in less amount include manganese (Mn), bromine (Br), sodium (Na), copper (Cu), rubidium (Rb), strontium (Sr), zinc (Zn), selenium (Se), fluoride (F), titanium (Ti) and chromium (Cr); Dobrzanski et al., 2020). Aliu et al. (2021) classified heavy metals into the essential (Mn, Iron [Fe], Cu, and Zn) and non-essential groups (Rb, Br, and Sr). Gombart et al. (2020) classified Fe, Zn, Cu, and Mn micronutrients as essential for humans. A slightly different classification of the trace element is given by Rehault-Godbert et al. (2019), that Ca, Cu, Fe, Mn, P, K, and Zn are found in raw whole eggs, egg yolk, and egg white. In contrast, Lim and Schoenun (2010) include Cu, Mn, Zn, and Cr as heavy metals with toxic properties depending on their doses consumed by the consumer.

The accumulation of essential and non-essential chemical trace elements in poultry eggs suggests that the composition and quantities of feed additives, as well as their chemical form, bioavailability, and bioactive substances or secondary metabolites, have a significant impact on the quality of poultry eggs (Abduljaleel, 2016; Ahmad et al., 2017; Heflin et al., 2018). This research was designed to address concerns with consumer health, egg quality, and the impacts of black pepper, turmeric, fennel, and their different combinations as a feed supplement in layer (*Gallus gallus*) diets, and the traceability of essential and non-essential nutrients in poultry eggs.

MATERIAL AND METHODS

Ethical approval

All experimental methods involving the use of live animals were conducted with the approval of the Centurion University, Jatni, India, Ethics Committee for Experiments with Animals.

Study design

The research was carried out from May 2022 to November 2022 at Centurion University of Technology and Management in Bhubaneswar, India. A total of 280 75-day-old chicks (*Gallus gallus*) with an average weight of 826.67 gm were purchased from the local hatchery of Jatni, India. After being weighed, the chicks were divided into one control and six experimental groups and randomly placed in a poultry house. Each group was further separated into five replicates, with eight chicks in each under controlled climate conditions. In the poultry house, an adaptation period of 2 weeks was maintained. Hens began laying eggs at week 25 of age, and 17 eggs were randomly chosen from each group for the study. Ever four chickens were roomed in 2 m^2 of floor area. The chicks were fed a conventional balanced diet as per NRC (1994) standard recommendations. An appropriate room temperature of daily high (29-32^oC) and low (25-22^oC) were regulated by an electronic control panel (Temptron-607 AgroLogic), and 14 hours of light and 10 hours of dark schedule for natural light were maintained throughout the experiment. The relative humidity was 65-76% throughout the investigation.

The daily feed consumption of layer chickens was calculated as g/day. To calculate feed consumption, the difference between the leftover feed and the given feed/week was determined (Abou-Elkhair et al., 2018). The percentage of hen-day production was calculated from week 25 of age using daily egg production records (Adebiyi et al., 2018).

Data on bird mortality, feed consumption, weight gain, and growth performance were recorded weekly. The chemical composition of the chickens' diet was analyzed according to the protocols of Cerrate et al. (2019), Barzegar et al. (2019), and AOAC (2005), in the Department of Applied Science, CUTM, BBSR, Odisha, India (Table 1). A control diet was given to the control group (D0), and three oral dietary phytobiotic essential oils (Eos) supplements with varied compositions were given to the other six groups. The chickens in the D0 received a basal diet with no additive (control), D1 was fed a basal diet plus 1% of black pepper (10 g/Kg of feed), D2 chickens were subjected to a basal diet plus 1% of turmeric (10g/Kg of feed), D3 received basal diet plus 1% of fennel (10g/Kg of feed), chickens in D4 were fed basal diet plus 0.5% of black pepper plus 0.5% of turmeric (10g/Kg of feed), D5 received basal diet plus 0.5% of black pepper plus 0.5% of fennel (10g/Kg of feed), and D6 was given basal diet plus 0.5% of turmeric plus 0.5% of fennel (10g/Kg of feed).

Table 1. Composition and chemical content of diets fed to layers for 6 months

Items (g/kg)	Control diet	Experimental groups (D_1- D_6)					
	D_0	D_1	D_2	D_3	D_4	D_5	D_6
Maize	355	353	352	353	351	355	347
Wheat	254	252	252	251	250	249	252
Soybean meal	244	242	243	243	245	243	245
Millet	40	39	40	39	40	39	38
Peanut meal	81	78	77	77	77	78	82.6
Black pepper	-	10	-	-	5	5	-
Turmeric	-	-	10	-	5	-	5
Fennel	-	-	-	10	-	5	5
DL-methionine	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Dicalcium phosphate	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Sodium chloride	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin-mineral complex[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Metabolizable energy (MJ/kg)[3]							
Crude protein	176	181	178	177	180	179	176
Lysine	8.4	8.5	8.5	8.5	8.6	8.5	8.4
Methionine	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Threonine	6.5	6.6	6.6	6.5	6.5	6.6	6.5
Calcium	39.0	40.1	40.2	39	40.1	40.1	39
Total protein	3.2	3.2	3.1	3.4	3.1	3.3	3.3
Sodium	1.8	1.8	1.9	1.9	1.8	1.9	2.0
DEB[4] (mEq)	175	177	178.1	178	177	179	179

[1]D0: Basal diet plus control diet, D1: Basal diet plus 1% of black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel; D4: Basal diet plus 0.5% of black pepper plus 0.5% of turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel. [2]The supplied premix/kg of diet: 3.45 mg retinyl acetate; 2 mg menadione (K3); 20 mg DL-alpha-tocopheryl acetate; 0.075 mg cholecalciferol; 2 mg thiamine; 2 mg riboflavin; 0.015 mg cyanocobalamin; 25 mg niacin; 8 mg 11 mg d-pantothenic acid; 1.1 mg folic acid; 0.13 mg biotin; 12,300 IU vitamin A, 4,500 IU vitamin D3; [3]Calculated according to (Barzegar et al., 2019; Cerrate et al., 2019) as a sum of ME content of components; [4]Dietary Electrolyte Balance.

Essential oils

Black pepper, turmeric, and fennel were purchased from the local market of Jatni, India, in March 2022. The EOs of the three phytobiotics were extracted using the supercritical CO_2 extraction method at the Centurion University Research and Development Laboratories in Paralakhemundi, India. The dried phytobiotics were processed to a fine powder using a grinder (SS Pulverizer, 3HP, India), and stored in an airtight vacuum-sealed bag. To reduce the loss of essential oils during the process, the phytobiotic powders were cooled (at 10°C) in a refrigerator (SSU-168, India) for two hours before grinding. Each phytobiotic powder (1000 g) was added to the high-pressure equilibration vessel. Liquid CO2 was supplied into the system via a reciprocating pump at a constant flow rate of 10 ml/minute and compressed to extraction pressures of 60°C and 300 bar for black pepper (Shityakov et al., 2019; Tran et al., 2019), 60°C and 250 bar for turmeric (Gopalan et al., 2000; Neves et al., 2020), and 32°C and 300 bar for fennel (Hammouda et al., 2013). For each phytobiotic EO, the precipitated fractions were collected in a trapping flask. The collected samples were kept in individual amber bottles at 4°C for further use. The extraction yields were calculated using gravimetric analysis.

X-Ray fluorescent spectrometer

The X-Ray fluorescent spectrometer operates on the principle that individual stimulated atoms by X-ray photons, wavelength, or external energy can count the number of photons released by the sample at each energy level (Figure 1). The elements can be measured and classified based on the excited characteristic x-ray emission rate. Freshly laid hen (*Gallus gallus*) eggs were procured from all experimental groups for 6 consecutive days at the pick (about 90%) of laying at week 30 of the age. Undamaged eggs (n = 17) of each experimental group with a weight between 42 and 44 g were randomly chosen, and each egg was labeled with the group name, number, and lay date (Figure 2).

The eggs were kept at 4°C and weighed using an analytical balance (Aczet, CY 224 by HPFS instruments India LLP, Vasai). Eggs were broken into a sheet of a plain whiteboard, and the edible part of the whole egg (albumin and yolk combined) was well stirred in a glass vessel. A metal container was dried to a constant weight in a hot air oven at 100°C. The egg samples were placed in these metal containers and heated to 100°C for 24 hours

until the sample could gain a constant weight. The dried egg samples were crushed with a mortar and pestle and kept in cleaned plastic bags. The X-ray fluorescence spectrometer (Epsilon 1 PAN analytical B. V., Netherlands) was used to quantify the elements present in samples. The X-Ray beam ranged 40-60 KV and 50-300 W (Jamaluddin et al., 2018).

Statistical analysis

The obtained data were analyzed using the Statistical analysis system (SAS, 2012). The significance of the findings was tested using one-way ANOVA. The Tukeys test was used to compare mean values at a significance level of $p < 0.05$. Box-whisker charting was chosen as the most appropriate descriptive statistical analysis approach for the comparative analysis of the data sets of macro element measurements. Box plots were used in this study to represent the means of various data sets as well as the range between the 25 and 75 percentiles. In contrast, outliers were considered to be insignificant and were not considered.

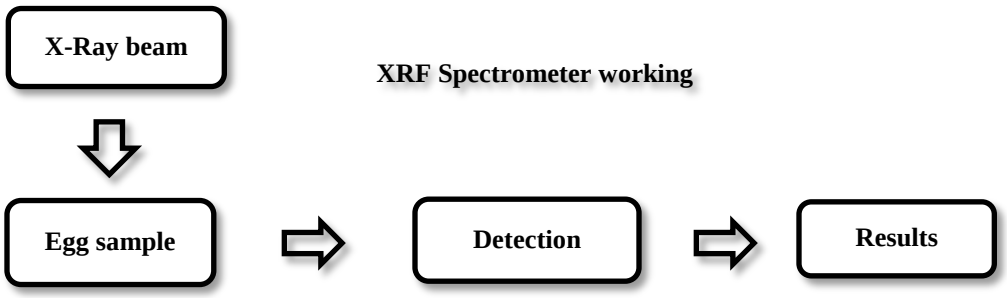


Figure 1. Working of the X-Ray fluorescent spectrometer (Epsilon-1) for the analysis of hen egg mineral contents

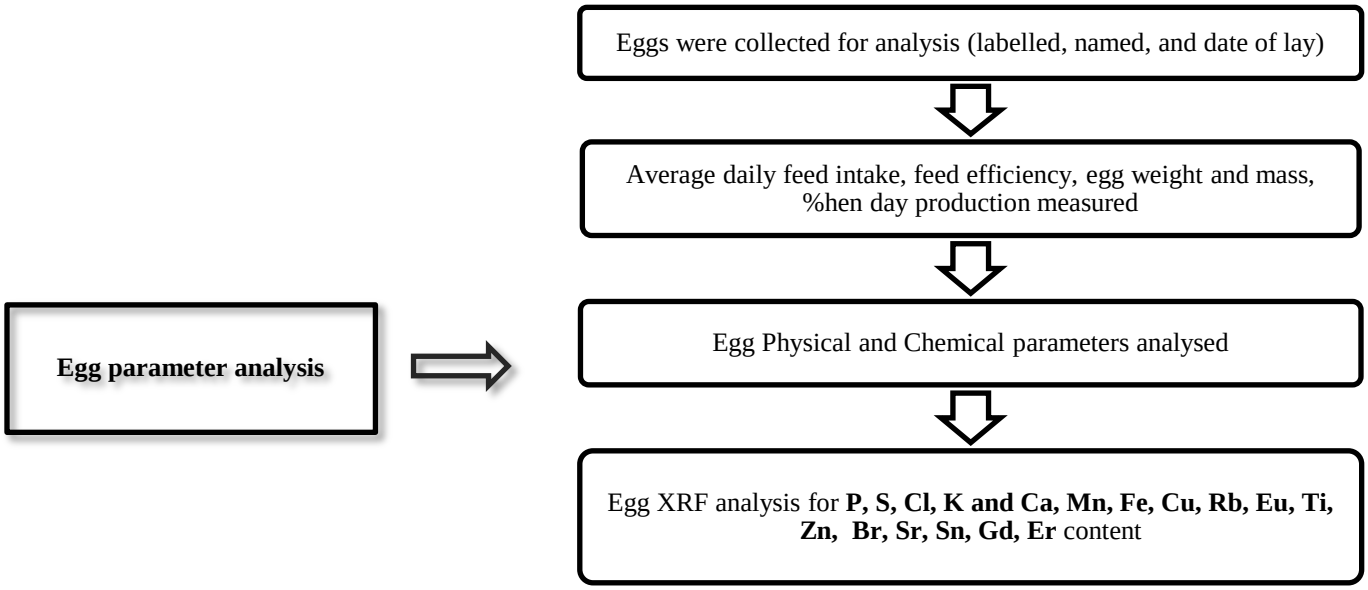


Figure 2. The schematic diagram for hen egg parameter analysis

RESULTS AND DISCUSSION

The Hen day production (HDP) of the layer chickens fed with D5 was 90.34% (0.5% black pepper and 0.5% fennel), and 86.67% for D4 (0.5% black pepper and 0.5% turmeric), which were significantly higher than that of the other experimental groups ($p < 0.05$). The obtained results were higher than those reported by Nuraini and Djulardi (2019). As can be seen in Table 2, D1, D3, and D6 groups were found to have a similar range of HDP and egg mass;

however, D0 (control) had the lowest HDP of 76.67% and egg mass. The D4, D5, and D2 diets substantially affected egg mass values, as Phuoc et al. (2019) supported.

The average egg weight was considerably higher in D4 when compared to the other experimental groups and the control ($p < 0.05$). Egg weight is a well-known factor that affects egg quality. Regarding the egg weight, the highest (44.80±0.02 g) was for D4 (Table 2). The findings indicated that the treatment-related values in D2 and D5 were statistically equivalent. In contrast, D1 and D6 have been proven to have a significantly similar effect on egg weight compared to D0, D3, and D4 diets ($p < 0.05$). There were no significant differences in feed efficiency with diets D1, D3, and D6, compared to D0, D2, and D5 ($p > 0.05$).

It has been demonstrated that albumin weight affects internal egg quality, with thicker and higher albumin content regarded as superior quality (Kowalska et al., 2021). Compared to the other experimental groups, the chickens fed the D2 and D4 diets indicated significantly greater albumin contents ($p < 0.05$), which is consistent with Abou-Elkhair et al. (2018). Indicators of the yolk quality include weight, texture, hardness, and scent (Wijedasa et al., 2020). According to Table 3, the yolk weight of the eggs from chickens in the D4 diet was found to be 18.87 ± 0.01 g, indicating higher yolk quality when compared to D0 (control) diet ($p < 0.05$), which is supported by Nuraini and Djulardi (2019). The D6 diet group showed no significant effect on yolk weight ($p > 0.05$), compared to the D0 (control) diet. The current findings are supported by Souza et al. (2020).

All 17 trace elements (P, S, Cl, K, Ca, Mn, Fe, Cu, Rb, Eu, Ti, Zn, Br, Sr, Gd, Er) were quantitatively found in layer eggs of all experimental groups. Figure 3 displays the highest P, S, Cl, K, and Ca contents. Other macronutrients were found in lower concentrations in the D0 (control), while K was found in higher concentrations in the control group, compared to other experimental groups. Layer chickens fed D5 diet had the highest Fe, P, and Ca levels. Those fed D6 diet had greater levels of S and Cl than the other experimental groups.

The proximate composition of eggs was similar, regardless of the dietary treatments (Table 4). As seen in Table 3, Mn was only found in diets D2 and D6. According to Gilani et al. (2021), hens can use the calcium in calcium carbonate to produce bones and eggshells. Ca-phytate complex formation was accelerated by high Ca concentrations relative to P. All experimental diets had no significant effect on Cu, K, or Rb ($p < 0.05$). As shown in Figure 3, the present findings for Ca content were greater in D4 and D5 diets than those reported by Dobrzanski et al. (2020) and Rehault-Godbert et al. (2019). The results of the present study for K content were similar to those reported by Ahmad et al. (2017); however, they were lower than the suggested content by Rehault-Godbert et al. (2019). According to Table 3, diets D2, D4, and D6 contain titanium (Ti), higher than those measured by Dobrzanski et al. (2020). Regarding D2 and D6 diets, the presence of Eu was below detectable levels. The amount of Zn was found to be significantly higher in diet D5 than in the control ($p < 0.05$), which is considerably less than the reported value of Zn by Aliu et al. (2021). The mineral profiles for Gd and Er were only detected in egg samples given with the D1 and D6 diets, respectively.

The mineral content of eggs can be used to evaluate the mineral status of animals (Gilani et al., 2021). The results of this study revealed that phytobiotics significantly increased Fe, Se, and Zn concentrations in hen eggs ($p < 0.05$), which is similar to the findings of recent research (Bonos et al., 2022; Vlaicu et al., 2022). Phytobiotics enhance Fe absorption in the intestine, allowing Fe to enter cells via the clathrin-mediated endocytosis pathway (Chithrani and Chan, 2007). The Zn defends cell membranes from oxidative damage and is a crucial component of the body's antioxidant defense mechanism (Aliu et al., 2021). This could happen due to Zn scavenging free radicals through enhanced metallothionein production. Additionally, Zn might facilitate metallothionein and function as a P53 co-transcriptional factor to repair DNA damage brought on by oxidative stress (Yin et al., 2022). This could explain the advantages of increased Zn concentrations shown in hen eggs provided with the D5, D4, and D1 diets, which contained combinations of black pepper and turmeric, black pepper and fennel, and black pepper, respectively ($p < 0.05$).

The thyroid metabolism depends heavily on trace minerals (Abdelli et al., 2021). It was shown that Fe supplementation could enhance T3 levels while decreasing T4 levels in livestock, which was significant since serum T4 levels can influence fat metabolism by controlling the production and breakdown of cholesterol (Dilawar et al., 2021). The current investigation found that phytobiotics significantly lowered the serum T4 concentration in diets D5, D4, and D3, compared to other groups ($p < 0.05$). By supplementing livestock diets with EOs of phytobiotics, the increased Fe content may activate 5'-deiodinase activity and increase T3 to T4 conversion (Abdelli et al., 2021). Dietary trace elements, particularly Zn, can stimulate carboxypeptidase B and stabilize many proteins to facilitate protein conversion (Vlaicu et al., 2022). Incorporating phytobiotics may have enhanced performance by upregulating the quality of the eggs produced by the layers (Samantaray and Nayak, 2022).

Copper (Cu) is recognized as essential and toxic to many biological processes. It can enter food materials

through mineralization by crops, food processing, or environmental contamination (Afsana et al., 2004; Rehault-Godbert et al., 2019). The detected Cu means value in egg samples was 0.289 µg/gm with diet D5 and D1, and Cu values in egg samples analyzed in the presented study were close to the level reported in the above-mentioned studies. However, Cu levels in the D0 control and D6 diet were significantly lower than the other experimental diets ($p < 0.05$), and with no significant differences between the D2 and D4 diets ($p < 0.05$). Fe is an essential mineral, and it is well known that including enough Fe in the diet is essential for lowering the risk of developing anemia (Yin et al., 2022). In this investigation, Fe was substantially higher in the D5 diet when compared to similar values in D3 and D4 diets, and it is significantly lower in the D6 diet, compared to D5 ($p < 0.05$). The mean Fe concentrations showed a significant difference ($p < 0.05$) between the D5 and D0 diets, which was not beyond the standard limit reported by Rehault-Godbert et al. (2019). The daily intake of essential elements, such as Ca, P, Cl, Mn, Fe, and Zn, were compared to the provisional and recommended daily intake of minerals and FAA/WHO for certain foods (FAO/WHO, 2002; RDI, 2020). As shown in Table 3 and Figure 3, the calculated data of the average daily intake of Cl, Fe, Mn, Cu, Zn, and P from the egg sample did not exceed the standard limit. Compared to the other group elements, Cu and Fe came closer to the limit of the essential elements.

Table 2. The effect of different phytobiotic combinations on layer performance over a 6-month period

Egg parameter	Control diet	Experimental diet[1]					
	D0	D1	D2	D3	D4	D5	D6
Average egg weight (gm)	34.12 ± 0.17^{c}	40.33 ± 0.11^{b}	42.72 ± 0.12^{ab}	39.71 ± 0.14^{b}	44.8 ± 0.02^{a}	42.92 ± 0.09^{ab}	40.85 ± 0.07^{b}
Hen day production (%)	76.67 ± 0.19^{d}	80.1 ± 0.011^{c}	83.33 ± 0.1^{b}	80.13 ± 0.12^{c}	86.67 ± 0.13^{a}	90.34 ± 0.14^{a}	80.4 ± 0.12^{c}
Egg mass (g/day/hen)	26.15 ± 0.45^{c}	32.33 ± 0.21^{b}	35.72 ± 0.12^{ab}	31.81 ± 0.17^{b}	38.82 ± 0.2^{a}	38.77 ± 0.14^{a}	32.84 ± 0.17^{b}
Yolk weight (gm)	14.25 ± 0.07^{c}	16.61 ± 0.04^{b}	17.56 ± 0.03^{a}	17.94 ± 0.1^{a}	18.87 ± 0.01^{a}	16.34 ± 0.04^{b}	14.63 ± 0.05^{c}
Albumin weight (gm)	19.7 ± 0.1^{c}	22.23 ± 0.12^{ab}	23.71 ± 0.00^{a}	23.21 ± 0.01^{a}	23.87 ± 0.01^{a}	22.25 ± 0.01^{ab}	21.37 ± 0.00^{b}
Haugh Unit	70.88 ± 0.1^{c}	79.99 ± 0.20^{ab}	88.24 ± 0.14^{a}	75.38 ± 0.11^{b}	78.64 ± 0.1^{b}	81.33 ± 0.12^{ab}	85.78 ± 0.09^{a}

[1]D0: Basal diet plus control diet, D1: Basal diet plus 1% of black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel; D4: Basal diet plus 0.5% of black pepper plus 0.5% of turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel. [abc]Different letters in the same row indicate significant differences at $p < 0.05$.

Table 3. The effect of phytobiotics on the mineral content of the edible portion of the hen eggs (yolk and albumen)

Nutrients/Elements	Elements (µg/gm)						
	Control diet	Experimental diet[1]					
	D0	D1	D2	D3	D4	D5	D6
Iron (Fe)	$6.37^{b} \pm 0.001$	$6.72^{b} \pm 0.01$	$5.99^{bc} \pm 0.002$	$7.3^{b} \pm 0.009$	$7.32^{b} \pm 0.001$	$10.71^{a} \pm 0.005$	$5.65^{c} \pm 0.001$
Zinc (Zn)	$2.22^{b} \pm 0.001$	$3.10^{a} \pm 0.003$	$2.61^{ab} \pm 0.002$	$2.85^{ab} \pm 0.001$	$3.31^{a} \pm 0.001$	$3.39^{a} \pm 0.001$	$2.29^{b} \pm 0.004$
Manganese (Mn)	-	-	$0.316^{a} \pm 0.003$	-	-	-	$0.042^{b} \pm 0.000$
Copper (Cu)	$0.204^{b} \pm 0.0001$	$0.289^{a} \pm 0.001$	$0.224^{b} \pm 0.0001$	$0.251^{ab} \pm 0.000$	$0.226^{b} \pm 0.001$	$0.289^{a} \pm 0.002$	$0.214^{b} \pm 0.000$
Rubidium (Rb)	-	$0.740^{a} \pm 0.01$	$0.683^{ab} \pm 0.021$	$0.644^{b} \pm 0.001$	$0.479^{c} \pm 0.003$	-	$0.176^{d} \pm 0.002$
Europium (Eu)	$0.761^{a} \pm 0.001$	$0.741^{a} \pm 0.000$	-	$0.599^{b} \pm 0.001$	$0.630^{ab} \pm 0.003$	$0.0001^{c} \pm 0.000$	-
Titanium (Ti)	-	-	$0.338^{b} \pm 0.008$	-	$0.293^{c} \pm 0.002$	$0.380^{a} \pm 0.004$	$0.266^{c} \pm 0.005$
Bromine (Br)	$0.44^{a} \pm 0.0001$	$0.235^{c} \pm 0.000$	$0.292^{c} \pm 0.001$	$0.363^{b} \pm 0.001$	$0.317^{bc} \pm 0.002$	$0.268^{c} \pm 0.000$	$0.3^{bc} \pm 0.002$
Strontium (Sr)	$0.206^{a} \pm 0.001$	-	$0.095^{b} \pm 0.004$	-	-	-	-
Tin (Sn)	$0.515^{a} \pm 0.0001$	-	-	$0.368^{b} \pm 0.000$	$0.395^{b} \pm 0.001$	$0.545^{a} \pm 0.003$	$0.364^{b} \pm 0.005$
Gadolinium (Gd)	-	$1.32^{a} \pm 0.001$	-	-	-	-	-
Erbium (Er)	-	-	-	-	-	-	$2.40^{a} \pm 0.01$

[1]D0: Basal diet plus control diet, D1: Basal diet plus 1% of black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel, D4: Basal diet plus 0.5% of black pepper plus 0.5% of turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel. [abcd]Different letters in the same row indicate significant differences at $p < 0.05$.

Table 4. The effect of phytobiotics on the chemical composition of the edible portion of the hen eggs (yolk and albumen)

Chemical composition / Experimental groups[1]	Moisture Content (%)	Crude protein (%)	Crude fat (%)	Total ash (%)	Carbohydrate (%)	Energy (Kcal/100g)
D0	74.12 ± 0.350^{b}	13.54 ± 0.04^{a}	13.18 ± 0.066^{a}	0.82 ± 0.128^{b}	0.87 ± 0.33^{b}	186.59 ± 1.27^{c}
D1	75.79 ± 0.278^{a}	13.16 ± 0.075^{a}	13.38 ± 0.171^{a}	1.26 ± 0.040^{a}	0.85 ± 0.209^{b}	195.65 ± 1.64^{a}
D2	74.95 ± 0.31^{b}	12.35 ± 0.10^{b}	13.28 ± 0.11^{a}	0.89 ± 0.08^{b}	0.93 ± 0.27^{a}	189.12 ± 1.45^{bc}
D3	75.14 ± 0.060^{a}	13.56 ± 0.068^{a}	12.26 ± 0.075^{b}	0.9 ± 0.044^{b}	0.88 ± 0.048^{b}	185.9 ± 0.801^{c}
D4	74.64 ± 0.225^{b}	12.36 ± 0.136^{b}	12.52 ± 0.111^{b}	0.76 ± 0.051^{bc}	0.94 ± 0.202^{a}	190.1 ± 1.686^{b}
D5	75.50 ± 0.153^{a}	12.5 ± 0.170^{b}	12.42 ± 0.136^{b}	0.88 ± 0.116^{b}	0.96 ± 0.224^{a}	193.92 ± 1.07^{ab}
D6	76.42 ± 0.14^{a}	13.80 ± 0.12^{a}	12.73 ± 0.10^{b}	0.88 ± 0.07^{b}	0.89 ± 0.15^{b}	193.30 ± 1.18^{ab}
D7	74.52 ± 0.11^{b}	12.56 ± 0.08^{b}	12.99 ± 0.06^{b}	0.68 ± 0.05^{c}	0.9 ± 0.04^{c}	188.55 ± 0.93^{bc}

[1]D0: Basal diet plus control diet, D1: Basal diet plus 1% of black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel, D4: Basal diet plus 0.5% of black pepper plus 0.5% of turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel. [abcd]Different letters in the same row indicate significant differences at $p < 0.05$.

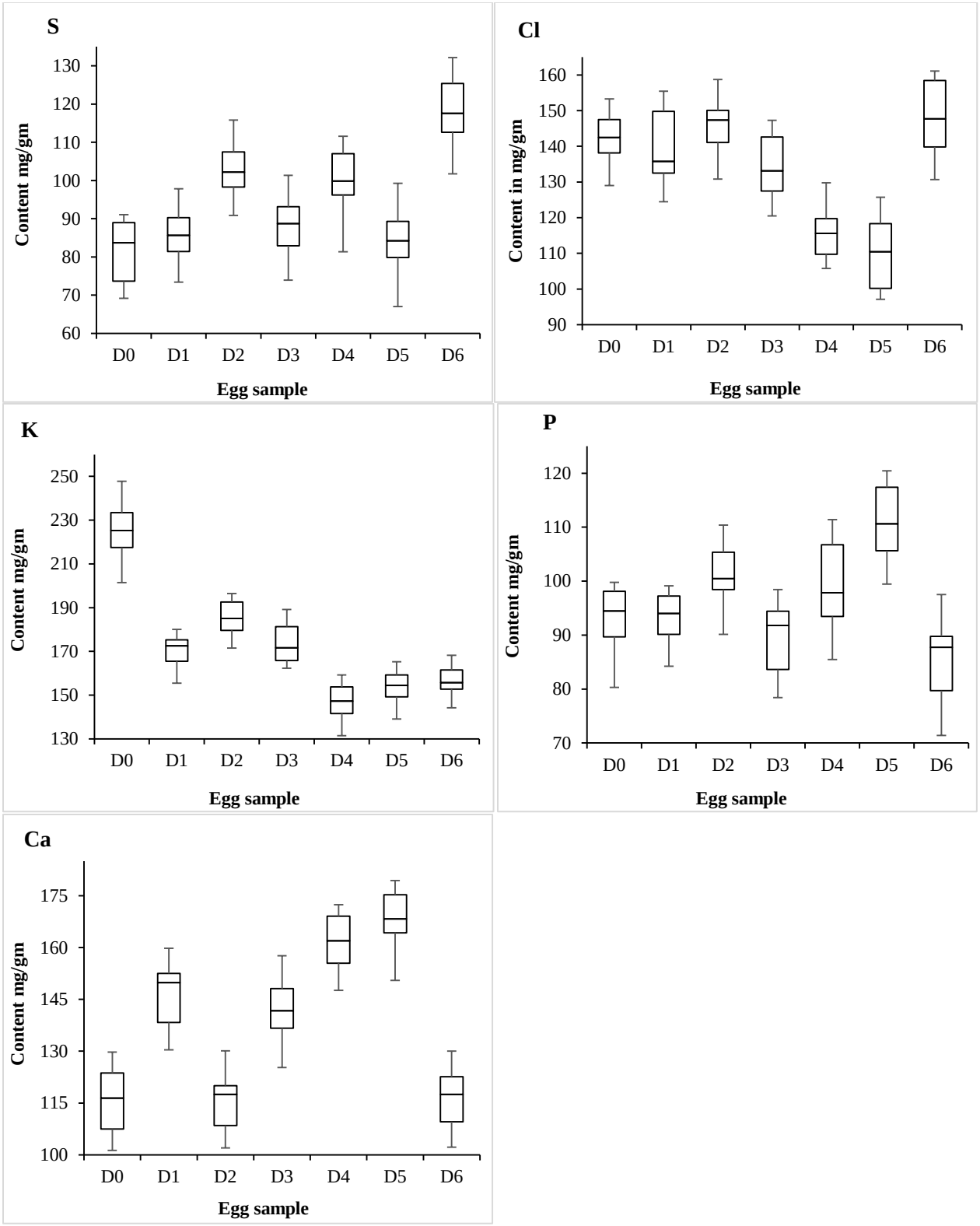


Figure 3. Box-whisker plots of (macro elements) phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), and calcium (Ca) content detected in hen eggs (week 30) with different experimental diets. D0: Basal diet plus control diet, D1: Basal diet plus 1% of Black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel; D4: Basal diet plus 0.5% of black pepper plus 0.5% of Turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel.

CONCLUSION

The results of the present study revealed that when phytobiotics were added to layer feed, they positively impacted the *Gallus gallus* laying performance and nutrient content of eggs. Layer chickens that received combined phytobiotics diet D4 (0.5% black pepper and 0.5% turmeric), D5 (0.5% black pepper and 0.5% fennel), and D6 (0.5% turmeric and 0.5% fennel) had better egg quality than layers that did not consume phytobiotics. Therefore, Eos of black pepper, turmeric, and fennel could become ideal feed additives for layer chickens to elevate egg nutrient content. However, more study is required to determine the ideal phytobiotic concentration for layer chickens' diets as a potential replacement for antibiotic growth promoters.

DECLARATION

Funding

This research received no external funding.

Authors' contributions

Lopamudra Samantaray conceptualized the paper, and fieldwork, contributed to writing, reviewed, and edited the manuscript and Yashaswi Nayak Compiled the information and prepared the draft for the manuscript. Both authors read and approved the final manuscript for publication in the present journal.

Competing interests

The authors declare no conflict of interest.

Ethical consideration

The authors thoroughly examined all ethical concerns surrounding plagiarism, consent to publish, misconduct, data fabrication, falsification, duplicate publishing or submission, and manuscript redundancy.

Availability of data and materials

This article includes all data generated or analyzed during this research.

REFERENCES

Abd El-Ghany WA (2020). Phytobiotics in poultry industry as growth promoters, antimicrobials, and immunomodulators – A review. Journal of World's Poultry Research, 10(4): 571-579. DOI: https://www.doi.org/10.36380/jwpr.2020.65

Abdelli N, Sola-Oriol, and Perez DJF (2021). Phytogenic feed additives in poultry: Achievements, prospective and challenges. Animals, 11(12): 3471. DOI: https://www.doi.org/10.3390/ani11123471

Abduljaleel SA (2016). Determination of some trace elements in chicken eggs from different sources. Journal of Pharmacognosy and Phytochemistry, 5(5): 417-420. Available at: https://www.phytojournal.com/archives/2016/vol5issue5/PartF/5-4-21-363.pdf

Abou-Elkhair R, Selim S, and Hussein E (2018). Effect of supplementing layer hen diet with phytogenic feed additives on laying performance, egg quality, egg lipid peroxidation and blood biochemical constituents. Animal Nutrition, 4(4): 394-400. DOI: https://www.doi.org/10.1016/j.aninu.2018.05.009

Adebiyi FG, Ologhobo AD, and Adejumo IO (2018). Raw Allium sativum as performance enhancer and hypocholesterolemic agent in laying. Asian Journal of Animal and Veterinary Advances, 13(3): 210-217. DOI: https://www.doi.org/10.3923/ajava.2018.210.217

Afsana K, Shiga K, Ishizuka S, and Hara H (2004). Reducing effect of ingesting Tannic acid on the absorption of Iron, but not of Zinc, Copper and Manganese by rats. Bioscience, Biotechnology, and Biochemistry, 68(3): 584-592. DOI: https://www.doi.org/10.1271/bbb.68.584

Ahmad S, Khalique A, Pasha TN, Mehmood S, Hussain K, Ahmad S, Shaheen MS, Naeem M, and Shafiq M (2017). Effect of moringa oleifera (Lam.) pods as feed additive on egg antioxidants, chemical composition and performance of commercial layers. South African Journal of Animal Science, 47(6): 865-874. DOI: http://www.doi.org/10.4314/sajas.v47i6.14

Aliu H, Dizman S, Sinani A, and Hodolli G (2021). Comparative study of heavy metal concentration in eggs originating from industrial poultry farms and free-range hens in Kosovo. Journal of Food Quality, 2021: 6615289. DOI: https://www.doi.org/10.1155/2021/6615289

Association of officiating analytical chemists (AOAC) (2005). Official method of analysis of AOAC international, 18th Edition. Washington. D.C. Method 935.14 and 992.24, 1-25. Available at: https://kupdf.net/download/aoac-2005_59b90b0808bbc57f21894ca4_pdf

Barzegar S, Shu-Biao W, Jean N, and Swick RA (2019). Metabolizable energy of corn, soybean meal and wheat for laying hens. Poultry Science, 98(11): 5876-5882. DOI: https://www.doi.org/10.3382/ps/pez333

Bonos E, Skoufos I, Giannenas I, Sidiropoulou E, Fotou K, Stylianaki I, Tsiftsoglou O, Lazari D, Venardou B, Galamatis D et al. (2022). Effect of an herbal mixture of oregano, garlic, sage and rock samphire extracts in combination with tributyrin on growth performance, intestinal microbiota and morphology, and meat quality in broilers. Sustainability, 14(20): 13565. DOI: https://www.doi.org/10.3390/su142013565

Castaldo L, Izzo L, De Pascale S, Narvaez A, Rodriguez-Carrasco Y, and Ritieni A (2021). Chemical composition, *in vitro* bioaccessibility and antioxidant activity of polyphenolic compounds from nutraceutical fennel waste extract. Molecules, 26(7): 1968. DOI: https://www.doi.org/10.3390/molecules26071968

Cerrate S, Ekmay R, England JA, and Coon C (2019). Predicting nutrient digestibility and energy value for broilers. Poultry Science, 98(9): 3994-4007. DOI: https://www.doi.org/10.3382/ps/pez142

Chithrani BD, and Chan WCW (2007). Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. Nano letters, 7 (6): 1542-50. DOI: https://www.doi.org/10.1021/nl070363y

Dilawar MA, Mun HS, Rathnayake D, Yang EJ, Seo YS, Park HS, and Yang CJ (2021). Egg quality parameters, production performance and immunity of laying hens supplemented with plant extracts. Animals, 11(4): 975. DOI: https://www.doi.org/10.3390/ani11040975

Dobrzanski Z, Chojnacka K, Trziszka T, Opalinski S, Bobak L, Konkol D, and Korczynski M (2020). The effect of dietary humic preparations on the content of essential and non-essential chemical elements in hen eggs. Animals, 10(18): 1252. DOI: https://www.doi.org/10.3390/ani10081252

Food and agriculture organization/ World health organization (FAO/WHO) (2002). Codex alimentarius-general standards for contaminants and toxins in food. General standard for contaminants and toxins in food and feed, 1-66.. Available at: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B193-1995%252FCXS_193e.pdf

Gilani SMH, Rashid Z, Galani S, Ilyas S, Sahar S, Zahoor-ul-Hassan, Al-Ghanim K, Zehra S, Azhar A, Al-Misned F et al. (2021). Growth performance, intestinal histomorphology, gut microflora and ghrelin gene expression analysis of broiler by supplementing natural growth promoters: A nutrigenomics approach. Saudi Journal of Biological Sciences, 28(6): 3438-3447. DOI: https://www.doi.org/10.1016/j.sjbs.2021.03.008

Gombart AF, Pierre A, and Maggini S (2020). A review of micronutrients and the immune system–working in harmony to reduce the risk of infection. Nutrients, 12(1): 236. DOI: https://www.doi.org/10.3390/nu12010236

Gopalan B, Goto M, Kodama A, and Hirose T (2000). Supercritical carbon dioxide extraction of turmeric (Curcuma longa). Journal of Agricultural and Food Chemistry, 48(6): 2189-2192. DOI: https://www.doi.org/10.1021/jf9908594

Hammouda FM, Saleh MA, Abdel-Azim NS, Shams KA, Ismail SA, Shahat AA, and Saleh IA (2013). Evaluation of the essential oil of foeniculum vulgare mill (fennel) fruits extracted by three different extraction methods by gc/ms. African Journal of Traditional, Complementary and Alternative Medicines, 11(2): 277-279. DOI: https://www.doi.org/10.4314/ajtcam.v11i2.8

Heflin LE, Malheiros R, Anderson KE, Johnson LK, and Raatz SK (2018). Mineral content of eggs differs with hen strain, age, and rearing environment. Poultry Science, 97(5): 1605-1613. DOI: https://www.doi.org/10.3382/ps/pey025

Ivanisova E, Vaskova D, Zagula G, Grynshpan D, Savitskaya TA, and Kacaniova M (2020). Phytochemical profile and biological activity of selected kind of medicinal herbs. Potravinarstvo Slovak Journal of Food Sciences, 14: 573-579. DOI: https://www.doi.org/10.5219/1370

Jamaluddin, Darwis A, Massinai MA (2018). X-ray fluorescence (XRF) to identify chemical analysis of minerals in Buton island, SE Sulawesi, Indonesia. IOP Conference Series: Earth and Environmental Science, 118: 012070. DOI: https://www.doi.org/10.1088/1755-1315/118/1/012070

Kumar S, Dobos GJ, and Rampp T (2017). The significance of ayurvedic medicinal plants. Journal of Evidence-Based Complementary and Alternative Medicine, 22(3): 494-501. DOI: https://www.doi.org/10.1177/2156587216671392

Lim SR and Schoenung JM (2010). Human health and ecological toxicity potentials due to heavy metal content in waste electronic devices with flat panel displays. Journal of Hazardous Materials, 177(1-3): 251-259. DOI: https://www.doi.org/10.1016/j.jhazmat.2009.12.025

Liu SJ, Wang J, He TF, Liu HS, and Piao XS (2021). Effects of natural capsicum extract on growth performance, nutrient utilization, antioxidant status, immune function, and meat quality in broilers. Poultry Science, 100(9): 101301. DOI: https://www.doi.org/10.1016/j.psj.2021.101301

Saeed M, Kamboh AA, Syed SF, Babazadeh D, Suheryani I, Shah QA, Umar M, Kakar I, Naveed M, Abd El-Hack ME, et al. (2018). Phytochemistry and beneficial impacts of cinnamon (*Cinnamomum zeylanicum*) as a dietary supplement in poultry diets. World's Poultry Science Journal, 74(2): 331-346. DOI: https://www.doi.org/10.1017/S0043933918000235

Mirzaei A, Razavi SA, Babazadeh D, Laven R, and Saeed M (2022). Roles of probiotics in farm animals: A review. Farm Animal Health and Nutrition, 1(1): 17-25. Available at: https://fahn.rovedar.com/article_160928_d4a1012ceced4c93aeb4ec9e5ab836a7.pdf

Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Chand RPB, Aparna SR, Mangalapandi P, and Samal A (2018). IMPPAT: A curated database of Indian medicinal plants, phytochemistry and therapeutics. Scientific Reports, 8: 4329. Available at: https://www.nature.com/articles/s41598-018-22631-z

Neves MIL, Strieder MM, Vardanega R, Silva EK, and Meireles MAA (2020). Biorefinery of turmeric (Curcuma longa L.) using non-thermal and clean emerging technologies: An update on the curcumin recovery step. RSC Advances, 10: 112-121. DOI: https://www.doi.org/10.1039/C9RA08265D

National Research Council (NRC) (1994). Nutrient requirements of poultry. 9th Revision. Ed. National Academy Press, Washington, DC.

Nuraini M and Djulardi A (2019). Effect of turmeric (Curcuma domestica, Val) extract as a feed additive on performance and egg quality of quail. International Journal of Poultry Science, 18(2): 88-92. DOI: https://www.doi.org/10.3923/ijps.2019.88.92

Phuoc TV, Dung NNX, Manh LH, and Tu NNV (2019). Effect of dietary turmeric (Curcuma longa) extract powderon productive performance and egg quality of black-bonechicken (Ac chicken). Livestock Research for Rural Development, 31(2): 23. Available at: http://www.lrrd.org/lrrd31/2/truong31023.html

Recommended daily intake of vitamins and minerals (RDI) (2020). Lentech. Available at: https://www.lenntech.com/recommended-daily-intake.html

Rehault-Godbert S, Guyot N, and Nys Y (2019). The golden egg: Nutritional value, bioactivities, and emerging benefits for human health. Nutrients, 11(3): 684. DOI: https://www.doi.org/10.3390/nu11030684

Samantaray L and Nayak Y (2022). Influence of phytobiotic essential oils on growth performance and hematological parameters of broiler chickens. Advances in Animal and Veterinary Sciences, 10(6): 1289-1295. DOI: https://www.doi.org/10.17582/journal.aavs/2022/10.6.1289.1295

Samantaray L and Nayak Y (2022). The influences of black pepper, turmeric and fennel essential oils supplementation in feed on egg quality characteristics of layers. Journal of Animal Health and Production, 10(4): 522-528. DOI: http://www.doi.org/10.17582/journal.jahp/2022/10.4.522.528

Shityakov S, Bigdelian E, Hussein AA, Hussain MB, Tripathi YC, Khan MU, and Shariati MA (2019). Phytochemical and pharmacological attributes of piperine: A bioactive ingredient of black pepper. European Journal of Medicinal Chemistry, 176: 149-161. DOI: https://www.doi.org/10.1016/j.ejmech.2019.04.002

Tran TH, Ha LK, Nguyen DC, Dao TP, Nhan LTH, Nguyen DH, Nguyen TD, NVo DV, Tran QT, and Bach LG (2019). The study on extraction process and analysis of components in essential oils of black pepper (*Piper nigrum* L.) seeds harvested in Gia Lai Province, Vietnam. Processes, 7(2): 56. DOI: https://www.doi.org/10.3390/pr7020056

Vlaicu PA, Untea AE, Turcu RP, Saracila M, Panaite TD, and Cornescu GM (2022). Nutritional composition and bioactive compounds of Basil, Thyme and Sage plant additives and their functionality on broiler thigh meat quality. Foods, 11(8): 1105. DOI: https://www.doi.org/10.3390/foods11081105

Welch RM and Graham RD (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany, 55(396): 353-364. DOI: DOI: https://www.doi.org/10.1093/jxb/erh064

Wijedasa WMRM, Wickramasinghe YHST, Vidanarachchi JK, and Himali SMC (2020). Comparison of egg quality characteristics of different poultry species. Journal of Agricultural Science, 12(11): 331-342. DOI: https://www.doi.org/10.5539/jas.v12n11p331

Yadav S, Teng PY, Santos TS, Gould RL, Craig SW, Fuller AL, Pazdro R, and Kim WK (2020). The effects of different doses of curcumin compound on growth performance, antioxidant status, and gut health of broiler chickens challenged with Eimeria species. Poultry Science, 99(11): 5936-5945. DOI: https://www.doi.org/10.1016/j.psj.2020.08.046

Yin D, Tong T, Moss AF, Zhang R, Kuang Y, Zhang Y, Li F, and Zhu Y (2022). Effects of coated trace minerals and the fat source on growth performance, antioxidant status, and meat quality in broiler chickens. The Journal of Poultry Science, 59(1): 56-63. DOI: https://www.doi.org/10.2141/jpsa.0200108

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 71-80, March 25, 2023

Research Paper, PII: S2322455X2300007-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.7

Effects of a Vitamins D and C Supplement on Performance, Hatchability, and Blood Profiles of Broiler Breeders

Nanik Setiyaningsih[1]*, Sumiati[2], Anuraga Jayanegara[2], and Wira Wisnu Wardani[3]

[1]*Graduate Student of Nutrition and Feed Science, IPB University, Jl. Agatis Kampus IPB Dramaga, Bogor 16680, Indonesia*
[2]*Departement of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Jl. Agatis Kampus IPB Darmaga, Bogor 16680, Indonesia*
[3]*Nutricell R&D, Cibis Nine 12th Floor Unit GI, Jl. TB Simatupang No. 2, Jakarta 12560, Indonesia*

*Corresponding author's Email: nafaky@gmail.com

Received: 21 December 2022
Accepted: 13 February 2023

ABSTRACT

Vitamin D is a fat-soluble vitamin that plays a crucial role in controlling Calcium and Phosphor homeostasis, bone mineralization, and modulation of immune responses. Vitamin C is a cofactor of enzymatic reactions with anti-inflammatory and antioxidant properties to prevent and repair damage to cells in the body from exposure to free radicals and the immune system. The current study aimed to investigate the effects of dietary supplementation of 25(OH)D3 with vitamin C at different doses on broiler breeders' blood profile, egg quality, and hatchability. The adaptation process before collecting the data was 2 weeks. A total of 6200 females and 620 male broiler breeders in the laying period aged 32-46 weeks were divided into 4 treatment groups with 5 replicates (each peach contained 310 female and 31 male breeders). The treatments included control as T0 (0 g/ton Nutricell HyC®), T1 (100 g/ton Nutricell HyC®), T2 (200 g/ton Nutricell HyC®), T3 (400 g/ton Nutricell HyC®) supplemented in feed. The observed variables were performance in breeding farms and hatcheries. The treatments with experimental doses indicated significant differences in the performance of broiler breeders, including feed intake, body weight, egg weight, egg mass, hen day production, hen house production, feed conversion ratio, and parameters of blood profile. The results showed a significant difference between the treatments and the control group in terms of hatch performance, clear eggs, exploding eggs, hatchability eggs, fertile eggs, salable chicks, and hatching of fertile eggs. However, no significant effects on fertility, culling of chicks, and embryonic mortality in the treatment groups were indicated. In conclusion, Nutricell HyC® with a dose of 400 g/ton in feed has indicated the best result in breeding farm and hatchery performance of broiler breeders in the laying period.

Keywords: Blood profile, Broiler breeder, Calcidiol (25(OH)D3), Nutricell HyC®, Performance, Vitamin C

INTRODUCTION

Broiler breeder operations aim to optimize the hatchability rate as well as saleable chicks and high-quality chicks. The success of a hatchery depends on the number of high-quality day-old chicks (DOC). Fertility and hatchability are the main factors that could affect the supply of DOC and are influenced by two factors, including breeder and hatchery management (Cobb, 2021). According to Lare et al. (2021), feed quality plays a vital role in broiler breeder performance, and it could affect egg quality, embryo growth, fertility, and egg hatchability.

Egg yolk provides a nutrient supply for embryo development during incubation and is a secondary nutrient resource for DOCs (Romanoff, 1960; Dayan et al., 2020). Meanwhile, eggshells provide minerals and trace elements supply for embryo development and become a major contributor of calcium (Ca) to support the skeletal mineralization of the embryo during the second half of the incubation or development phase (Yair and Uni, 2011; Halgrain et al., 2022). One of the egg criteria related to the incubation process and time to hatch is the porosity of the eggshell, which affects the exchange of oxygen (O_2), carbon dioxide (CO_2), and dihydrogen monoxide (H_2O) vapor in the shell (Narushin et al., 2002). The amount and size of the pores determine the eggshell quality. Eggshell thickness affects the activity of the embryo and the loss of

moisture conditions in the external environment of the egg (Bergoug et al., 2013). Extreme porosity and shell thickness have the potential to inhibit embryo development through pathogenic bacteria contamination or might be lethal to embryo development even with little contamination of pathogenic bacteria (Dzoma, 2010).

Vitamin D has an important role in the absorption of Ca and phosphorus (P), regulation of parathyroid hormone (PTH), immune system, mineralization, bone mobilization, and control concentrations of Ca in the blood and muscles (Garcia et al., 2013). The Ca and P storage depends on the availability of minerals in the diet that affects bone development and mineralization (Regassa et al., 2015). The formation of eggshells and maintenance of plasma Ca levels in chickens depend on the availability and use of Ca in the diet and minerals in bone, especially the medullary bone of breeders (Kerschnitzki et al., 2014).

The body response that is not specific and adaptive to any condition is a symptom of stress in chickens that is influenced by various factors, from external and internal pressure, such as climate change, transportation, as well as changes in drinking water and types of feed and infections (Etim et al., 2013). Stress conditions in chickens might activate the pituitary-adrenal axis and cause the secretion of adrenocorticotropin hormone (ACTH), which affects the blood homeostasis system (Scanes, 2016). The ratio of heterophile to lymphocytes (H/L) describes the body's response to various stress conditions as a marker to assess poultry's welfare and health status (Huth and Archer, 2015).

Vitamin C is a water-soluble vitamin synthesized by liver cells, functions as a cofactor for several enzymes, is an antioxidant, and can improve immune function (Abidin and Khatoon, 2013). Ascorbic acid is a powerful natural antioxidant that could reduce stressful conditions' adverse effects and improve performance (Rao et al., 2011; Whitehead and Keller, 2019). Under normal conditions, poultry could synthesize AA in their liver cell to meet their needs, but if under stress conditions, supplementation in feed was required (Van Hieu et al., 2022). when chickens are under stress, supplementation of AA in the feed could improve health and oxidative status (Gouda et al., 2020). Fletcher et al. (2021) reported that intake of AA during stressful conditions could control many brain activities due to the increased production of neurotransmitters and norepinephrine. The purpose of this study was to evaluate the effect of combined supplementation of 25(OH)D_3 with vitamin C in Nutricell HyC® products at different doses on broiler breeder performance, blood profile, egg quality, and hatchability.

MATERIALS AND METHODS

Formulation of experimental diets

The experimental diets are formulated and presented in Table 1.

Table 1. The analysis of experimental diets of broiler breeders Cobb strain aged 32-46 weeks

	T0	T1	T2	T3
Raw material (%)				
Yellow corn	45	45	45	45
Wheat flour	15	15	15	15
Soybean meal	16.6	16.6	16.6	16.6
Rice bran	4	4	4	4
Wheat pollard	2	2	2	2
Full fat soya	5	5	5	5
Salt	0.3	0.3	0.3	0.3
Limestone grit	7.1	7.1	7.1	7.1
Monodicalcium phosphate	2	2	2	2
Vitamin E	0.2	0.2	0.2	0.2
Choline chloride	0.5	0.5	0.5	0.5
Vitalink	0.3	0.3	0.3	0.3
Crude Palm Oil	0.2	0.2	0.2	0.2
Methionine	0.1	0.1	0.1	0.1
Lysine	0.2	0.2	0.2	0.2
Threonine	0.1	0.1	0.1	0.1
Calculation in formulation				
Metabolizable Energy (kcal/kg)	2835	2835	2835	2835
Vitamin D3 (IU/g)	0	3750	4000	4500
Vitamin C (g)	0	95	190	380
Nutrition composition (%)				
Crude protein	16.54	16.88	16.46	16.37
Ash	11.14	11.12	10.30	10.43
Crude fat	6.05	5.92	5.83	5.79
Crude fiber	3.42	3.48	3.63	3.54
Moisture	11.01	10.98	10.90	11.20
Starch	34.29	33.40	34.85	36.69
Calcium	2.79	3.04	2.95	2.61
Phosphor	0.41	0.44	0.46	0.42

T0/Control: 0 g/ton Nutricell HyC®; T1:100 g/ton Nutricell HyC®; T2:200 g/ton Nutricell HyC®; T3:400 g/ton Nutricell HyC®

Ethical approval

The study was conducted under the supervision of the animal health manager in PT Peternakan Ayam Manggis, Indonesia. Following the SOP and Guidelines of Cobb, so no need the ethical approval.

Experimental design

The study was carried out from July to October 2021 in a broiler breeder farm and hatchery of PT Peternakan Ayam Manggis in Tenjo Ayu, Sukabumi, West Java, Indonesia. Feed was produced at the feed mill PT Megah

Prayasa Sentosa, Cikupa, Tangerang, Banten, Indonesia. The DOCs were purchased from the hatchery PT Hybro Indonesia, Cianjur, Indonesia. The study was conducted using 6200 female broiler breeders Cobb strain aged 32-46 weeks. The chickens were distributed randomly into 20 pens (310 female and 31 male broiler breeders were added). The trial feed adaptation period was performed in two weeks. The treatments included basal diet without Nutricell HyC® supplementation as control (T0), basal diet with 100 g/ton (T1), 200 g/ton (T2), 400 g/ton (T3) of Nutricell HyC®. Each treatment consisted of five replication (Cobb, 2021). Nutricell HyC® contains a combination of 25(OH)D3 and vitamin C to improve broiler breeder performance.

Trial feed was produced by mixing Nutricell HyC® supplied by PT Nutricell Pasific with soybean meal in a premix mixer. Mixed additives were then transferred into the main mixer to be mixed with the whole formulation, then pelleted and crumbled under feed mill standard operation. The formulation included HyD 62.500 µg, vitamin C 950.000 mg, and filler.

The cages used were openside with size 8 x 9 (m), lined with fibrocement tiles, had a concrete floor with rice husk litter, and were equipped with polypropylene side curtains, feeder tubs for females and tube feeders for males, and nipple drinking holder. Drinking water was given *ad libitum* and fed once daily at 05.45 am. The light was supplied by natural light from the sun and artificial light by lamps for 17 hours from 03 pm until 08 am with lux 15. The ambient temperature was around 25-29°C, and relative humidity was around 72-75%.

Blood profile analyses were conducted at IPB University Bogor, Indonesia, and proximate analysis was conducted at PT Nutricell Pasific, Jakarta, Indonesia.

Production performance

Eggs were collected four times a day, and the number of released and rejected eggs from each pen was recorded every day until the end of the experiment. Random sampling was performed weekly for each cage to monitor the body weight (BW) of broiler breeders. Briefly, BW of broiler breeder in the laying period was evaluated twice a week to determine the broiler breeder restricted feeding based on its BW relative to its target BW. Broiler breeder performance parameters, such as egg mass, feed conversion ratio (FCR), hen day production (HDP), and hen house production (HHP) were calculated as follows:

$$\text{Egg mass (g)} = \text{HDP (\%)} \times \text{Average egg weight (g)} \quad \text{(Formula 1)}$$

$$\text{FCR (per g egg mass)} = \frac{\text{Feed intake (g)}}{\text{Egg production (g)}} \quad \text{(Formula 2)}$$

$$\text{HDP (\%)} = \frac{\text{Number of egg produced during the period}}{\text{Number of hens at the early egg produced}} \times 100 \quad \text{(Formula 3)}$$

$$\text{HHP (\%)} = \frac{\text{Number of egg produced during the period}}{\text{Number of hens at the early egg produced}} \times 100 \quad \text{(Formula 4)}$$

Incubation responses

The hatching process lasted for 21 days, where the incubation process was 18 days in the setter machine and 3 days hatching in the hatcher machine. Eggs were collected 3-4 times daily and transferred to the hatchery when graded a satisfactory quality. In the hatchery, eggs were classified into two groups, namely indubitable and non-indubitable. The indubitable eggs were fumigated at 25°C with 70% humidity for 15 minutes. After that, they were stored in an environmentally controlled room (18-21°C and 60-70% relative humidity) for a maximum period of 5 days. Indubitable eggs were transferred into a pre-heating room for 8-12 hours at a temperature of 25-26°C before being transferred into the setter machine. The non-indubitable eggs, such as cracked, shell-less, double yolk, abnormally shaped, were recorded and separated.

A total of 9900 eggs from broiler breeders within the age range of 34 to 46 weeks were set for incubation twice a week. The eggs were set with the same temperature, humidity, and turning conditions in the setter machine. The temperature was 37.5°C, relative humidity was 83%, and turning occurred automatically every hour at a 90° angle. After ten days of incubation, several eggs from each treatment were candled to determine fertility and embryonic mortality. The unclear eggs were returned to the setter machine to follow incubation. Clear eggs were separated and recorded. A random sampling of each treatment (< 7 days) was done to determine early embryo mortality and infertility. The candling process was carried out after 18 days of incubation to determine clear eggs and unclear eggs. The clear eggs were then separated and recorded. The unclear eggs detected during the candling process were then transferred to the hatch basket and kept in the hatchery machine at a temperature of 36.5°C and relative humidity of 85% until 21 days of incubation.

To determine percentages of early embryo mortality (1-7 days), middle embryo mortality (8-14 days), and late embryo mortality (15-21 days), the eggs which could not be hatched were sampled. The clear eggs were assumed

infertile, and the rest were recorded separately as fertile eggs with the early dead embryo and dead embryonic eggs.

Fertility data were collected, and fertility percentage was obtained from the number of fertile eggs to the number of eggs set in the incubator and was calculated as Formula 5.

$$\text{Fertile eggs (\%)} = \frac{\text{Number of fertile eggs}}{\text{Number of eggs incubated}} \text{x}100$$

(Formula 5)

After 21 days of incubation, DOCs were pulled out from the incubator and visually selected according to commercial hatchery standards. Abnormal DOCs, including missing eyes, legs with cuts, splayed legs, cross beak, and poor feathering, as well as chicks that could not flip over in less than 10 seconds, were counted as culling chicks and separated according to the Cobb hatchery management guide (Cobb, 2021). All unhatched eggs were subjected to random sampling to predict estimated days of embryo mortality.

Hatchability data were collected, and hatchability percentage was obtained from the number of hatched eggs to the number of eggs set in the incubator and was calculated as Formula 7.

$$\text{Hatchability (\%)} = \frac{\text{Number of chick hatched}}{\text{Number of eggs incubated}} \text{x}100$$

(Formula 6)

The percentage of the hatch of fertile is an indicator of the effectiveness of the hatchery. The calculation method is indicated in Formula 7.

$$\text{Hatch of fertile (\%)} = \frac{\text{Percentage of hatchability}}{\text{Percentage of fertile}} \text{x}100$$

(Formula 7)

The embryo mortality was observed and divided into three groups, namely early embryo mortality (1-7 days), middle embryo mortality (8-14 days), and late embryo mortality (15-21 days) incubation. The calculation of the percentage of embryonic mortality is indicated in Formula 8.

$$\text{Embryonic mortality (\%)} = \frac{\text{Number of embryo mortality}}{\text{Number of fertile eggs}} x100$$

(Formula 8)

Hematology determination

The process of taking blood samples was carried out on broiler breeders aged 45 weeks on the underside of the wings. The blood sample was taken using a syringe of as much as 2 ml and put in an anticoagulant tube. The collecting blood sample was at 06.00 am, and the anticoagulant tube was included in the ice flask, after finished direct sent to the laboratory of Bogor Agriculture University (IPB), Bogor, Indonesia. For analysis of Ca and P, blood plasma was separated in a tube containing fresh blood spiked with anticoagulant substances. Centrifuged until the red blood cells fell to the bottom of the tube, the white blood cells were on top and formed a layer of buffy coat, and the blood plasma was on top of the layer yellowish. Hematological analysis of blood consisted of the hemoglobin, hematocrit, leukocyte, erythrocyte, eosinophil, basophil, monocyte, heterophile, lymphocyte, Ca, and P.

The erythrocyte measurement method used was microvisual. Accordingly, the blood was diluted in an erythrocyte pipette with an isotonic solution, then put into the counting chamber. The number of erythrocytes was calculated in a certain volume using a conversion factor. The leukocyte measurement was the same with erythrocyte, and differential white blood cell (WBC) counts were made on monolayer blood films, fixed and stained with erythrocyteGiemsa-Wright's stain. The total WBC count was determined by a manual method using a hemacytometer. The method used to measure hemoglobin was Sahli's method (Van Lerberghe et al., 1983). According to Sahli's method, hemoglobin was converted into acid hematin with the help of 0.1 N HCL solution then the resulting color was compared visually with the standard color. The tool used is Sahli's hemoglobinometer. The method used to measure hematocrit was a microtube. Therefore, when the blood was centrifuged, heavier cells (erythrocytes) fell to the bottom of the tube, while the lighter cells (leukocytes and platelets) were above the heavier cells. The tool used in this method was a microcapillary tube with heparin and a microhematocrit centrifuge (Sastradipradja et al., 1989).

Statistical analysis

The IBM statistical SPSS version 26 was used for data analysis. The observed data showed as mean ± standard deviation in the table. One-way analysis of variance (ANOVA) was used to analyze the data. The differences among treatments at a significant level of ($p < 0.05$) followed by the Tukey test. All percentage data were perform as quadratic transformation for statistical analysis.

RESULTS AND DISCUSSION

Broiler breeder performance fed Nutricell HyC® are shown in Table 2. Supplementation of Nutricell HyC® at various levels (T1-T3) significantly decreased feed intake of broiler breeder in comparison to control group ($p < 0.05$,

Table 2). With regard to body weight, egg weight, egg mass, HDP and HHP, only T3 was significantly higher than that of T0 ($p < 0.05$). Similarly, T3 decreased FCR of the broiler breeders significantly as compared to T0 ($p < 0.05$). Meanwhile, a non-significant effect was observed on the parameter of broiler breeder depletion. It could be seen that the BW of broiler breeders is still within the standard range, and they were not overweight. The older brooders need to reduce their feed intake to minimize reproductive problems during the egg-laying phase; therefore, it is necessary to limit feed because body weight gain in broiler breeders must be limited throughout production (Richards et al., 2010). Fritts and Waldroup (2003) observed that male broiler strain cobb fed 25(OH)D3 could better support body weight gain than vitamin D_3. The supplementation of Nutricell HyC® increased the egg weight, compared to control feed, but all treatments showed egg weight in the range required for hatching. Standard egg weight at the age of 34-46 weeks was 60.7-66.5 g. According to Ummer-Franco et al. (2010), egg weight was not affected by fertility, early and middle embryo death, culling of chicks, feed intake, or FCR. Wang et al. (2020) indicated that 25(OH)D3 increased the relative weight and thickness of the eggshells. The corticosterone hormone increases energy supply when chickens experience stress, where vitamin C has a vital role in the biosynthesis of this hormone (Siegel and Kampen, 1984). Heat stress conditions at various levels significantly affect egg production, egg weight, eggshell quality, weight gain, and mortality (Lin et al., 2006). The supplementation of Nutricell HyC® in this study might increase feed efficiency. According to Araujo et al. (2019), supplementary feed canthaxanthin + 25(OH)D3 increased egg production and FCR but did not affect feed intake.

Table 2. Effect of Nutricell HyC® supplementation supplementation added in feed on broiler breeder performance at the age of 34-46 weeks

Treatments / Production Performance	T0 (control)	T1	T2	T3	p-value
Feed intake (g/day)	159.07 ± 0.04^{b}	158.96 ± 0.04^{a}	158.90 ± 0.07^{a}	158.93 ± 0.02^{a}	0.001
Body weight (g)	3727.45 ± 4.81^{a}	3729.37 ± 4.82^{ab}	3734.26 ± 4.12^{ab}	3736.34 ± 4.03^{b}	0.021
Egg weight (g)	65.63 ± 0.53^{a}	65.89 ± 0.33^{ab}	66.03 ± 0.37^{ab}	66.58 ± 0.24^{b}	0.009
Egg mass (g)	49.35 ± 0.91^{a}	49.62 ± 0.41^{a}	50.13 ± 0.35^{ab}	50.81 ± 0.44^{b}	0.005
HDP[1] (%)	75.18 ± 0.86^{a}	75.31 ± 0.53^{ab}	75.92 ± 0.24^{ab}	76.31 ± 0.56^{b}	0.026
HHP[1] (%)	71.85 ± 0.82^{a}	71.96 ± 0.50^{a}	72.65 ± 0.23^{ab}	73.02 ± 0.54^{b}	0.013
Depletion[1] (%)	0.28 ± 0.02	0.25 ± 0.04	0.25 ± 0.02	0.23 ± 0.03	0.065
FCR	3.23 ± 0.07^{b}	3.21 ± 0.02^{ab}	3.17 ± 0.07^{ab}	3.14 ± 0.03^{a}	0.012

[1:] The data were performed as quadratic transformation; [a,b:] Value in the same row with different superscript letters significantly differ ($p < 0.05$). T0/Control: 0 g/ton Nutricell HyC®; T1:100 g/ton Nutricell HyC®; T2:200 g/ton Nutricell HyC®; T3:400 g/ton Nutricell HyC® ; HDP: Hen day production, HHP: Hen house production; FCR: Feed conversion ratio.

Table 3. Effect of Nutricell HyC® supplementation in feed on broiler breeder eggshell porosity at the age of 34-46 weeks[1]

Treatments / Eggshell porosity	T0 (control)	T1	T2	T3	p-value
Score 1 (%)	0	0	0.22 ± 0.31	0.11 ± 0.25	0.261
Score 2 (%)	0.11 ± 0.25^{a}	1.56 ± 0.82^{b}	4.22 ± 1.45^{c}	6.22 ± 2.16^{c}	<0.001
Score 3 (%)	8.11 ± 3.01^{a}	22.22 ± 4.76^{b}	34.00 ± 3.08^{c}	40.11 ± 3.25^{c}	<0.001
Score 4 (%)	91.78 ± 2.87^{d}	76.11 ± 5.42^{c}	61.55 ± 4.55^{b}	53.56 ± 3.82^{a}	<0.001

[1:] All data were performed as quadratic transformation. [a,b,c:] Value in the same row with different superscripts letters a significantly differ ($p < 0.05$); T0/Control: 0 g/ton Nutricell HyC®; T1: 100 g/ton Nutricell HyC®; T2: 200 g/ton Nutricell HyC®; T3: 400 g/ton Nutricell HyC®; Score 1 : Excellent eggshell quality; Score 2: Good eggshell quality; Score 3: Average eggshell quality; Score 4: Poor eggshell quality.

The Nutricell HyC® supplementation in broiler breeder diet generally demonstrated an improvement in the eggshell quality (Table 3). The supplementation of Nutricell HyC® could significantly decreased the poor eggshell quality (score 4), increased the average eggshell quality (score 3), and also enhanced the good eggshell quality (score 2) compared to control group ($p < 0.05$). However, Nutricell HyC® supplementation did not cause a significant effect on the excellet eggshell quality (score 1, $p > 0.05$). The combination of 25(OH)D3 with vitamin C contained in Nutricell HyC® might increase Ca and P absorption in the small intestine and Ca deposition at

bones which played a role in the formation of $CaCO_3$, during the formation of the eggshell inside the uterus which lasts for 20 to 21 hours (Fritts and Waldroup, 2003). Calcium and P intake could not decrease eggshell quality, ascorbic acid availability also play an important role in the regulation of Ca metabolism, and eggshell formation, which is required for the conversion of 25(OH)D3 to $1.25(OH)2D_3$ was limited (Bains, 1995; Lohakare et al., 2005). Nutricell HyC® contains vitamin C formed as sodium ascorbate, which is very important for synthesizing the eggshell's organic matrix. The calcified eggshell play as a barrier of eggs and embryos against physical damage and contamination from pathogenic bacteria. The eggshell serves as a Ca resource in embryo development and the exchange of water and gases between the embryo and environment during extra-uterine development through the eggshell structure (Nys et al., 2004). Saunders-Blades and Korver (2014) mentioned Vitamin D_3 is very important for the absorption of Ca and P from the intestine during eggshell formation. Although Ca be absorbed passively from the intestine, vitamin D3 allows chickens to absorb Ca in sufficient quantities to maintain eggshell formation and normal medullary bone reserves. Wang et al. (2020) observed that supplementation of 25(OH)D3 in a laying hen diet increased serum 25(OH)D3, the content of carbonic anhydrase (CA) serum, which is an enzyme that plays a major role in the deposition of calcium carbonate in eggshell formation. Supplementation of vitamins C and E in feed can prevent the deterioration of eggshell quality and reduce the effects of stress due to high environmental temperatures in broiler breeders (Chung et al., 2005).

Table 4. Effect of Nutricell HyC® supplementation in feed on performance hatchability of broiler breeders at the ages of 34-46 weeks[1]

Treatments / Performance	T0 (control)	T1	T2	T3	p-value
Clear eggs (%)	4.07 ± 0.11^{c}	3.87 ± 0.12^{ab}	3.92 ± 0.06^{bc}	3.72 ± 0.12^{a}	0.001
Exploding eggs (%)	0.49 ± 0.05^{c}	0.42 ± 0.02^{b}	0.29 ± 0.03^{a}	0.28 ± 0.02^{a}	<0.001
Dis eggs (%)	4.82 ± 0.05^{b}	4.72 ± 0.16^{ab}	4.81 ± 0.11^{b}	4.54 ± 0.18^{a}	0.014
Hatchability eggs (%)	90.62 ± 0.13^{a}	90.99 ± 0.12^{b}	90.97 ± 0.16^{b}	91.46 ± 0.11^{c}	<0.001
Fertile eggs (%)	95.48 ± 0.11^{a}	95.79 ± 0.13^{b}	95.81 ± 0.07^{b}	96.01 ± 0.12^{c}	<0.001
Infertile egss (%)	1.62 ± 0.54	1.29 ± 0.16	1.49 ± 0.16	1.27 ± 0.37	0.353
Culling chicks (%)	0.55 ± 0.07	0.58 ± 0.10	0.57 ± 0.10	0.48 ± 0.05	0.236
Chicksalable (%)	90.13 ± 0.15^{a}	90.51 ± 0.26^{ab}	90.41 ± 0.2^{b}	91.03 ± 0.16^{c}	<0.001
Hatching of fertile eggs (%)	94.91 ± 0.08^{a}	94.99 ± 0.16^{a}	94.96 ± 0.12^{a}	95.27 ± 0.18^{b}	0.005
Early embryo mortality (%)	2.22 ± 0.48	1.76 ± 0.22	2.22 ± 0.39	1.88 ± 0.46	0.200
Intermediate embryo mortality (%)	0.55 ± 0.24	0.68 ± 0.28	0.56 ± 0.33	0.60 ± 0.35	0.902
Late embryo mortality (%)	3.15 ± 0.82	3.22 ± 0.64	4.12 ± 0.43	2.98 ± 0.70	0.063

[1]: All data were performed as quadratic transformation. [a,b,c]: Value in the same row with different superscripts letters a significantly differ ($p < 0.05$); T0/Control: 0 g/ton Nutricell HyC®; T1: 100 g/ton Nutricell HyC®; T2:200 g/ton Nutricell HyC®; T3:400 g/ton Nutricell HyC®

The treatments (T1, T2, and T3) showed higher ($p < 0.01$) hatchability, fertile eggs, hatching fertile, chicksalable, and reduce clear eggs, exploding eggs, and dis eggs compared with T0 ($p < 0.05$). However, Nutricell HyC® supplementation had no significant effect on infertile, culling chicks, early, intermediate, and late embryonic death ($p > 0.05$). The supplementation with Nutricell HyC®, which contains 25(OH)D3 could repair the quality of eggshell pores so the rate of O_2 and CO_2 transport during the incubation period becomes better, which might reduce clear eggs, exploding eggs and dis eggs (Akbari Moghaddam et al., 2019). Coto et al. (2010) and Saunders-Blades and Korver (2015) observed an improvement in eggshell thickness and hatchability of fertile eggs fed 25(OH)D3 supplementation. The result of the present study showed a significant difference in increasing hatchability of eggs, fertile eggs, chick salable, and hatching of fertile eggs ($p < 0.05$). Dietary supplementation of Nutricell HyC® contains 25(OH)D3, metabolically active vitamin D3, which does not need to be hydroxylated in the liver. Then, it enters into the bloodstream and bounds to Vitamin D protein (BDP), and undergoes hydroxylation in kidneys, epithelial cells, and immune cells are converted into $1,25\ (OH)_2D_3$ (calcitriol), which works like a hormone (Pande et al., 2015). According to Brown et al. (1999) and Brandi (2008), active metabolite synergizes with parathyroid hormone (PTH) to increase intestinal Ca absorption and Ca deposition in bone, decreases Ca excretion in the excreta and maintain bone homeostasis, cell differentiation and

proliferation, central nervous system, and modulates immune response so that it can influence on hatchability, eggshell thickness, bone structure, and DOC immunity. Vitamin C affects many tissues because of its primary function as an important component of collagen synthesis and an antioxidant. Ascorbit acid is involved in the synthesis of collagen by influencing the function of prolyl hydroxylase domain (PHD) protein as an antioxidant and important cofactor for catalyzing many biochemical reactions (Aghajanian et al., 2015). In bone formation, Vitamin C increases the production of hydroxyproline, which is required for collagen synthesis. A network of collagen fibrils is necessary for proper bone and eggshell formation Lohakare et al. (2005). Vitamin D3 supplementation in the form of 25(OH)D3 metabolism involved in Ca and P metabolism could increase bone growth, egg-laying rate, shell quality, and reproduction (Torres et al., 2009; Rosa et al., 2012). Feeds supplemented with canthaxanthin + 25(OH)D3 showed an increase in hatchability, hatchability of fertile and decreased early embryo death (Araujo et al., 2019). However, the current study showed that the results were not significantly different ($p > 0.05$) for infertile eggs, culling chicks, and early, intermediate, and late embryo mortality.

Table 5. Effect of Nutricell HyC® supplementation in feed on broiler breeder blood profile in laying period (32-46 weeks of age)

Performance \ Treatments	T0 (control)	T1	T2	T3	p-value
Hemoglobin (g/dl)	11.76 ± 1.01[ab]	10.71 ± 0.45[a]	10.64 ± 1.05[a]	12.08 ± 0.76[b]	0.035
Hematocrit[1] (%)	30.00 ± 2.83	30.20 ± 1.92	30.00 ± 3.32	33.00 ± 1.87	0.218
Leukocyte[1] (x 10^3/mm^3)	23.10 ± 4.76	23.78 ± 2.70	20.60 ± 3.12	22.04 ± 3.88	0.607
Erythrocyte (x 10^6/mm^3)	2.35 ± 0.59	2.25 ± 0.78	2.72 ± 0.95	2.37 ± 0.60	0.765
Eosinophil[1] (%)	4.79 ± 1.17	5.62 ± 2.90	8.14 ± 2.51	6.52 ± 3.54	0.268
Basophil[1] (%)	3,83 ± 1,89	3.39 ± 1.03	5.29 ± 1.45	3.55 ± 1.07	0.167
Monocyte[1] (%)	1.84 ± 0.77	1.13 ± 0.41	2.45 ± 1.50	2.03 ± 1.15	0.280
Heterophile[1] (%)	34.39 ± 6.52	38.59 ± 3.66	35.89 ± 4.22	38.64 ± 3.43	0.406
Lymfocyte[1] (%)	55.16 ± 4.58	51.28 ± 3.75	48.23 ± 2.49	49.25 ± 5.33	0.081
Heterophil/Lymphocyte[1] (%)	0.69 ± 0.17	0.77 ± 0.13	0.79 ± 0.14	0.69 ± 0.081	0.500
Calcium (mg/dl)	10.76 ± 1.48	9.88 ± 0.83	10.89 ± 0.11	9.78 ± 0.97	0.197
Phosphor (mg/dl)	6.53 ± 1.80	4.56 ± 0.67	5.50 ± 0.46	5.43 ± 1.49	0.138

[1:] Data were performed as quadratic transformation. [a,b,c;] Value in the same row with different superscripts letters a significantly differ ($p < 0.05$); T0/Control: 0 g/ton Nutricell HyC®; T1: 100 g/ton Nutricell HyC®; T2:200 g/ton Nutricell HyC®; T3:400 g/ton Nutricell HyC®

Table 5 showed that Nutricell HyC® supplementation had no significant effect on most of blood parameters ($p > 0.05$). However, the amount of hemoglobin for T3 was significantly higher than that of T1 and T2 ($p < 0.05$). In this research, the hemoglobin levels among treatments ranged from 10.64 g/dl to 12.08 g/dl, where the hemoglobin level was still within normal limits. According to Weiss et al. (2010), the amount of hemoglobin in chickens (*gallus domesticus*) ranged from 7 to 13 g/dl. This is similar to the research conducted by Roy and Mishra (2011) examining the effects of antistress agents on broiler breeders. The findings indicated that hemoglobin levels in different groups ranged from 9 to 10.13 g/dl.

The findings of the current study showed the ratio of heterophile/lymphocyte (H/L) in broiler breeders ranged from 0.69% to 0.79%, indicating that the levels were still normal. Hachesoo et al. (2011) reported that H/L ratio of indigenous broiler breeders was 0.70 %, and H/L ratio of Ross-308 was 0.71%. According to Gross and Siegeh (1983), the level of stressors in poultry could be indicated by the ratio of Heterophils/Lymphosytes, about 0.2% (low level), 0.5% (normal level), and 0.8% (high level) to environmental adaptation. Therefore, it can be concluded that the levels of blood parameters were in normal ranges and H/L ratio of broiler breeders in the production phase obtained in this research can be interpreted as appropriate health and immune status. This shows that the supplementation of Nutricell HyC® with different levels can maintain the H/L ratio of broiler breeders in the production phase in the normal range. Table 5 shows that the supplementation of Nutricell HyC® in the feed has no significant difference in Ca and P levels of the broiler breeders' blood plasma ($p > 0.05$). In this research, blood plasma Ca levels ranged from 9.78 to 10.76 mg/dl, and P levels in blood plasma ranged from 4.56 to 6.53 mg/dl.

The results of the present study are in the same line with the findings of Hachesoo et al. (2011), who reported Ca and P levels in the blood plasma of indigenous chickens were 9.36 mg/dl and 4.23 mg/dl, respectively, while Ca and P levels for Ross-308 chickens were 9.28 mg/dl and 4.44 mg/dl, respectively.

CONCLUSION

In conclusion, the obtained results of the current study indicated that Nutricell HyC® supplementation with doses of 400 g/ton (T3) gave the best results in increasing egg production, eggshell porosity, hatchability, and immunity of broiler breeders aged 34-46 weeks. Future studies can investigate the effect of supplementation of Vitamin D3 more than 4500 IU/g on broiler breeder performance.

DECLARATIONS

Acknowledgments

The authors would like to thank PT Peternakan Ayam Manggis, where the study was carried out, PT Megah Prayasa Sentosa, where the feed was produced for this study, and we would like to thank PT Nutricell Pasific for its financial support during this study. We would like to thank all colleagues and those who contributed to this study.

Competing interests

The authors declare no conflict of interest for this manuscript

Authors' contributions

Nanik Setiyaningsih conducted the study in a breeding farm and hatchery contributed to data collection, analyzed data, processed statistical data, and prepared manuscripts. Sumiati, Anuraga Jayanegara, Wira Wisnu Wardani contributed to the supervision of the study, the analysis of the result, and the writing of the manuscript. All authors confirmed the statistical result and approved the final version of the manuscript.

Ethical consideration

The authors declare that they have checked the manuscript for plagiarism, double publication, and no data fabrication or redundancy.

Availability of data and materials

The data relating to the article will be sent by the corresponding author according to reasonable requests.

REFERENCES

Abidin Z and Khatoon A (2013). Heat stress in poultry and the beneficial effect of ascorbic acid (vitamin C) supplementation during periods of heat stress. World's Poultry Science Journal, 69(1): 135-152. DOI: https://www.doi.org/10.1017/S0043933913000123

Aghajanian P, Hall S, Wongworawat MD, and Mohan S (2015). The roles and mechanisms of actions of vitamin C in bone: New development. Journal of Bone and Mineral Research, 30(11): 1945-1955. DOI: https://www.doi.org/10.1002/jbmr.2709

Akbari Moghaddam Kakhki R, Heuthorst T, Mills A, Neijat M, and Kiarie E (2019). Interactive effects of calcium and top-dressed 25-hydroxy vitamin D3 on egg production, egg shell quality, and bones attributes in aged Lohmann LSL-lite layers. Poultry science, 98(3): 1254-1262. DOI: https://www.doi.org/10.3382/ps/pey446

Araujo LF, Araujo CSS, Pereira RJG, Bittencourt LC, Silva CC, Cisneros F, Hermes RG, Sartore YGA, and Dias MT (2019). The dietary supplementation of canthaxanthin in combination with 25(OH)D3 results in reproductive, performance, and progeny quality gains in broiler breeders. Poultry Science, 98(11): 5801-5808. DOI: https//www.doi.org/10.3382/ps/pez377

Bains BS (1995). The role of vitamin C in stress management. World's Poultry, 12(4): 38-41.

Bergoug H, Burel C, Guinebretiere M, Tong Q, Roulston N, Romanini CEB, Exadaktylos V, Mcgonnell IM, Demmers TGM, Verhelst R, et al. (2013). Effect of pre-incubation and incubation conditions on hatchability, hatch time and hatch window, and effect of post-hatch handling on chick quality at placement. World's Poultry Science Journal, 69(2): 313-334. DOI: https://www.doi.org/10.1017/S00043933913000329

Brandi L (2008). 1alpha (OH)D_3 One-alpha-hydroxy-cholecalciferol-an active vitamin D analog. Clinical studies on prophylaxis and treatment of secondary hyperparathyroidism in uremic patients on chronic dialysis. Danish Medical Bulletin, 55(4): 186-210. Available at: https://pubmed.ncbi.nlm.nih.gov/19232159/

Brown AJ, Dusso A, and Slatopolsky E (1999) Vitamin D. American Journal of Physiology, 277, F157-F175. DOI: https://www.doi.org/10.1152/ajprenal.1999.277.2.f157

Cobb (2021). Breeder management guide. pp. 1-153. Available at: https://www.cobbafrica.com/wp-content/uploads/Cobb-500-Breeder-Managment-Guide.pdf

Coto C, Cerate S, Wang Z, Yan F, Min Y, Costa FP, and Waldroup PW (2010). Effect of source e level of vitamin D on the performance of breeder hens and the carryover to the progeny. International Journal of Poultry Science, 9(7): 623-633. DOI: https://www.doi.org/10.3923/ijps.2010.623.633

Chung MK, Choi JH, Chung, and Chee KM (2005). Effects of dietary vitamin C and E on egg shell quality of broiler breeder hens exposed to heat stress. Asian-Australasian Journal of Animal Sciences, 18(4): 545-551. DOI: https://www.doi.org/10.5713/ajas.2005.545

Dayan J, Reicher N, Melkman-Zehavi T, and Uni Z (2020). Incubation temperature affects yolk utilization through changes in expression of yolk sac tissue functional genes. Poultry Science, 99(11): 6128-6138. DOI: https://www.doi.org/10.1016/j.psj.2020.07.037

Dzoma BM (2010). Some factors affecting fertility and hatchability in the farmed ostrich: A review. Journal of Animal and Veterinary Advances, 9(2): 229-239. DOI: https://www.cabdirect.org/cabdirect/abstract/20103053159

Etim NN, Enyenihi GE, Williams ME, Udo DM, and Offiong EE (2013). Haematological parameters: Indicators of the physiological status of farm animals. British Journal of Science, 10(1): 33-45.

Fletcher BD, Flett JAM, Wickham SR, Pullar JM, Vissers MCM, and Conner TS (2021). Initial evidence of variation by ethnicity in the

relationship between vitamin C status and mental states in young adults. Nutrients, 13(3): 792. DOI: https://www.doi.org/10.3390/nu13030792

Fritts CA and Waldroup PW (2003). Effects of source and level of vitamin D on live performance and bone development in growing broilers. Journal of Applied Poultry Research, 12(1): 45-52. DOI: https://www.doi.org/10.1093/japr/12.1.45

Garcia AFQM, Murakami AE, do Amaral Duarte CR, Rojas ICO, Picoli KP, and Puzolti MM (2013). Use of vitamin D3 and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. Asian-Australasian Journal of Animal Sciences, 26(3): 408-415. DOI: https://www.doi.org/10.5713/ajas.2012.12455

Gouda A, Amer SA, Gabr S, and Tolba SA (2020). Effect of dietary supplemental ascorbic acid and folic acid on the growth performance, redox status, and immune status of broiler chickens under heat stress. Tropical Animal Health and Production, 52(6): 2987-2996. DOI: https://www.doi.org/10.1007/S11250-020-02316-4

Gross WB and Siegel HS (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Disease, 27(4): 972-979. DOI: https://www.doi.org/10.2307/1590198

Hachesoo BA, Talebi A, and Rezai SA (2011). Comparative study on blood profiles of indigenous and Ross-308 broiler breeders. Global Veterinaria, 7(3): 238-241. Available at: https://www.idosi.org/gv/GV7(3)11/6.pdf

Halgrain M, Bernardet N, Crepean M, Meme N, Narcy A, Hincke M, and Godbert SR (2022). Eggshell decalcification and skeletal mineralization during chicken embryonic development: Defining candidate genes in the chorioallantoic membrane. Poultry Science, 101(2): 101622. DOI: https://www.doi.org/10.1016/j.psj.2021.101622

Huth JC and Archer GS (2015). Comparison of two LED light bulbs to a dimmable CFL and their effect on broiler chicken growth, stress, and fear. Poultry Science, 94(9): 2027-2036. DOI: https://www.doi.org/10.3382/ps/pev215

Kerschnitzki M, Zander T, Zaslansky P, Fratzl P, Shahar R, and Wagermaier W (2014). Rapid alterations of avian medullary bone material during the daily egg-laying cycle. Bone, 69: 109-117. DOI: https://www.doi.org/10.1016/j.bone.2014.08.019

Lohakare JD, Kim JK, Ryu MH, Hahn TW, and Chae BJ (2005). Effects of vitamin C and vitamin D interaction on the performance, immunity, and bone characteristics of commercial broilers. Journal of Applied Poultry Research, 14(4): 670-678. DOI: https://www.doi.org/10.1093/japr/14.4.670

Lare L, Talaki E, Dzogbema KF, Sodjedo C, and Lombo Y (2021). Influence of dietary protein on embryonic development and hatching traits of indigenous quinea fowls (*numida meleagris*) breeders in Togo. Journal of World Poultry Research, 11(4): 422-430. Available at: http://eprints.science-line.com/id/eprint/236/1/JWPR%2011%284%29%20422-430%202021.pdf

Lin H, Jiao HC, Buyse J, and Decuypere E (2006). Strategies for preventing heat stress in poultry. World's Poultry Science Journal, 62(1): 71-86. DOI: https://www.doi.org/10.1079/WPS200585

Narushin VG and Romanov MN (2002). Egg physical characteristics and hatchability. World's Poultry Science Journal, 58(3): 297-303. DOI: https://www.doi.org/10.1079/WPS20020023

Nys Y, Gautron J, Garcia-Ruiz JM, and Hincke MT (2004). Avian eggshell mineralization: Biochemical and functional characterization of matrix proteins. Comptes Rendus Palevol (Palevol Reports), 3(6-7): 549-562. DOI: https://www.doi.org/10.1016/j.crpv.2004.08.002

Pande VV, Chousalkar KC, Bhanugopan MS, and Quinn JC (2015). Super pharmacological levels of calcitriol (1,25-(OH)2D3) inhibits mineral deposition and decreases cell proliferation in a strain dependent manner in chicken mesenchymal stem cells undergoing osteogenic differentiation *in vitro*. Poultry Science, 94(11): 2784-2796. DOI: http://www.doi.org/10.3382/ps/pev284

Romanoff LA (1960). Avian diseases. In: H.E. Evans (Editor), The avian embryo: Structural and functional development. American Association of Avian Pathologists., 4(4): 541-542. DOI: https://www.doi.org/10.2307/1587707

Rao PS, Kalva S, Yerramilli A, and Mamidi S (2011). Free radicals and tissue damage: Role of antioxidants. Free Radicalss and Antioxidants, 1(4): 2-7. DOI: https://www.doi.org/10.5530/ax.2011.4.2

Regassa A, Adhikari R, Nyachoti CM, and Kim WK (2015). Effects of 25-(OH)D3 on fecal Ca and P excretion, bone mineralization, Ca and P transporter mRNA expression and performance in growing female pigs. Journal of Environmental Science and Health, Part B, 50(4): 293-299. DOI: https://www.doi.org/10.1080/03601234.2015.999612

Richards MP, Rosebrough RW, Coon CN, and McMurtry JP (2010). Feed intake regulation for the female broiler breeder: In theory and in practice. Journal of Applied Poultry Research, 19(2): 182-193. DOI: https://www.doi.org/10.3382/japr.2010-00167

Roy S and Mishra SC (2011). Effect of antistress agents on haemato-biochemical profiles of broiler and breeder hen during summer. Veterinary World, 4(2): 60-63. Available at: http://www.veterinaryworld.org/Vol.4/February%20-%202011/Effect%20of%20Antistress%20agents%20on%20Haemato-Biochemical%20profiles%20of%20broiler%20and%20breeder%20hen%20druing%20summer.pdf

Rosa AP, Scher A, Sorbara JOB, Boemo LS, Forgiarini J, and Londero A (2012). Effects of canthaxanthin on the productive and reproductive performance of broiler breeders. Poultry Science, 91(3): 660-666. DOI: https://www.doi.org/10.3382/ps.2011-01582

Sastradipradja D, Sikar SHS, Widjayakusuma R, Maad A, Unandar T, Nasution H, Suriawinata R, and Hamzak K (1989). Penuntun Praktikum Fisiologi Veteriner. Pusat Antar Universitas Ilmu Hayati. Bogor.

Saunders-Blades JL and Korver DR (2014). The effect of maternal vitamin D source on broiler hatching egg quality, hatchability, and progeny bone mineral density and performance. Journal of Applied Poultry Research, 23(4): 773-783. DOI: https//www.doi.org/10.3382/japr.2014-01006

Saunders-Blades JL and Korver DR (2015). Effect of hen age and maternal vitamin D source on performance, hatchability, bone mineral density, and progeny *in vitro* early innate immune function. Poultry Science, 94(6): 1233-1246. DOI: https://www.doi.org/10.3382/ps/pev002

Scanes CG (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poultry Science, 95(9): 2208-2215. DOI: https://www.doi.org/10.3382/ps/pew137

Siegel HS and Kampen MV (1984). Energy relationships in growing chickens given daily injections of corticosterone. British Poultry Science, 25(4): 477-485. DOI: https://www.doi.org/10.1080/00071668408454889

Torres CA, Vieira SL, Reis RN, Ferreira AK, Silva PXD, and Furtado FVF (2009). Productive performance of broiler breeder hens fed 25-hydroxycholecalciferol. Revista Brasileira de Zootecnia, 38(7): 1286-1290. DOI: https://www.doi.org/10.1590/S1516-35982009000700018

Ulmer-Franco AM, Fasenko GM, and O'Dea Christopher EE (2010). Hatching egg characteristics, chick quality, and broiler performance at 2 breeder flock ages and from 3 egg weights1. Poultry Science, 89(12): 2735-2742. DOI: https://www.doi.org/10.3382/ps.2009-00403

Van Hieu T, Guntoro B, Qui NH, Quyen NTK, and Al-Hafiz FA (2022). The application of ascorbic acid as a therapeutic feed additive to boost immunity and antioxidant activity of poultry in heat stress environment. Veterinary World, 15(3): 685-693. DOI: http://www.doi.org/10.14202/vetworld.2022.685-693

Van Lerberghe W, Keegels G, Cornelis G, Ancona C, Mangelschots E, and Van Balen H (1983). Haemoglobin measurement: The reliability of some simple techniques for use in a primary health care setting. Bulletin of the World Health Organization, 61(6): 957-965. Available at: https://apps.who.int/iris/bitstream/handle/10665/264977/PMC2536237.pdf?sequence=1&isAllowed=y

Yair R and Uni Z (2011). Content and uptake of minerals in the yolk of broiler embryos during incubation and effect of nutrient enrichment. Poultry Science, 90(7): 1523-1531. DOI: https://www.doi.org/10.3382/ps.2010-01283

Wang J, Qiu L, Gong H, Celi P, Yan L, Ding X, Bai S, Zeng Q, Mao X, Xu S, et al. (2020). Effect of dietary 25-hydroxycholecalciferol supplementation and high stocking density on performance, egg quality, and tibia quality in laying hens. Poultry Science, 99(5): 2608-2615. DOI: https://www.doi.org/10.1016/j.psj.2019.12.054

Schalm OW, Jain NC, and Carol EJ (2010). Schalm's veterinary hematology. In: Weiss DJ and Wardrop KJ (Editors), 6th Edition. Blackwell Publishing Ltd., Oxford. Available at: https://www.vet-ebooks.com/schalms-veterinary-hematology-6th-edition/

Whitehead CC and Keller T (2019). An update on ascorbic acid in poultry. World's Poultry Science Journal, 59(2): 161-184. DOI: https://www.doi.org/10.1079/WPS20030010

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 81-88, March 25, 2023

Research Paper, PII: S2322455X2300008-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.8

Mycotoxins and Heavy Metals of Poultry Feeds from the Centre, Littoral, and Western Regions of Cameroon

Fabrice De Paul Tatfo Keutchatang[1,2*], Borelle Mafogang[2], Alex Dimitri Tchuenchieu Kamgain[1], Evelyne Nguegwouo[1], Hippolyte Mouafo Tene[1], Isabelle Sandrine Bouelet Ntsama[3], Gabriel Medoua Nama[1], and Germain Kansci[2]

[1]*Centre for Food, Food Security and Nutrition Research, IMPM, PO Box 13033, Yaoundé, Cameroon*
[2]*Department of Biochemistry, Laboratory of Food Science and Metabolism, Faculty of Sciences, University of Yaoundé 1, PO Box 812, Yaoundé, Cameroon*
[3]*Advanced Teacher's Training College for Technical Education, University of Douala, PO Box 1872, Douala, Cameroon*

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

Received: 12 December 2022
Accepted: 25 January 2023

ABSTRACT

Heavy metals are a group of elements that could be found in poultry feeds and influence poultry production. Poultry feed generally consists of agricultural products, such as maize, groundnuts, and wheat, which may also be contaminated by mycotoxins. The use of mycotoxins and heavy metals contaminated feed in the poultry sector might represent a potential source of cross-contamination for humans. This study aimed to analyze total aflatoxins (AFs), aflatoxin B_1 (AFB_1), ochratoxin A (OTA), chromium, copper, nickel, zinc, arsenic, cadmium, lead, and mercury in poultry feed from the Centre, Littoral, and Western regions of Cameroon. In this order, six local broiler feeds, six local layer feeds, and three imported layer feeds were randomly collected from each region and analyzed using inductively coupled plasma spectrometry for heavy metals and competitive indirect ELISA for mycotoxins. The results indicated that all feed samples contained the analyzed mycotoxins and heavy metals. The ranges for the mean concentrations of mycotoxins were 3.5-19.7, 2.7-19.3, 0.8-1.1 µg/kg for AFs, AFB_1, and OTA, respectively. They were globally below the established regulated limits (20 µg/kg for AFs, 10 µg/kg for AFB_1 and 5 µg/kg for OTA). The bulk layer feed from the Littoral region had the highest lead (995.8 ± 0.4 µg/kg) and cadmium (3.3 ± 0.0 µg/kg) concentrations. The average concentration of lead was above the permissible limit (10 µg/kg). Bulk broiler feed from the Littoral region scored the highest concentration of arsenic (2819.4 ± 0.1 µg/kg) above the permissible limit (500 µg/kg). Bulk broiler feed from the Centre region showed the highest concentration of mercury (5.6 ± 0.0 µg/kg) although lower than the permissible limit of 100 µg/kg. This study demonstrates that there are potential safety issues associated to poultry feeds used in some regions of Cameroon. It suggests a possible low productivity of poultry and health issues for consumers.

Keywords: Aflatoxin, feed, Contamination, Heavy metals, Ochratoxin A, Poultry

INTRODUCTION

The poultry sector is known as an important source of protein and other useful nutrients for human nutrition and health in Cameroon (Guetiya Wadoum et al., 2016). Chickens are easy to rear (Paryad and Mahmoudi, 2008), available at low prices, and known as food for billions of people, including those who live in low-middle-income countries (Aral et al., 2013), such as Cameroon. Poultry production represents 42% of meat production. In Cameroon, chicken meat and eggs consumption increased from 2.2 kg to 5.6 kg and 16 to 52 eggs between 2006 and 2016, respectively. In addition, poultry represents 4% of the gross domestic product (Höffler, 2018). Poultry feeds are generally a mixture of agricultural products, such as maize, groundnuts, and wheat, which may be contaminated by mycotoxins and heavy metals (Abia et al., 2013a, Akinmusire et al., 2018). The use of such contaminated feed in the poultry sector may result in poultry productivity and in a source of human contamination. It is recommended to initially analyze the different contaminants found in these feeds to limit the

risks of feed quality on the poultry productivity directly and indirectly on human health. However, studies on poultry feed contamination are scarce, and it refers to 2013 in Cameroon. Mycotoxins are secondary metabolites produced by three genera (*Aspergillus*, *Penicillium*, and *Fusarium*) of fungi which can produce more than 500 toxins. Among these mycotoxins, Aflatoxins and Ochratoxin A (OTA), exhibit pathogenic characteristics (Becer and Filazi, 2010; Kaya, 2014). Toxic heavy metals are mineral elements with a specific weight greater than $5g/cm^3$ (Demirezen and Uruc, 2006). These mineral elements are a serious concern due to their impacts (toxicity, bioaccumulation, and biomagnifications) in the food chain (Demirezen and Uruc, 2006; Hazrat et al., 2019). Considering the fact that contamination of poultry feed by contaminants, such as mycotoxins and toxic metals, cannot be entirely avoided due to favorable climatic conditions for their development (Tatfo Keutchatang et al., 2021) and the availability of pollutants in the environment, there is a need for such contamination to be minimized, and to reduce theirs side effects on animal and human by one health approach (WHO, 2017). This study was initiated to enrich the data already available on the contamination of poultry feed by mycotoxins and heavy metals in Cameroon. It aimed to analyze mycotoxins (total aflatoxins, aflatoxin B_1, and OTA) as well as eight heavy metals in poultry feeds collected in the Centre, Littoral, and West regions using enzyme-linked immunosorbent assay (ELISA) and inductively coupled plasma spectrometry (ICP- OES), respectively.

MATERIALS AND METHODS

Study area

The current study was conducted on poultry farms located in three regions of Cameroon: Centre, Littoral, and West. These regions are the large areas of production and consumption of chickens and eggs (Teleu and Ngatchou, 2006). The study was conducted from January to December 2019.

Sampling design

A total number of 15 samples of chicken feed, constituted of 6 local broiler feed (2 per region), 6 local layer feed (2 per region), and 3 imported layer feed (1 per region) eaten by broiler and layer chickens were collected from poultry farms. Local feed samples were collected from layer and broiler farms, while imported layer feeds were collected from imported feed outlets. Indeed, a preliminary study reported the classification of chicken farms in these three regions into two groups (moderate and high risk of biosecurity) according to biosecurity score (Tatfo Keutchatang et al., 2021). Feed sampling was done as described by the European Commission (2006) Directive No. 401/2006 (EC, 2006). In each selected farm or outlet, 4 kg of feed was sampled. Different points of four randomly selected feeds (50 Kg bags) from those available were duplicated. A total of four bags randomly selected for feed sampling were chosen from the same strip to reduce variability and ensure the effective representativeness of the strip. Each sub-sample of 1 kg consisted of three portions of 300 to 350 g. The sub-samples were collected manually using a probe at three points top, middle, and bottom of feed bags. The feeds taken from each point were homogenized in bags, and 1/4 of each was collected to provide 15 representative samples as 4 feed samples in the Centre, 4 in the Littoral, 4 in the West, and 3 outlets. The samples were conditioned in polystyrene bags and transported to the laboratory for quality control at the Centre for Food and Nutrition Research of the IMPM, Yaoundé, Cameroon, where the feed samples were ground with a blender (Black & Decker®, England), weighed in several aliquots of 5g using a scale (Mettler Toledo, USA), and stored in sterile plastic bags at -20°C for analysis. The samples were kept in the laboratory for a maximum of 7 days.

Sample preparation and analysis

Water content of different samples

Water content was determined using the reference methods of the Association of Official Analytical Chemists (AOAC, 2000) for bulk chicken feeds. An amount of 5 g of each sample in triplicate was dried at 105°C (Rolabo, Germany) until constant weight in an aluminum foil previously dried and weighed the dried samples were cooled in a desiccator (Borosilicate Glass 3.3, Indane Chemical Company, Borivali, Mumbai, Maharashtra) for 30 minutes and reweighed. The water content of each sample was determined by calculating the differences between the masses of the fresh and dried samples (AOAC, 2000).

Determination of mycotoxin content

Total Aflatoxins (AFs), Aflatoxin B_1 (AFB_1), and OTA concentrations in the samples were determined using quantitative enzyme-linked immune sorbent assay kits (ELISA, BIOO Scientific Corporation, MaxSignal®, USA). Samples containing 2 g of ground bulk chicken feed were mixed with 25 mL of 70% methanol (HPLC grade, Merck, Germany) in 50 mL falcon tubes for 10

minutes using a vortex, centrifuged at 4000 × g for 10 minutes using the Rotofix 32 A, centrifuge (Germany). Then, 100 µL of the supernatant was collected and diluted with 700 µL 70% methanol (HPLC grade, Merck, Germany). The mixture was used for total AFs, AFB_1, and OTA analyses following the kit manufacturer's instructions and as described by Tatfo Keutchatang et al. (2022). The concentrations of determined mycotoxins were inversely proportional to the color intensity established using an automated microplate reader (EL × 800, BIOTEK, Instruments Inc., Winooski, VT, United States) at 450 nm and estimated based on a calibration curve.

Determination of heavy metal content

Sample preparation

The dried samples were cooled in a desiccator for 30 minutes and reweighed. The different bulk samples were dried and ground with a blender (Black & Decker®, England), then weighed in several aliquots of 500 mg by using a scale (Mettler Tolero, USA). Then, 500 mg of each powder bulk sample and 50 mL of nitric acid were introduced into the container to obtain a mixture left to stand overnight (Broekaert, 2005).

Inductively coupled plasma with optical emission spectrometry

The analysis was conducted as described by Broekaert (2005). The selected heavy metals contained Arsenic (As), Copper (Cu), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Nickel (Ni), Lead (Pb), and Zinc (Zn) contents were determined. These metals were selected based on their benefits and toxicity in living organisms. The detection of the elements present in the analyte was performed by emission. The nebulized analyte was driven by a peristaltic pump to obtain an aerosol that was transported in the plasma, where it was desolvated, vaporized, atomized, or ionized. The return to a lower energy state was accompanied by the emission of radiation characteristic of the elements. A monochromator separated the different wavelengths. The wavelengths of the analyzed elements and the preparation of their standards are presented in Table 1.

Table 1. Standard solution used during the determination of metals

	Volumes (mL)						
Solutions étalons	1	2	3	4	5	6	7
Solutions Cd, Ni, Pb, Cr, As, Hg, Zn, Cu	125	150	175	200	2 25	250	–
HNO_3 conc.	125	100	75	50	25	–	250
Volume final	250	250	250	250	250	250	

Cd: Cadmium, Ni: Nickel, Pb: Lead, Cr: Chromium, As: Arsenic, Hg: Mercury, Zn: Zinc, Cu: Copper, HNO_3 Conc: Concentrated nitric acid

Mineralization for heavy metals determination

An amount of 500 mg of sample was weighed and introduced for digestion in a DigiTUBE containing a mixture of 5 mL of nitric acid and 10 mL concentrated hydrogen peroxide for 16 hours at 25°C. Then, the mixture was brought to 95°C for 2 hours in a graphite heating block before being filtered. In each series of tubes at least three blanks were placed and three controls prepared. After the installation of the tracks on DigiPREP, the tubes were rotated. For this purpose, the locating lugs matched the notches and the bottom of the tubes was in contact with the bottom of the graphite block. The blanks were covered with perforated plugs to be able to insert the DigiPROBE temperature probe inside. The probe was placed low enough to be immersed in the liquid without touching the bottom of the tube. The temperature controller was switched on and the temperature program was selected. After allowing the tubes to cool to 25°C, the volume of each sample was adjusted to 20 mL (Broekaert, 2005).

Quality control

The analytical test for mycotoxins was conducted using the internal quality control (IQC) approach and validated before usage. The quality control was performed by choosing five different IQCs as follows, calibration, blanks, mid-range standard, spiked standard solution, certified references material, and duplicates. Results were discarded and the sample was if a sample did ~~was~~ not met the acceptance criteria, and the sample was reanalysed. The limit of detection (LOD) of the analysed samples was within the range of 0.06-0.3 µg/kg for AFs and 0.3-0.6 µg/kg for OTA, while the limit of quantification (LOQ) was in the range of 0.2-1 µg/kg for AFs and 1-2 µg/kg for OTA. Samples with values below LOQ were recorded as non-detectable (CEAEQ, 2015). The calibration standards for metals were prepared from certified standards. A total

number of four external reference samples and one standard reference sample from the National Institute of Standards and Technology (NIST) were introduced into each series for analysis.

Statistical analysis

Data obtained were transferred into Microsoft Excel for the calculation of the concentrations of µg kg−1. The obtained data were then subjected to analysis of variance (ANOVA) and Student's T test for paired samples at the significance level of 5% for means comparison using a statistical package, SPSS version 20.0 for windows. Results were expressed as mean ± standard deviation.

RESULTS

Water content of chicken feed samples

Table 2 presents the water content g/100 of fresh matter (FM) of chicken feed samples. Water content varied from 20.8 ± 16.6 g/100g of FM in the Centre and Littoral to 24.0 ± 5.1 g/100g of FM in the West for local bulk broiler feed samples. A significant difference was observed between water content for local bulk broiler feed from both the Centre and Littoral regions and West region ($p < 0.05$). Local bulk layer feeds showed water content varying from 12.4 ± 0.2 g/100g of fresh matter in the Littoral and West to 16.8 ± 6.6 g/100g of FM in the Centre. A significant difference was observed between water content from both Littoral and West regions and the Centre region ($p < 0.05$) concerning local bulk layer feeds. All the imported feed samples for the three regions presented a water content of 12.4 ± 2.7 g/100 g of FM with no significant difference ($p > 0.05$).

Total aflatoxin, aflatoxin B_1, and Ochratoxin A content in chicken feeds

Table 3 presents total aflatoxin (AFT), aflatoxin B_1 (AFB_1), and Ochratoxin A (OTA) contents in broiler and laying chicken feed from the Centre, Littoral, and West regions. The AFT content of broiler feed varies from 3.9 ± 0.2 (Littoral region) to 19.6 ± 0.3 µg/kg (Centre region). The AFB_1 content of broiler feed varies from 1.6 ± 0.1 (West region) to 19.3 ± 0.2 µg/kg (Central region). The OTA content of broiler feed ranges from 1.1 ± 0.01 to 0.8 ± 0.01µg/kg. In the layer feed, the AFT content varies from 3.5 ± 0.1 to 12.6 ± 0.2 µg/kg, the AFB_1 content from 2.8 ± 0.1 to 11.4 ± 0, 2µg/kg, and OTA content from 0.8 ± 0.01 to 1.1 ± 0.001µg/kg.

The total aflatoxin (AFs) content of broiler and layer feed is higher in the Centre region and low in the Littoral region. Aflatoxin B_1 (AFB_1) content is always higher in the Centre and low in the Littoral for broiler feed, raised in the Centre region and lower in the Littoral region for layer feed. In terms of Ochratoxin A (OTA) content, the highest value is presented by broiler feed from the Centre and Littoral regions, while the Littoral region had the highest value in layer feed (Table 3). Table 3 shows a variation between the values of the levels of different toxins from one region to another. This variation results in some cases in a significant difference ($p < 0.05$). This variation in the contents of AFT, AFB_1, and OTA can be explained by the different level of contamination of the different ingredients used in the composition of chicken feed and climatic conditions. However, significant differences were also observed between ochratoxin A contents in sample feeds from each region ($p < 0.05$).

Heavy metal content of chicken feed samples

Heavy metals analyzed were of two groups, including essential (Zn, Cu, Chromium, and Nickel) and toxic metals (Lead, Arsenic, Cd, and Hg). Average contents of each metal of each group in different bulk chicken feeds are presented in Tables 4, 5, and 6. Concerning essential metals, Zn showed the highest average content (1587168.5 ± 49.5 µg/kg), while Nishowed the lowest content (8275.7 ± 21.5 µg/kg) as presented in Table 4. Significant differences were not observed between heavy metal contents in imported bulk broiler feed from each region ($p > 0.05$). Table 5 presents the average contents of non-toxic heavy metals in both bulk local layer and broiler feeds from the Centre, Littoral and West regions. As shown in Table 5, significant differences were observed between heavy metal contents of non-toxic heavy metals in bulk local layer and broiler feeds from the three regions ($p < 0.05$). However, bulk local layer and broiler feeds showed the highest average concentration of Zn while Cr presented the lowest average content. Furthermore, significant differences were observed between Nickel, Zn, Cu, and Cr contents in the bulk feed from the Littoral and West regions ($p < 0.05$). These differences were probably due to the diverse sources of the raw materials of the ingredients used to produce feeds.

Table 6 presents the average contents of toxic heavy metals in bulk layer and broiler feeds from the three regions. Significant differences were observed between the average contents for each metal and from each region ($p < 0.05$). The Pb showed the highest average content in bulk local layer feed samples from each region, while Arsenic (As) obtained the lowest average content. In bulk local broiler feed samples, As showed the highest average

contents and Cd had the lowest average contents. This clearly shows that the content levels of bulk feed samples are different in terms of chicken type ($p < 0.05$).

Table 2. Water content (g/100 g) of fresh matter of different bulk samples in the three regions of Cameroon

Sample \ Regions	Centre	Littoral	West
Local broiler feed (g/100 g of FM)	20.8 ± 0.6^{aA}	20.8 ± 0.5^{aA}	24.0 ± 0.1^{bA}
Local layer feed (g/100 g of FM)	16.8 ± 0.6^{B}	12.4 ± 0.2^{B}	12.4 ± 0.2^{bB}
Imported layer feed (g/100 g of FM)	12.4 ± 0.7^{aC}	12.4 ± 0.7^{aC}	12.4 ± 0.7^{ab}

FM: Fresh matter, ^{a,b,c} Significant difference in the same column ($p < 0.05$), $^{A, B, C}$ Significant difference in the same row ($p < 0.05$)

Table 3. Total aflatoxins, Aflatoxin B_1, and Ochratoxin A contents in bulk chicken feed samples collected in some poultry farms from the Centre, the Littoral and the West regions of Cameroon

Mycotoxins	Region	Mycotoxin content (µg/kg)								
		Local broiler feed			Local layer feed			Imported layer feed		
		Mean ± SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max
Total Aflatoxin (AFs)	Centre	19.6^{a}±0.3	17.2	20.7	12.6^{b}±0.2	11.9	13.3	8.2^{c}±1.4	6.4	9.6
	Littoral	3.9^{a}±0.2	2.7	5.2	3.5^{a}±0.1	3.2	3.8	8.2^{b}±1.4	6.4	9.6
	West	7.4^{a}±0.1	6.3	8.4	4.7^{b}±0.1	2.4	8.1	8.2^{c}±1.4	6.4	9.6
Aflatoxin B_1 (AFB_1)	Centre	19.3^{a}±0.2	17.6	21.0	11.4^{b}±0.2	8.7	14.1	3.6^{c}±0.4	3.1	4
	Littoral	3.7^{a}±0.1	3.4	4.1	$2..8^{b}$±0.1	1.6	3.9	3.6^{a}±0.4	3.1	4
	West	1.6^{a}±0.2	1.4	1.8	3.3^{b}±0.1	2.9	4.9	3.6^{b}±0.4	3.1	4
Ochratoxine A (OTA)	Centre	1.1^{a}±0.01	1.0	1.4	0.9^{a}±0.01	0.8	1.1	2.6^{b}±0.4b	1.8	3
	Littoral	1.1^{a}±0.01	1.0	1.3	1.1^{a}±0.01	1.0	1.2	2.6^{b}±0.4	1.8	3
	West	0.8^{a}±0.01	0.6	0.9	0.8^{a}±0.01	0.6	0.9	2.6^{b}±0.4	1.8	3

Significant difference between different letters in the same row ($p < 0.05$)

Table 4. Essential and toxic heavy metals in a bulk imported layer feed (µg/kg)

Heavy metals		Average concentration (µg/kg)
Essentials	Cu	248967.8 ± 78.1
	Cr	7760.8 ± 47.7
	Ni	8275.7 ± 21.5
	Zn	1587168.5 ± 49.5
Toxics	As	1.0 ± 0.2
	Cd	2.5 ± 0.3
	Pb	3229.8 ± 3.0
	Hg	4.9 ± 0.6

Cd: Cadmium, Ni: Nickel, Pb: Lead, Cr: Chromium, As: Arsenic, Hg: Mercury, Zn: Zinc, Cu: Copper

Table 5. Average concentrations of non-toxic heavy metals (µg/kg) in bulk local broiler and layer feeds from the three regions of Cameroon

Non-toxic heavy metals	Centre		Littoral		West	
	Bulk broiler feed	Bulk layer feed	Bulk broiler feed	Bulk layer feed	Bulk broiler feed	Bulk layer feed
Ni	22575 ± 35.4^{aA}	6942.4 ± 0.1^{aA}	22522.3 ± 0.4^{bB}	7349.3 ± 0.4^{bB}	22561 ± 55.2^{cC}	7145.8 ± 287.4^{cC}
Zn	82791.7 ± 0.2^{aA}	51789.4 ± 0.1^{aA}	82834.4 ± 0.1^{bB}	54537.4 ± 0.1^{bB}	82813 ± 30.4^{cC}	4150.8 ± 0.4^{cC}
Cu	17760.2 ± 0.5^{aA}	7963.4 ± 0.1^{aA}	17737.5 ± 0.0^{bB}	8370.8 ± 0.4^{bB}	17749 ± 16.3^{cC}	8167.3 ± 288.1^{cC}
Cr	1867.8 ± 0.4^{aA}	3957.2 ± 0.2^{aA}	1882.2 ± 0.07^{bB}	4150.8 ± 0.4^{bB}	1875.1 ± 10.0^{cC}	4054 ± 137.2^{cC}

^{a,b,c} Significant difference in the same column ($p < 0.05$), $^{A, B, C}$ Significant difference in the same row ($p < 0.05$), Ni: Nickel, Zn: Zinc, Cu: Copper Cr: Chromium

Table 6. Average concentrations of toxic heavy metals (µg/kg) in bulk local broiler and layer feeds from the three regions of Cameroon

Toxic heavy metals	Centre		Littoral		West	
	Bulk broiler feed	Bulk layer feed	Bulk broiler feed	Bulk layer feed	Bulk broiler feed	Bulk layer feed
Pb	10 ± 0.0^{aA}	10 ± 0.0^{aA}	7.5 ± 0.0^{bB}	995.8 ± 0.4^{bB}	8.8 ± 1.8^{cC}	503 ± 209.3^{cC}
As	2818.8 ± 0.4^{aA}	2.2 ± 0.0^{aA}	2819.4 ± 0.1^{bB}	2.0 ± 0.07^{bB}	2819.3 ± 0.4^{cC}	2.1 ± 0.1^{cC}
Cd	2.8 ± 0.07^{aA}	2.4 ± 0.0^{aA}	2.7 ± 0.0^{bB}	3.3 ± 0.0^{bB}	2.7 ± 0.0^{cC}	2.9 ± 0.6^{cC}
Hg	4.6 ± 0.07^{aA}	5.6 ± 0.0^{aA}	4.3 ± 0.0^{bB}	5 ± 0.0^{bB}	4.4 ± 0.1^{cC}	5.3 ± 0.4^{cC}

A significant difference between identical letters in the same column and different letters in the same line ($p < 0.05$), Pb: Lead, As: Arsenic, Cd: Cadmium, Hg: Mercury

DISCUSSION

Total aflatoxins, Aflatoxin B_1, and Ochratoxin A in chicken feed samples

This study was conducted in the Centre, Littoral, and West regions of Cameroon, namely Centre, Littoral and West. Total aflatoxins (AFs), Aflatoxin B_1 (AFB_1), and Ochratoxin A (OTA) contents in chicken feed samples, their average content globally respected the recommended standard. The recommended concentrations of AFs, AFB_1, and OTA in poultry feeds (20 µg/kg, 10 µg/kg, and 5µg/kg, respectively, FAO/WHO, 2017; Mokubedi et al., 2019) were higher than concentrations found for different feed samples in this study. This is probably because these feeds were well stored at the farm. During sample collection, it was observed that feeds are stored in places that are not humid and are mostly made for immediate use (2 to 3 days). However, the results of this study are different from previous studies in Guyana ($27380 \pm 82120 \times 10^{-3}$ µg/kg) by Mokubedi et al. (2019), in Nigeria (127400×10^{-3} µg/kg) by Akinmusire et al. (2018), and Cameroon (30000×10^{-3} and 22000 x 10^{-3} µg/kg) by Abia et al. (2013a) for AFs. Aflatoxin B_1 (AFB_1) content of broiler and layer feed in the Centre region is higher than in other regions. In addition, this content is also higher than the maximum limit for AFB_1 in chicken feed (10µg/kg) set by the Commission of the European Union and the Food and Drug Administration of the United States of America in 2010 (FAO/WHO, 2017). This AFB_1 content represents 193% of 10µg/kg in broiler feed and 114% in layer feed from the Centre region. The obtained results might probably be the consequence of conditions in which feed samples are produced or stored, which promote this toxin production by molds, such as *Aspergillus* whose presence in feed has already been reported (FAO/WHO, 2017). In fact, in the Center region, it was observed that food took longer on the farm, compared to the other two regions. The concentrations of OTA detected in all chicken feed were below the maximum tolerable limit of 5µg/Kg (Morrison et al., 2017). Previous studies in Nigeria and Cameroon reported the contamination of chicken feed or poultry by OTA at variable concentrations of 1200×10^{-3} and 2100×10^{-3} µg/kg (Abia et al., 2013b) and 5400×10^{-3} µg/kg (Akinmusire et al., 2018). Mycotoxins can be carried over from feed to animal body and be bio-accumulated (Mokubedi et al., 2019). Hence, although values are globally lower than the norm, it is suggested that measures should be taken to minimize mold contamination of poultry feeds.

Heavy metals in feed samples

Analysis of heavy metals was carried out in two groups of essential and toxic metals. The concentrations of Zn in different local bulk feed samples were above the maximum acceptable Zn concentration of 3 mg/kg (3000 µg/kg) established by the World Health Organization (WHO, 2011). Compared to the permissible concentration of 2 mg/kg (2000 µg/kg) for Cu in feed asserted by the WHO (2011), the mean concentrations of Cu in all feeds were above. Similar to Zn, Cu is required for many biological processes, including enzyme functions as well as a positive influence on livestock growth and reproduction. Due to the variation of their bioavailability, supplementation of Zn and Cu is necessary for most livestock species (EC, 2003a; EC, 2003b). A similar result was reported by Okoye et al. (2011) in Nigeria. Nickel average concentrations were higher than those reported by Okoye et al. (2011) in Nigeria, ranging from 2250 to 4875 µg/kg and higher than 70 µg/kg in feeds (WHO, 2011). The imported layer feed showed the highest mean concentration for Cr (7760.8 ± 47.7 µg/kg) than any other feed sample. Chromium concentrations in

different feed samples were above the permissible limit set by WHO (2011) of 50 µg/kg in feeds.

Bulk broiler feed samples from the three regions showed an average concentration of Arsenic above the permissible concentration (500 µg/kg, Nachman et al., 2005). The level of Cd in the bulk layer feed from the two poultry farms in the Littoral region and the second poultry farm in the West was above the permissible concentration of 3 µg/kg in feed (WHO, 2011). The Commission Directive 2005/8/EC permits a maximum Hg 0.1 mg/kg (100µg/kg) for complete feedstuffs (EC, 2005). The current study indicated that all bulk feed samples showed Hg average concentrations above this maximum allowed limit. Islam et al. (2007) reported the presence of Hg at the concentration of 57.9 µg/kg and 11.6 µg/kg in different types of poultry feed produced in Bangladesh. The permissible Pb limit set by WHO (2011) is 10 µg/kg. Bulk layer feed from the Littoral and West region was above the permissible limit. These low values of heavy metals, particularly toxic metals, could be bio-accumulated in chicken tissues and eggs during their life and be responsible for health concerns as reported by the CFIA (2017) and Tatfo Keutchatang et al. (2022). Contaminants can be accumulated in chicken tissues and eggs used for human consumption.

CONCLUSION

Feeds used in chicken farming for broilers and egg production were contaminated by mycotoxins (total aflatoxins, aflatoxin B_1, and ochratoxin A) and both essential and toxic metals in the study area (Centre, Littoral, and Western regions of Cameroon). The contents of these contaminants were, in a few cases, above the recommended or permissible limits. This situation could lead to the presence of their residues in chicken tissues and eggs responsible for health concerns and the low productivity of the poultry sector in Cameroon.

DECLARATIONS

Acknowledgments

The authors would like to express their gratitude to farmers for their cooperation and support. The authors also thank Mr. Guy Albert NGOUFACK, a Zootechnichian, for his assistance during the conduct of this study.

Funding

This study was self-funded.

Authors' contributions

Fabrice De Paul Tatfo Keutchatang, and Isabelle Sandrine Bouelet Nstama drafted the research protocol, collected data, and drafted the manuscript under the guidance of Gabriel Medoua Nama and Germain KANSCI. Data were analyzed by Borelle Mafogang Alex Dimitri Tchuenchieu Kamgain, Evelyne Nguegwouo, Hippolyte Mouafo Tene, and Fabrice De Paul Tatfo Keutchatang. All activities were supervised by Gabriel Medoua Nama and Germain KANSCI. All the authors edited the manuscript and approved its final content.

Competing interests

The authors declare that they have no conflicts of interest.

Ethical considerations

All ethical issues, including concerns to publish, data falsification, reuse of data already polished, misconduct, plagiarism, and redundancy were taken into consideration and have been verified and checked by the authors

Availability of data and materials

The data of the article will be sent by the corresponding author according to reasonable requests.

REFERENCES

Abia WA, Simo GN, Warth B, Suyolk M, Krska R, Tchana A, and Moundipa PF (2013a). Determination of multiple mycotoxins levels in poultry feeds from Cameroon. Japan Journal of Veterinary Research, 61(Suppl): S33-S39. Available at: https://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/52353/3/JJVR61-S_PROC.pdf

Abia WA, Warth B, Sulyok M, Krska R, Tchana AN, Njobeh PB, Dutton MF, and Moundipa PF (2013b). Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). Food Control, 31(2): 438-453. DOI: https://www.doi.org/10.1016/j.foodcont.2012.10.006

Akinmusire OO, El-Yuguda AD, Musa JA, Oyedele OA, Sulyok M, Somorin YM, Ezekiel CN, and Krska R (2018). Mycotoxins in poultryfeed and feedingredients in Nigeria. Mycotoxin Research, 35(2): 149-155. DOI: https://www.doi.org/10.1007/s12550-018-0337-y

Aral Y, Aydin E, Demir P, Akin AC, Cevger Y, Kuyululu ÇY, and Arikan MS (2013). Consumer preferences and consumption situation of chicken meat in Ankara province, Turkey. Turkish Journal of Veterinary and Animal Sciences, 37(5): 582-587. Available at: https://journals.tubitak.gov.tr/cgi/viewcontent.cgi?article=1980&context=veterinary

Association of official analytical chemists (AOAC) (2000). Official methods of analysis. USA, p. 25.

Becer UK and Filazi A (2010). Aflatoxins, nitrates and nitrites analysis in the commercial cat and dog foods. Fresenius Environmental Bulletin, 19(11): 2523-2527.

Broekaert JAC (2005). Analytical atomic spectrometry flames and Plasmas, 2nd Edition. DOI: https://www.doi.org/10.1002/3527606653

Canadian food inspection agency (CFIA) (2017). RG-8 regulatory guidance: Contaminants in feed (Formerly RG-1, Chapter7) Section 4: Metal contamination. Available at: https://www.inspection.gc.ca/animal-health/livestock-feeds/regulatory-guidance/rg 8/eng/1347383943203/1347384015909

Centre d'expertise en analyse environnementale du Québec (CEAEQ) (2015). Available at: www.ceaeq.gouv.qc.ca/index.asp

Demirezen D and Uruc K (2006). Comparative study of trace elements in certain fish, meat and meat products. Meat Science, 74(2): 255-260. DOI: http://www.doi.org/10.1016/j.meatsci.2006.03.012

European Commission (EC) (2003a). Opinion of the scientific committee on animal nutrition on the use of zinc in feedstuffs. European commission, health and consumer protection directorate, Brussels, Belgium. Available at: https://lavasoft.gosearchresults.com/?q=%3B+EC%29+European+Commission+%282003a%29.&tt=vmn__webcompa__1_0__go__lvs__webcompa__1_0__go__ch_WCYID10420_190423__yrff__yrff&pid=5ac784309091147a162b4431

European commission (EC) (2003b). Opinion of the scientific committee on animal nutrition on the use of copper in feedstuffs. European commission, health and consumer protection directorate. Brussels, Belgium. Available at: https://food.ec.europa.eu/system/files/2020-12/sci-com_scan-old_report_out115.pdf

European commission (EC) (2006). Commission regulation No. 401/2006 means commission regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs as amended by commission regulation (EU). Available at: https://www.legislation.gov.uk/eur/2006/401/contents

Food and agricultural organization/ world health organization (FAO/WHO) (2017). Joint FAO/WHO food standards programme, codex committee on contaminants in foods, WHO, Geneva, Switzerland. Available at: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-735-11%252FREPORT%252FREP17_CFe.pdf

Guetiya Wadoum RE, Zambou NF, Anyangwe FF, Njimou JR, Coman MM, Verdenelli MC, Cecchini C, Silvi S, Orpianesi C, Cresci A et al. (2016). Abusive use of antibiotics in poultry farming in Cameroon and the public health implications. British Poultry Science, 57(4): 483-493. DOI: https://www.doi.org/10.1080/00071668.2016.1180668

Hazrat A, Ezzat K, and Ikram I (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. Journal of Chemistry, 2019: 673030. DOI: https://www.doi.org/10.1155/2019/6730305

Höffler H (2018). Poultry production in Cameroon: How the import restriction affects the Cameroonian poultry sector. Deutsche Gesellschaftfür Internationale Zusammenarbeit (GIZ) GmbH, sector project agricultural trade and value chains, SNRD-Asia, Bangkok, Thailand, p. 12. Available at: https://www.giz.de/de/downloads/GIZ_SVAAA_Policy-Brief-Cameroon-Chicken_EN.pdf

Islam MS, Kazi MA, Hossain MM, Ahsan MA, and Hossain AM (2007). Propagation of heavy metals in poultry feed production in Bangladesh. Bangladesh Journal of Scientific and Industrial Research, 42(4): 465-474. DOI: https://doi.org/10.3329/BJSIR.V42I4.755

Kaya S (2014). Mycotoxins. In: S. Kaya (Editor), Veterinary toxicology, 3rd Edition. Medisan Publisher., Ankara, Turkey. pp. 393-433.

Mokubedi SM, Phoku JZ, Changwa RN, Gbashi SPB, and Njobeh PB (2019). Analysis of mycotoxins contamination in poultry feeds manufactured in selected provinces of South Africa using UHPLG-MS/MS. Toxins, 11(8): 452. DOI: https://www.doi.org/10.3390/toxins11080452

Morrison DM, Ledoux DR, Chester LFB, and Samuels CAN (2017). A limited survey of aflatoxins in poultry feed and feed ingredients in Guyana. Veterinary Sciences, 4(4): 60. DOI: https://www.doi.org/10.3390/vetsci4040060

Nachman KE, graham JP, Price Lb, and Silbergeld KL (2005). Arsenic: A roadblock to potential animal waste management solutions. Environmental Health Perspectives, 113(9): 1123-1124. DOI: https://www.doi.org/10.1289/ehp.7834

Okoye COB, Ibeto CN, and Ihedioha JN (2011). Assessment of heavy metals in chicken feeds sold in south eastern Nigeria. Advances in Applied Science Research, 2(3): 63-68. Available at: https://www.primescholars.com/articles/assessment-of-heavy-metals-in-chicken-feeds-sold-in-south-eastern-nigeria.pdf

Paryad A, and Mahmoudi M (2008). Effect of different levels of supplemental yeast (Saccharomyces cerevisiae) on performance, blood constituents and carcass characteristics of broiler chicks. African Journal of Agricultural Research, 3: 835-842. Available at: https://academicjournals.org/journal/AJAR/article-stat/AD3847E38476

Scientific committee for animal nutrition (SCAN) (2003). Summary record of the 149th scan plenary meeting. Available at: https://ec.europa.eu/food/system/files/2020-12/sci-com_scan-old_sum_out131.pdf

Tatfo Keutchatang FDP, Sandrine IBN, MedouaNG, and Kansci G (2021). Biosecurity practices and characteristics of poultry farms in three regions of Cameroon. Journal of World Poultry Research, 11(1): 64-72. DOI: https://www.doi.org/10.36380/jwpr.2021.9

Tatfo Keutchatang FDP, Tchuenchieu KA, Nguegwouo E, Tene MH, Sandrine IBN, Kansci G, and Medoua NG (2022**).** Occurrence of total aflatoxins, aflatoxin B1, and ochratoxin A in chicken and eggs in some Cameroon urban areas and population dietary exposure. Journal of Environmental and Public Health, 2022: 5541049. DOI: https://www.doi.org/10.1155/2022/5541049

World health organization (WHO) (2011). Influenza at the human-animal interface. Summary and assessment, 5 December 2011. Available at: https://www.who.int/publications/m/item/influenza-at-the-human-animal-interface-summary-and-assessment-5-dec-2011

World health organization (WHO) (2017). What is one health? Available at: https://www.onehealthcommission.org/en/why_one_health/what_is_one_health/

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 89-95, March 25, 2023

Research Paper, PII: S2322455X2300009-13
License: CC BY 4.0

DOI: https://dx.doi.org/10.36380/jwpr.2023.9

Carcass and Internal Organs Characteristics of Broiler Chickens Fed Soybean Diet

Ekeocha Anthony Henry[1*], Aganga Ademiju Adeolu[1], Emerue Patrick Chinedu[2], and Akinsoyinu Oluwadamilola Victoria[1]

[1]*Department of Animal Production and Health, Federal University, Oye-Ekiti, Nigeria*
[2]*Department of Livestock Improvement Management, Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Oyo State, Nigeria*

Corresponding author's E-mail: anthony.ekeocha@fuoye.edu.ng

Received: 07 January. 2023
Accepted: 21 February 2023

ABSTRACT

Soybean (Glycine max) is a principal vegetable protein source in the animal feed industry in Nigeria, including the poultry feed industry, but because of the fact that it contains various anti-nutritional factors, the raw full-fat cannot be used in poultry nutrition. The present study was carried out to examine the carcass, and internal organ characteristics of broiler chickens fed a soybean diet. A total of eight hundred and four unsexed one-day-old broiler chickens (Arbor Acre) with an average weight of 45 ± 1.1 g were used in 56 days feeding trial to observe the effect of different processing methods of soybean on broiler chicken carcass and organs characteristics including; liver, spleen, lungs, crop, bile, pancreas, heart, intestine and empty gizzard. There were four dietary treatments (T1-T4), each containing soybean meal as the control diet, dried-boiled soybean, roasted soybean and dried-fermented soybean. Each treatment was replicated three times with 67 broiler chickens per replicate, giving 201 broiler chickens per treatment. The experiment was arranged in a completely randomized design. The starter diet was fed for 4 weeks and the finisher diet for 4 weeks. At the end of the feeding trial, 15 broiler chickens were randomly selected for sampling and collecting the data. The weight of the internal organs and carcass characteristics showed no significant difference in the treatment groups, but the fermented method showed the highest value in the live weight (2075.00 g), eviscerated weight (1532.46 g), and breast weight (483.72 g) compared to other carcass parameters in other treatments including the control diet. It is, therefore, concluded that for optimal broiler growth, the fermented processing method of soybean is recommended.

Keywords: Broilers chicken, Carcass characteristic, Diet, Internal organ, Processed soybean

INTRODUCTION

Soybean is a major source of protein widely used in the diet of broiler chickens (Guo et al., 2020). However, the use of soybean in young chickens is limited and contraindicated because of the anti-nutritional factors (ANF) and content of trypsin inhibitors (TI) and lectins, oligosaccharides, and phytate (Wu et al. 2020). Extrusion has a positive effect on the level of anti-nutrients (for example, by reducing the activity of trypsin inhibitors); however, it improves the digestibility of protein and starch (Konieczka et al., 2014). Several soybean processing techniques that are aimed at increasing the nutritional quality of soybean and other legumes can be accomplished by methods, such as toasting, cooking, extruding, salt treatment, fermentation, germination pressure cooking, cooking, soaking, urea treatment (Ayanwale, 1999; Okagbare and Akpodiete, 2006; Akande and Fabiyi, 2010). These soybean processing techniques uniquely present different opportunities and challenges in both the nutritional profile and nutrient availability of soybean for utilization by broiler chickens.

Different processing methods are used in processing soybean for their different protein products. Processing methods have an impact on the quality of the products, but it all depends on the method used (Araba and Dale, 1990). The heating process has been identified as the only method that affects the protein quality of soybean. Anti-nutritive factor (trypsin inhibitors and lectins) can be rendered inactive when

heating conditions, such as moisture content, heating temperature and heating time are properly used (Araba and Dale, 1990). Using high heating temperature leads to denaturing of the amino acid and protein content of the soya bean (Hurrell, 1990; Parsons et al., 1992). Therefore, the current study aimed to examine the effect of different soybean processing methods on broiler chicken carcass and internal organs characteristics.

MATERIALS AND METHODS

Ethical approval

The experiment was approved by the Federal University of Oye Ekiti (FUOYE) Faculty of Science Research Ethics Committee (RECOM), and reviewed and considered the submitted research protocol and hereby gives ethical approval (FUOYEFSC 201122-REC2022/006) to carry out the research.

Collection and processing of test ingredients

The experiment was conducted at the Federal University Oye Ekiti, animal production and health department, Ikole campus, Nigeria, located on latitude 7.7979^{o}N and longitude 5.3286^{o}E with bimodal rainfall peaks in July and August at the experimental location. The average ambient temperature during the experiment was 24.2^{o}C with high humidity of over 75%. The soybean seeds (Glycine max, East Asia), were procured from a local market in Ikole, Ekiti State, Nigeria. The collected seeds were cleaned by winnowing and hand-picking of stones and debris and were subjected to three processing methods (Akande and Fabiyi, 2010).

Roasting method

Soybean seeds were grilled in a metal saucepan over firewood. Soybean seeds were constantly and continuously stirred using a stir rod to avoid burning while roasting until a golden brown color was achieved. Soybean seeds were later spread on a concrete floor to cool, then ground into a meal referred to as dried roasted soybean (DRS) meal (Akande and Fabiyi, 2010).

Boiled soybean

Dried soybean seed was cooked by bringing water in a metal pot to a boiling point and pouring it in the boiling water for thirty minutes to produce the cooked full-fat and then sun-dried for 4 days and ground to produce the corresponding full-fat soybean designated as dried, boiled soya bean (DBS) meal (Akande and Fabiyi, 2010).

Fermented soybean

The soya beans seed was cooked in water for 30 minutes, left to cool, and then fermented for 48 hours. The soybean was then sieved, sundried for 4 days, and powdered to make dried fermented soybeans (DFS), (Akande and Fabiyi, 2010).

Management of experimental animals

A total of eight hundred and four one-day-old (804) unsexed broiler chickens (Arbor Acre) with an average weight of 45 ± 1.1 g were sourced from a reputable commercial hatchery at Ikole-Ekiti and were randomly allocated to 4 treatments and 3 replicates of 67 broiler chicks per individual cage of (6.5 x 3.2 x 6) ft. Each cage was adjusted with installed nipple drinks and free access water (*ad libitum*) and was raised on a deep litter system with wood shavings as bedding materials and a controlled environment. The feed was given twice daily in the morning (07.00 a.m.) and afternoon (04.00 p.m.). The treatments used in the research were four dietary treatments (T1-T4) with each diet containing T1 (soybean meal) as control diet, T2 (dried-boiled soybean), T3 (roasted soybean) and T4 (dried-fermented soybean) in a completely randomized design. The investigated parameters include growth performance, carcass and internal organ characteristics. The formulated feed with processed soybean at 33.56% in the starter mash diet and finisher mash diets with processed soybean at 30.57% and nutrient composition in the experimental diet are presented in tables 1 and 2, as the feeding was based on just starter and finisher diet and no grower diet was given. Routine vaccination and standard management practices were carried out (Kalam et al., 2021) and a 17-20 hours lightening schedule was observed, the feeding trial lasted for 56 days.

Carcass yield

At the end of the experiment, 15 broiler chickens were randomly selected from each replicate (45 broiler chickens per treatment). The broiler chickens were starved of feed overnight and then slaughtered by severing the jugular vein with a sharp knife and allowing blood to drain for five minutes. Slaughtered chickens were scalded in hot water (about 50^{o}C) for one minute, then de-feathered and eviscerated manually. The live weights and dressed weights were recorded and the internal organ (liver, kidney, heart, gizzard and intestine) were recorded and expressed as a percentage of live weight. The dressing percentage was calculated as the percent of live weight after bleeding and de-feathering. Eviscerated carcass weight was determined after removing blood, feather, shank, head, heart, liver, gizzard, kidney, lung, pancreas, crop, pro-ventricles, and the intestine.

Statistical analysis

For the statistical analysis, analysis of variance (ANOVA) using a general linear model (GLM) was carried out using SAS software (version 2012) on Demand for Academics (ODA, Cary, NC, USA). Moreover, means of treatment were analyzed using Tukey's honestly significant difference test at 5% probability test.

Table 1. Gross composition of starter and finisher experimental diets for the period of four weeks in broiler chickens

Ingredients (%)	Starter (Days 1-28)				Finisher (Days 29-56)			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Maize	54.96	54.96	54.96	54.96	56.96	56.96	56.96	56.96
Soybean-meal	33.56	-	-	-	30.57	-	-	-
Dried boiled soybean	-	33.56	-	-	-	30.57	-	-
Roasted soybean	-	-	33.56	-	-	-	30.57	-
Dried fermented soybean	-	-	-	33.56	-	-	-	30.57
Wheat offal	8.51	8.51	8.51	8.51	9.15	9.15	9.15	9.15
DCP	1.50	1.50	1.50	1.50	1.70	1.70	1.70	1.70
Limestone	0.30	0.30	0.30	0.30	0.50	0.50	0.50	0.50
Lysine	0.25	0.25	0.25	0.25	0.23	0.23	0.22	0.22
Methionine	0.20	0.20	0.20	0.20	0.18	0.18	0.18	0.18
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Enzyme	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Total	100	100	100	100	100	100	100	100

T1: Commercial soybean meal (control), T2: Dried boiled soybean, T3: Dried roasted soybean, T4: Dried fermented soybean, DCP: Dicalcium phosphate, Premix: Vitamin minerals (methionine, lysine, biotin, niacin, folic acid)

Table 2. Nutrient composition of starter and finisher period of experimental diets fed to broiler chickens

Nutrients	Starter (Days 1-28)				Finisher (Days 29-56)			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
ME (KCAL/KG)	2937	2937	2939	2812	2937	2958	2938	2823
Crude protein (%)	21.5	21.54	21.15	23.8	20.45	20.48	20.12	22.54
Crude fibre (%)	6.00	7.80	5.72	6.50	5.80	8.20	7.10	6.30
Ash content (%)	7.65	5.33	6.85	7.23	7.83	5.69	7.26	7.39
Fat content (%)	1.98	2.07	2.27	2.98	2.15	2.23	2.35	3.15
Moisture content (%)	9.74	10.12	10.29	8.5	8.49	8.34	9.69	8.99

T1: Commercial soybean meal (control), T2: Dried boiled soybean, T3: Dried roasted soybean, T4: Dried fermented soybean, ME: Metabolizable energy

RESULTS AND DISCUSSION

Weight and percentage of carcass parameters

The weights of broiler chickens fed processed soybean diets are presented in Tables 3, and the percentage of broiler chicken carcasses are tabulated in Table 4. The live weights of chicken had no significant difference in the entire treatment group ($p > 0.05$). However, broiler chickens fed diet with fermented soybean had the highest live weight (2075 g) and the lowest (1766.67 g) was recorded in broiler chickens fed with roasted soybean meal. Fermented soybean had the best effect on the slaughter weight (1983.33 g). This may be due to the high level of crude protein that fermented soybean contained (51.85%) and with the lowest recorded in broiler chickens fed roasted soybean meal (1708.33 g). Dressed weight had no significant difference in all the treatment groups ($p > 0.05$), but the lowest was recorded in broiler chickens fed dried roasted soybean meal (1624.32 g). The findings of Abu et al. (2015) in broiler chickens are consistent with this finding of where the dressed weight of the broilers expressed as percentage live weights were similar between treatment groups. The were no significant differences ($p > 0.05$) in the eviscerated and wing weights across the treatment group. However, broiler chickens fed DFS had the highest eviscerated weight (1532.46 g), while those fed dried roasted soybean mean meal had the lowest (1299.13 g). The high eviscerated weight might result from an increase in the bioavailability of nutrients (Hotz and Gibson, 2007) and a decrease in the amounts of anti-nutritional agents (Egounlety and Aworh, 2003). Broiler chickens fed DRS had the heaviest wings (94.53 g), whereas the control group had the lightest wings (80.87 g). This finding is in line with the findings of Roberts et al. (1999), who demonstrated that broiler chickens that were fed roasted soybeans had better performance.

There was a significant difference among the groups regarding the weight of the thighs ($p < 0.05$) except for broiler chickens on a control diet (100.03 g) and those fed a dried boiled soybean diet whose weights were similar (100.80 g). This might be attributed to a better processing method that promotes digestibility. The thigh weight in the fermented soybean group showed a significant difference ($p < 0.05$), having the highest thigh weight of 111.92 g. The weight of thigh and breast, which signifies carcass superiority, was higher in DFS, compared to the weight of thigh and breast of broiler chickens fed DBS and the control diet, while broiler chickens fed roasted soybean had the lowest (87.97 g) thigh weight (Medugu et al., 2010). There was no significant difference ($p > 0.05$) in the weight of the drumstick across the treatment groups and the control except for broiler chickens fed a fermented soybean diet.

There was no significant difference ($p > 0.05$) observed on the chicken's back, neck, shank, and head weight across the treatment group. The feather weight for treatments 1-3 showed no significant difference ($p > 0.05$) except for treatment 4 (DFS), which had the highest weight of 56.73 g, compared to other treatment groups. The chicken back of the broiler chickens was weightier (273.46 g) in the fermented diet group, while the chicken back weight was last recorded in broiler chickens fed boiled and roasted soybean, respectively. There was a significant difference ($p < 0.05$) shown in the breast weight across the treatment group with treatment 4 (DFS) which had the highest (483.72 g), compared with those recorded in broiler chicken fed roasted soybean (582.48 g), boiled Soybean (410.25 g), and commercial soybean meal (399.15 g). Studies have shown that the fermentation method of processing soybean can degrade anti-nutritive and allergenic soybean compounds (Dimidi et al., 2019). The neck was also weightier in birds fed the fermented soybean diet, the control had the best shank weight, and the head was weightier in the roasted and fermented soybean diets, respectively, while the feather was found to have more weight in birds fed the fermented soybean diet.

The percentage of the chicken's back, breast, neck, shank, and head were also similar in all the treatment groups and the diets. The percentage of the feather in the live weight was similar in the control (2.13%), and broiler chicken fed boiled soybean (2.16%); however, significantly different ($p < 0.05$), compared to broiler chickens fed roasted (1.90%) and fermented (2.70%).

Table 3. Weight of carcass parameters of broiler chicken fed processed soybean diets for 56 days

Parameters (g)	T1	T2	T3	T4
Live weight	1791.67 ± 222.30	1816.67 ± 116.90	1766.67 ± 125.17	2075.00 ± 267.86
Slaughter weight	1740.00 ± 223.16	1766.67 ± 116.90	1708.33 ± 131.97	1983.33 ± 294.39
Dressed weight	1669.12 ± 233.12	1695.12 ± 85.58	1624.32 ± 123.01	1875.14 ± 257.37
Eviscerated weight	1330.12 ± 211.18	1371.43 ± 57.01	1299.13 ± 99.23	1532.46 ± 210.13
Wing weight	80.87 ± 10.63	86.54 ± 6.76	94.53 ± 28.89	91.97 ± 7.11
Thigh weight	100.03 ± 13.24^{ab}	100.80 ± 12.96^{ab}	87.97 ± 9.62^{b}	111.92 ± 16.58^{a}
Drumstick weight	103.55 ± 19.11	104.73 ± 9.42	103.15 ± 13.81	103.28 ± 15.07
Back weight	244.86 ± 60.65	233.27 ± 13.54	233.39 ± 31.42	273.46 ± 52.91
Breast weight	399.15 ± 68.16^{ab}	410.25 ± 30.84^{ab}	382.48 ± 35.85^{b}	483.72 ± 87.55^{a}
Neck weight	84.87 ± 18.52	89.37 ± 9.66	80.41 ± 12.37	90.33 ± 19.32
Shank weight	47.39 ± 8.37	44.53 ± 5.07	44.64 ± 7.12	43.29 ± 8.50
Head weight	54.89 ± 4.03	56.91 ± 7.32	59.54 ± 12.51	59.15 ± 10.53
Feather weight	38.22 ± 13.74^{b}	39.26 ± 7.19^{b}	33.45 ± 6.24^{b}	56.73 ± 15.75^{a}

[ab]Different superscripts letters across a row are significantly different from each other at $p < 0.05$. T1: Commercial soybean meal (control), T2: Dried boiled soybean, T3: Dried roasted soybean, T4: Dried fermented soybean

Table 4. Weight of carcass in total live weights of broiler chickens fed processed soybean diets for 56 days

Parameters (%)	T1	T2	T3	T4
Dressed	93.04 ± 2.44	93.38 ± 1.79	91.93 ± 2.27	90.34 ± 2.71
Eviscerated	74.01 ± 1.65	75.59 ± 0.88	73.53 ± 0.77	73.83 ± 0.96
Wing	4.53 ± 0.46	4.76 ± 0.14	5.29 ± 1.17	4.47 ± 0.42
Thigh	5.58 ± 0.21	5.56 ± 0.67	4.98 ± 0.48	5.41 ± 0.57
Drumstick	5.75 ± 0.50^{a}	5.76 ± 0.37^{a}	4.76 ± 0.14	4.99 ± 0.54^{b}
Back	13.52 ± 2.07	12.88 ± 1.06	5.56 ± 0.67	13.16 ± 1.61
Breast	22.20 ± 1.56	22.61 ± 1.53	5.76 ± 0.37^{a}	23.23 ± 2.16
Neck	4.71 ± 0.71	4.94 ± 0.67	4.54 ± 0.54	4.34 ± 0.59
Shank	2.59 ± 0.30	2.46 ± 0.29	2.52 ± 0.30	2.10 ± 0.42
Head	3.08 ± 0.21	3.13 ± 0.29	3.35 ± 053	2.86 ± 0.42
Feather	2.13 ± 0.28^{ab}	2.16 ± 0.13^{ab}	1.90 ± 0.14^{b}	2.70 ± 0.22^{a}

[ab]Different superscript letters across a row are significantly different from each other at $p < 0.05$. T1: Commercial soybean meal (control), T2: Dried boiled soybean, T3: Dried roasted soybean, T4: Dried fermented soybean

Table 5. Weight of internal organs of broiler chickens fed processed soybean diets for 56 days

Parameters (g)	T1	T2	T3	T4
Liver weight	33.34 ± 8.66	29.36 ± 2.68	27.70 ± 3.94	33.33 ± 8.23
Spleen weight	1.45 ± 0.59	1.20 ± 0.26	1.19 ± 0.30	2.24 ± 1.31
Lung weight	8.92 ± 1.87	8.42 ± 1.53	8.87 ± 1.30	9.99 ± 1.69
Heart weight	7.75 ± 1.70	9.36 ± 1.63	7.47 ± 0.57	7.99 ± 0.76
Intestine weight	73.96 ± 6.47	72.66 ± 9.84	70.07 ± 7.37	77.38 ± 23.17
Crop weight	12.79 ± 1.41	13.57 ± 2.25	9.98 ± 2.68	14.45 ± 4.84
Bile weight	2.27 ± 0.56	2.10 ± 1.06	2.66 ± 0.67	3.78 ± 2.26
Pancreas weight	3.09 ± 0.47	2.90 ± 0.51	3.30 ± 0.47	3.52 ± 0.60
Empty Gizzard weight	41.39 ± 4.76	40.18 ± 6.82	38.77 ± 1.37	41.01 ± 3.84

T1: Commercial soybean meal (control), T2: Dried boiled soybean, T3: Dried roasted soybean, T4: Dried fermented soybean

Table 6. Weight of internal organs in live weight of broiler chickens fed processed soybean diets for 56 days

Parameters (%)	T1	T2	T3	T4
Liver	1.85 ± 0.13	1.62 ± 0.09	1.57 ± 0.08	1.59 ± 0.09
Spleen	1.45 ± 0.24	1.20 ± 0.11	1.19 ± 0.12	2.24 ± 0.54
Lung	0.50 ± 0.03	0.46 ± 0.03	0.51 ± 0.03	0.48 ± 0.03
Heart	0.43 ± 0.03^{b}	0.51 ± 0.03^{a}	0.42 ± 0.01^{b}	0.39 ± 0.02^{b}
Intestine	4.14 ± 0.09	3.99 ± 0.13	3.98 ± 0.19	3.71 ± 0.34
Crop	0.72 ± 0.04	0.75 ± 0.05	0.56 ± 0.05	0.70 ± 0.08
Bile	0.15 ± 0.01	0.17 ± 0.02	0.15 ± 0.01	0.18 ± 0.05
Pancreas	0.17 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.17 ± 0.01
Empty Gizzard	2.33 ± 0.14	2.21± 0.14	2.20 ± 0.07	1.99 ± 0.09

^{ab}Different superscript letters across a row are significantly different from each other at $p < 0.05$. T1: Commercial soybean meal (control), T2: Dried boiled soybean, T3: Dried roasted soybean, T4: Dried fermented soybean

Weight and percentage of internal organs

The weights and percentages of the internal organs of birds fed processed soybean diets were measured and presented in tables 5 and 6. The internal organs measured were the liver, spleen, lungs, heart, intestine, crop, bile, pancreas, and empty gizzard and their weights were similar in all the basal diets. The values of the organs are within the range reported by Ayanwale (2006) on processed soybean. The mean liver weight was the highest in chickens fed dried fermented soya bean (33.33 ± 8.66 g) and in control group (33.34 ± 8.66 g). The weights of the spleen, lung, heart, intestine, crop, bile, and pancreas were higher in broiler chickens fed dried fermented soybean. Broiler chickens fed dried roasted soybean had the least weight of spleen, heart, intestine, crop, and empty gizzard, while broiler chickens fed dried boiled soybean had the lowest lung weight (8.42 ± 1.53 g). The percentage weight of the internal organs in the live weight indicated no significant differences in the percentage of the lung, intestine, crop, bile, pancreas, and empty gizzards across the treatment diets and the control ($p > 0.05$; Table 6). The percentage of heart weight in live weight d in broiler chickens fed Dried boiled soybean differ significantly compared to control and other treatment groups ($p < 0.05$; Table 5). The internal organs of broiler chickens fed processed soybean seed show no significant difference across the treatment group ($p > 0.05$).

CONCLUSION

In conclusion, DFS enhanced live weight, eviscerated weight, breast weight and internal organ characteristics of broiler chicken. It should be recommended for poultry farmers consider soybeans as the protein source by attention that the processed soybean had no deleterious effect in the present study.

DECLARATIONS

Acknowledgments

Special thanks go to Mrs. Gode Dakuna for the technical assistance in the field and authors who personally funded the research.

Authors' contribution

Akinsoyinu Oluwadamilola collected the samples and carried out the fieldwork and wrote the first draft. Ekeocha Anthony Henry, Aganga Ademiju Adeolu, Emerue Patrick Chinedu and Akinsoyinu Oluwadamilola Victoria supervised the overall research, and statistical analysis and revised the draft of the manuscript. All authors approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors declare that they have no competing interests.

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 who command respect in Nigeria by the ethically committed monitory team.

Availability of data and materials

All related data of the published article can be available according to a reasonable request from the corresponding author.

REFERENCES

Abu OA, Olaleru IF, and Omojola AB (2015). Carcass characteristics and meat quality of broilers fed cassava peel and leaf meals as a replacement for maize and soyabean meal. Journal of Agriculture and Veterinary Science, 8(3): 41-46. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=2bed7c70a2333fa865c0098c427a016fbe57733a

Akande KE and Fabiyi EF (2010). Effect of processing methods on some antinutritional factors in legume seeds for poultry feeding. International Journal of Poultry Science, *9:* 996-1001. DOI: https://scialert.net/abstract/?doi=ijps.2010.996.1001

Araba M and Dale NM (1990). Evaluation of protein solubility as an indicator of over processing of soyabean meal. Poultry Science, 69(1): 76-83. DOI: https://www.doi.org/10.3382/ps.0690076

Ayanwale BA (1999). Performance and carcass characteristic of broiler chickens fed sodium sesquicarbonate processed soybean diets. Tropical Journal of Animal Science, 2(1): 85-93. Available at: https://www.ajol.info/index.php/tjas/article/view/49653/35982

Ayanwale BA (2006). Growth and characteristics of broiler fed alkali processed Soyabean. Nigerian Journal of Animal Production, 33(1): 40-44. DOI: https://www.doi.org/10.51791/njap.v33i1.2144

Dimidi E, Cox SR, Rossi M, and Whelan K (2019). Fermented foods: Definitions and characteristics, impact on the gut microbiota and effects on gastrointestinal health and disease. Nutrients, 11(8): 1806. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6723656/

Egounlety M and Aworh O (2003). Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max merr.), cowpea (Vigna unguiculata l. Walp) and ground bean (Macrotyloma geocarpa harms). Journal of Food Engineering, 56(2-3): 249-254. DOI: https://www.doi.org/10.1016/S0260-8774(02)00262-5

Guo S, Zhang Y, Cheng Q, Xv J, Hou Y, Wu X, Du E, and Ding B (2020). Partial substitution of fermented soybean meal for soybean meal influences the carcass traits and meat quality of broiler chickens. Animals, 10(2): 225. DOI: https://www.doi.org/10.3390/ani10020225

Hotz C and Gibson R (2007). Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. The Journal of Nutrition, 137(4): 1097-1100. DOI: https://www.doi.org/10.1093/jn/137.4.1097

Hurrell RF (1990). Influence of the Maillard reaction on nutritional value of foods. In: P. A. Finot, H. U. Aschbacher, R. F. Hurrell, and R. Liardon (Editors), The Maillard reaction in foods processing, human nutrition and physiology. pp. 245-258. DOI: https://www.doi.org/10.1007/978-3-0348-9127-1_27

Kalam M, Alim M, Shano S, Khan Nayem R, Badsha R, Al Mamun A, Hoque A, Tanzin AZ, Ali Khan S, Islam A et al. (2021). Knowledge, attitude, and practices on antimicrobial use and antimicrobial resistance among poultry drug and feed sellers in Bangladesh. Veterinary Sciences, 8(6): 111. DOI: https://www.doi.org/10.3390/vetsci8060111

Konieczka P, Nowicka K, Madar M, Taciak M, and Smulikowska S (2018). Effects of pea extrusion and enzyme and probiotic supplementation on performance, microbiota activity and biofilm formation in the broiler gastrointestinal tract. British Poultry Science. 59(6): 654-62.

Konieczka P, Smulikowska S, Czerwinski J, and Mieczkowska A (2014). Raw vs extruded coloured-flower pea as an ingredient in broiler diets: Effects on performance, ileal digestibility, gut morphology, and intestinal microbialactivity. Journal of Animal Feed Science, 23: 244-252. Available at: https://pdfs.semanticscholar.org/e23a/e2ba3c69797c28cc805616245555d2ecc7ea.pdf

Kuźniacka J, Adamski M, Czarnecki R, and Banaszak M (2014). Results of rearing broiler chickens under various systems. Journal of Agricultural Science, 6(4): 19-25. DOI: https://www.doi.org/10.5539/JAS.V6N4p19

Medugu CI, Kwari ID, Igwebuke JU, Nkama I, Mohammed ID, and Hamaker B (2010). Carcass and blood components of broiler chickens fed sorghum or millet as replacement for maize in the semi arid zone of Nigeria. Agriculture and Biology Journal of North America, 1(3): 326-329. Available at: http://www.scihub.org/ABJNA/PDF/2010/3/1-3-326-329.pdf

Okagbare GO and Akpodiete OJ (2006). Soybean in pre ruminant nutrition: A review. Nigeria Journal of Animal Production, 33(1): 83-86. DOI: https://www.doi.org/10.51791/njap.v33i1.2216

Parsons CM, Hashimoto K, Wedekind KJ, and Baker DH (1992). Soybean protein solubility in potassium hydroxide: An *in vitro* test of *in vivo* protein quality. Journal of Animal Science, 69(7): 2918-2924. DOI: https://www.doi.org/10.2527/1991.6972918x

Qin G, Ter Elst ER, Bosch MW, and Vander Poel AFB (1996). Thermal processing of whole soyabean: Studies inactivation of anti-nutritional factors and effect on lead digestibility in piglets. Animal Feed Science Technology, 57(4): 313-324. DOI: https://www.doi.org/10.1016/0377-8401(95)00863-2

Roberts R K, Pendergrass R, and Hayes R M (1999). Economic analysis of alternative herbicide regimes on Roundup Ready soybeans. Journal of Production Agriculture, 12(3), 449-454. DOI: https://www.doi.org/10.2134/jpa1999.0449

Wu P, Golly MK, Guo Y, Ma H, He R, Luo X, Luo S, Zhang Ch, Zhang L, and Zhu J (2020). Effect of partial replacement of soybean meal with high-temperature fermented soybean meal in antibiotic-growth-promoter-free diets on growth performance, organ weights, serum indexes, intestinal flora and histomorphology of broiler chickens. Animal Feed Science and Technology, 269: 114616. DOI: http://www.doi.org/doi:10.1016/j.anifeedsci.2020.114616

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 96-102, March 25, 2023

Research Paper, PII: S2322455X2300010-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.10

Investigating the Preventive Effect of Herbal Medicine (*Allium sativum*, *Artemisia annua*, and *Quercus infectoria*) against Coccidiosis in Broiler Chickens

Abolfazl Ghaniei, Seyed Ali Ghafouri*, Soheil Sadr, and Negar Hassanbeigi

Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Corresponding author's E-mail: saghafouri@um.ac.ir

Received: 21 December 2022
Accepted: 16 February 2023

ABSTRACT

Coccidiosis is a critical disease in the poultry industry worldwide. Producers apply different strategies to control and prevent this disease. Herbal drugs are suitable remedies for reducing losses associated with coccidiosis in poultry. The present study aimed to evaluate the effectiveness of an herbal mixture in preventing coccidiosis. A total of 160 broiler chickens were divided into four treatment groups, with five replicates for each. Experimental infection of all groups, except group D, was carried out with mixed *Eimeria* species (*E. tenella*, *E. necatrix*, *E. brunetti*, and *E. maxima*) on day 14. Broiler chickens in group A were given an herbal mixture (75% *Quercus infectoria*, 16% *Artemisia annua*, and 9% *Allium sativum*) as feed additives during the rearing period, and group B was treated with Monensin. No treatment was applied to group C after the chickens were experimentally infected with mixed *Eimeria* spp. Group D was used as the negative control since chickens in this group were not infected or sick during the experiment. Body weight gain, feed conversion ratio (FCR), mortality rate, intestinal lesion scoring, and oocyst count per gram (OPG) were evaluated in this study. The results of the present study revealed that the highest mean body weight was gained in group D, followed by chickens in group A. The best FCR results were attributed to chickens in group D, followed by group B. In this study, both drugs decreased mortality rate, intestinal lesion scores, and OPG in the treated chickens. In conclusion, this herbal mixture can reduce coccidial lesions.

Keywords: Broiler, Coccidiosis, Herbal mixture, Prevention

INTRODUCTION

Avian coccidiosis is an infectious parasitic disease of the intestinal tract caused by a protozoan of the genus *Eimeria*, including (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*; Abd El-Hack et al., 2021). Coccidiosis results in mortality, poor growth, impaired performance, and increased susceptibility to secondary infections (De Gussem, 2007). World poultry production continues to be negatively affected by this parasitic condition (Noack et al., 2019). Residual effects and increased resistance are associated with conventional anticoccidials. The development of vaccines against poultry coccidiosis has benefited technology and immunology, but they still need to meet farmers' expectations as effective, safe, and economical (Alfaro et al., 2007; Kim and Lillehoj, 2019). Due to resistance, consumer concerns, increased regulations, and the possibility of a ban on anticoccidial drugs as feed additives, alternative control strategies are needed. (Acharya and Acharya, 2017). Numerous studies indicated that natural products could be an effective means to combat coccidiosis (Brisibe et al., 2008; Idris et al., 2017). There is evidence that biologically active compounds from multiple plant types are effective alternatives to conventional methods of controlling coccidiosis (Peek and Landman, 2011). Plant extracts contain natural compounds containing metabolites capable of inhibiting the life cycles of various *Eimeria* species (Cobaxin-Cárdenas, 2018). Artemisinin and its derivatives in the *Artemisia annua* were shown to be gametocytocidal and reduced the transmission potential of falciparum malaria (Willcox et al., 2004). Several studies have tried to investigate the efficacy of this herb against *Eimeria* parasites, and the results were promising (del Cacho et al., 2010; Dragan et

al., 2010; Jiao et al., 2018). Artemisinin induces oxidative stress in parasite cells and directly inhibits sporulation and cell wall formation in Eimeria species (Muthamilselvan et al., 2016). *Quercus infectoria* has medicinal effects such as anti-inflammatory, astringent, antidiabetic, antimicrobial, and gastroprotective. *Quercus infectoria* anti-parasitic activity against *Entamoeba histolytica, Blastocystis hominis, and Leishmania major* was studied before (Thangavel et al., 2020). It contains compounds such as gallic acid and its derivative, gallotannins and hydrolyzable tannins, which have been found to control coccidiosis in poultry (Arina and Harisun, 2019). *Allium sativum* (*A. sativum*) has been utilized as a spice and a native drug for years. It includes antifungal, antibacterial, antiparasitic, antiviral, antioxidant, anti-thrombotic, anti-cancerous, anticholesteremic, and vasodilator effects (Ogbuewu et al., 2019). *Allium sativum*, often used in alternative medicine, has attracted immense interest in the medical literature (Ibrahim et al., 2021). The *A. sativum* holds the highest concentration of sulfur, such as allicin, and other biological activities (Waqas et al., 2018). Thiosulfate allicin has effective antiprotozoal activity, and other non-sulfur components, including saponins, proteins, and phenolic compounds, can also be helpfull with its antiparasitic effects (Al-Massad et al., 2018).

An herbal formulation, including extracts of *Allium Sativum, Artemisia Annua,* and *Quercus infectoria,* was evaluated based on a comparative model to determine its effectiveness as an anticoccidial agent in infected broiler chickens.

METHODS AND MATERIALS

Ethical approval

Guidelines for animal care and use were followed at all international, national, and institutional levels (IR.UM.REC.1400.217). This experiment was performed following the guidelines approved by the Animal Ethics Committee of the Ferdowsi University of Mashhad.

Study design

A total of 160 day-old Ross 308 broiler chickens were purchased and reared under standard management practice with free access to feed (Table 1) and water in a specialized clinic of the Faculty of the Veterinary Medicine of the Ferdowsi University of Mashhad, Iran. All broilers were raised in floor pens and placed in cages after 14 days (Four broiler chickens in each cage). During the first week of the trial, the temperature was kept at 33°C, gradually decreased to 21°C on day 21, and remained constant until the end of the clinical trial.

Table 1. The diet and chemical analysis of broiler chickens during 45 days of study

Ingredient	Weight (Kg)
Corn	604
Wheat	35
Soy	315
NaCl	2
Carbonate	8
Diphosphate	8
Oil	15
Liquid methionine	2.2
Lysine	1
Choline	1.5
Cocci Phyt	1
Supplement	5
Phytase	0.1
Bicarbonate	2
Multienzyme	0.05
Threonine	0.15
Total	1000 kg
Pr	19.00%
Ca	0.78%
P	0.43%
MET	0.43%
LYS	1.00%
Na	0.18%
Cl	0.17%
K	0.83%
Energy	3012 kcal

Parasite

A mixture of sporulated oocysts of *Eimeria spp.* was purchased from the Faculty of Veterinary Medicine, University of Tehran, Iran. Microscopic analysis showed that the suspension contains 50% *E. tenella*, 25% *E. maxima*, and 25% other species, including *E. acervulina* and *E. necatrix*. On day 14 of age, each broiler in groups A, B, and C were challenged via oral gavage with 200,000 sporulated oocysts of the prepared *Eimeria* spp.

Grouping and treatment

At the same time as the chickens got sick, the broilers were moved to cages following the completion of the second week and randomly gathered into groups. Four groups were isolated, with five repeats included eight chickens in each replicate. Herbal mixtures (Cocci Phyt®, Iran) are given to group A. It contains 75% *Quercus infectoria* with a minimum of 30% total tannin, 16%

Artemisia annua with a minimum of 0.02% artemisinin, and 9% *Allium sativum* with a minimum of 0.4% total phenol contents. Group B was treated with Monensin. Group A received an herbal mixture and group B received Monensin at the daily dose of 1 kg/ton of feed from the first day of age. Group C did not receive any treatment. Group D was not infected experimentally, and the chickens were clinically healthy during the experiment.

Weighing chickens, measuring feed consumption, and calculating the feed conversion ratio

All broiler chickens were weighed individually on the experiment's first day and weekly until day 42. The daily feed consumption of each replicate was recorded after the challenge weekly, and the feed conversion ratio was calculated at the end of study as the proportion of average feed intake to average daily gain.

Clinical signs and casualties

All broiler chickens were monitored for clinical manifestations of coccidiosis, including reduced daily feed intake, lethargy, depression, feces status, and recumbency. Also, mortality rates throughout the experiment were recorded.

Oocyst count

To assess coccidial oocyst shed by chickens, oocyst per gram of feces (OPG) was counted using the McMaster method on days 5, 7, 9, and 11 after the challenge (Haug et al., 2006).

Lesion score

On day seven after the challenge, lesion scoring was performed using the method of Johnson and Reid (1970). For this reason, three broiler chickens from each replicate were slaughtered and evaluated for gastrointestinal lesions at the end of study period. Because the ingested *Eimeria* solution mostly contained *Eimeria tenella*, the cecum was selected for the autopsy and scoring lesions. The scoring system ranged from 0 to 4, in which 4 indicates the most damage to the intestinal. To get a score of zero, the chickens had to be in the best possible health without lesions.

Statistical analysis

The data relating to body weight of 42-day-old, FCR, and OPG were processed using ANOVA, coupled with the Tukey test (SPSS ® Software version 16). P-value < 0.05 was considered statistically significant for all the analyzed data.

RESULTS

Weight gain

The mean body mass gain during the rearing period and feed conversion ratio are shown in (Table 2). As expected, the highest weights were observed in group D, which did not get infected. There was no statistical difference between the mean body weights of chickens in groups A and B up to 35 days of age. Still, the mean was significantly higher in the herbal mixture-treated group at the end of the experiment on day 42 ($p < 0.05$). The feed conversion ratio (FCR) was significantly lower in groups A, B, and D compared to group C ($p < 0.05$), but no difference was observed between treated groups ($p > 0.05$).

Clinical signs

The broiler chickens were perfectly healthy, and the feces consistency was standard on the day of the challenge. Two-day post challenge (DPC), blood clots were seen in the broiler chickens' feces, but in terms of clinical signs and appetite, the broilers' condition was normal. Bleeding in feces increased gradually for 2 days, and it was seen as bloody diarrhea finally. Bloody diarrhea and lethargy were observed in broilers in three DPC. Nevertheless, still, almost a third of the broiler chickens were clinically healthy. Reduced feed intake was observed on the fifth DPC and returned to normal on the 9th DPC. Most clinical signs and bloody diarrhea in chickens were improved in treated groups on the 9th DPC.

Mortality rate

Mortality rates in treated groups were identical, and the highest rate was related to the untreated challenged group (Table 3). Present results indicated that preventing coccidiosis could decrease mortality.

Oocyst count

Oocyst count was significantly lower in treated groups ($p < 0.05$), and no significant difference was seen between these groups ($p > 0.05$). The herbal mixture has been observed to reduce oocysts per gram of feces, as determined by counting the number of oocysts per gram of feces. A sharp decrease in fecal oocysts is observed in treated groups after 11 days, which is essential to stop parasite shedding in infected chickens.

Lesion score

The results showed no lesion in the intestines of group D. This group D scored zero, representing a healthy

gut. In group C, the majority of injuries, like hemorrhages and ulcers, were observed, as would be expected. Notably, the intestinal lesions score decreased in group A compared to group B, which showed perfect performance of the herbal mixture.

Table 2. The mean body weight gains and feed conversion ratio of broiler chickens, before and after challenged with *Eimeria* spp.

Average weight of group	Age (Day)							FCR
	1*	7*	14*	21**	28**	35**	42**	
A (g)	43.19±1.58	115±2.58	238.2±5.20	303.2±8.18	739.4±14.58	1403±20.15	2194±31.62^{a}	2.65±0.16^{a}
B (g)	44.5±1.58	113±2.76	271.6±5.16	489.4±12.23	850.8±15.27	1327±18.79	2010±30.45^{b}	2.55±0.15^{a}
C (g)	46.7±1.58	149±3.57	309±6.14	454.2±10.45	796.5±13.78	1202±17.89	1871±29.67^{b}	2.92±0.18^{b}
D (g)	46.7±1.58	149±3.57	316±6.32	524.4±14.31	916.8±15.69	1482±21.70	2214±35.50^{a}	2.46±0.14^{a}

A: Herbal mixture treated group. B: Monensin treated group. C: Untreated group. D: Unchallenged group. Values with different superscripts in a column differ significantly ($p < 0.05$). *: Days 1, 7, and 14 are before challenging broiler chickens with *Eimeria* spp. **: Days 21, 28, 35, and 42 are after challenging broiler chickens with *Eimeria* spp.

Table 3. Oocyst per gram of feces, lesion score, and mortality rate of broiler chickens after challenging with *Eimeria* spp.

Groups	OPG (DPC)				Mean score of lesion	Mortality (%)
	5	7	9	11		
A	530±16^{a}	934±11^{a}	1080±16^{a}	505±4^{a}	1.1±0.6^{b}	3
B	550±40^{a}	1048±81^{a}	950±36^{a}	475±20^{a}	0.8±0.4^{ab}	3
C	15600±816^{b}	17000±1061^{b}	17500±816^{b}	12340±277^{b}	2.6±.08^{c}	10
D	N	N	N	N	N	0

A: Cocci Phyt® treated and challenged group. B: Monensin treated and challenged group. C: Untreated and challenged group. D: Untreated and unchallenged group. Values with different superscripts in a column differ significantly ($p < 0.05$). N: Not seen; OPG: Oocyst per gram; DPC: Day post challenge.

DISCUSSION

Since 2006, the prophylactic use of anticoccidial chemicals as feed additives has been strictly limited in European countries. In this context, scientists examined natural compounds as potential anti-coccidiosis agents daily (Tewari and Maharana, 2011). Coccidial infections are associated with reduced weight gain and poor feed conversion ratio (Lillehoj and Lillehoj, 2000; Abebe and Gugsa, 2018). The findings showed that herbal mixtures could improve weight gain and feed conversion ratio (FCR), as no significant differences exist between treated and unchallenged groups. Furthermore, the highest mean weight gain in challenged groups was related to the herbal treatment group. Previous studies indicated the potential of *Artemisia annua* leaves in better weight gain and feed consumption due to high levels of crude protein, amino acids, vitamins, minerals, flavonoids, and antioxidants, which resulted (Brisibe et al., 2008; Brisibe et al., 2009). In addition, these compounds could help birds maintain their commensal microflora, enhancing digestion and absorption and improving immune response (Septembre-Malaterre et al., 2020; Ekiert et al., 2021). Furthermore, the beneficial effects of *Allium sativum* on poultry performance have been shown in several studies (Navidshad et al., 2018; Ogbuewu et al., 2019). *Allium sativum* could improve feed conversion ratio (FCR) by modifying small intestine morphology in broiler chickens (Lee et al., 2016). There has been some evidence that tannins promote growth in monogastric animals by balancing their adverse effects on feed palatability and nutrient digestion with their antimicrobial, antioxidant, and anti-inflammatory activities and promoting the health status of an intestinal ecosystem (Starčević et al., 2015; Huang et al., 2018). Immune responses during infection cause free radical production as a defense mechanism against *Eimeria* parasites; however, it damages host cells (Masood et al., 2013). The antioxidant capacity of *A. annua*, *A. sativum*, and tannins could alleviate detrimental effects of oxidative stress in the gut and thus improve animal performance during coccidiosis in broiler chickens (Chung, 2006; Nahed et al., 2022). In the current study, the efficiency of herbal mixtures in reducing oocyst shedding and lesion score was comparable to Monensin, a widely used coccidiosis drug. Herbal formulation reduced oocysts shed in feces compared to the control group. These results align with the previous finding about the anticoccidial effects of *Artemisia annua* on broiler and layer chickens (Allen et al., 1997; Quiroz-Castañeda and Dantán-González, 2015; Muthamilselvan et al., 2016). The

A. annua anticoccidial activity was attributed to artemisinin, a sesquiterpene lactone containing an endoperoxide bridge (Acharya and Acharya, 2017). Del Cacho et al. (2010) reported a lower oocyst sporulation rate of *E. tenella* in chickens treated with pure artemisinin due to inhibition of sarcoplasmic–endoplasmic reticulum calcium ATPase (SERCA) expression in macrogametes (del Cacho et al., 2010). The SERCA plays a role in calcium homeostasis and affects the secretion of wall-forming bodies (Dragan et al., 2014). The inhibition effect of artemisinin on SERCA was shown in *Toxoplasma gondii* previously (Nagamune et al., 2007). Also, artemisinin facilitates the apoptosis of infected host cells and inhibits the inflammatory response against *E. tenella* infection (Jiao et al., 2018). There is little information about the anticoccidial effects of hydrolyzable tannins. In Tosi et al. (2013) study, chestnut hydrolyzable tannins could reduce *Clostridium perfringens* in the gut of broiler chickens challenged with *E. tenella*, *E. acervulina*, and *E. maxima* (Tosi et al., 2013). The anticoccidial activity of *A. sativum* was attributed to allicin. Allicin was shown to inhibit *E. tenella* replication *in vitro* (Alnassan et al., 2015). Various thiol-containing enzymes in microorganisms are inhibited by allicin when it interacts with the SH group on cysteine residues.

CONCLUSION

In conclusion, herbal drug containing *Artemisia Annua*, *Quercus infectoria*, and *Allium Sativum* could improve performance and have anti-coccidiosis effects on the broiler chickens challenged by *Eimeria* spp. To determine how effective this drug is for controlling coccidiosis in breeders, turkeys, and layer chickens, more research is needed, especially for drug residue in meat. It may be possible to develop new anticoccidial drugs by extracting active ingredients of *Allium sativum*, *Artemisia annua*, and *Quercus infectoria*.

DECLARATIONS

Acknowledgments

In appreciation of prepared supports, the authors would like to extend their thanks to the deputy research director at the Ferdowsi University of Mashhad. The authors would like to thank Amir Ali Amiri and Behnoush Dianat (Makian Dam Pars Science-Based Company, Tehran, Iran) for providing us herbal mixture.

Funding

No funding was received for conducting this study.

Authors' contributions

Seyed Ali Ghafouri contributed to the conceptualization, methodology, formal analysis, writing, and editing the original draft. Abolfazl Ghanei took part in the methodology, formal analysis, writing, and editing the original draft. Soheil Sadr contributed to the methodology, formal analysis, writing and editing the original draft. Negar Hassanbeigi participated in the research design, and writing the original draft. Ali Ghafouri supervised the whole study. All authors checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors confirm that there was no conflict of interest with any financial, personal, or other relationships with other people or organizations related to this paper

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 the authors.

Availability of data and materials

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

REFERENCES

Abd El-Hack ME, Alaidaroos BA, Farsi RM, Abou-Kassem DE, El-Saadony MT, Saad AM, Shafi ME, Albaqami NM, Taha AE, and Ashour EA (2021). Impacts of supplementing broiler diets with biological curcumin, zinc nanoparticles and Bacillus licheniformis on growth, carcass traits, blood indices, meat quality and cecal microbial load. Animals, 11(7): 1878. DOI: https://www.doi.org/10.3390%2Fani11071878

Abebe E and Gugsa G (2018). A review on poultry coccidiosis. Abyssinia Journal of Science and Technology, 3(1): 1-12. Available at: https://abjol.org.et/index.php/ajst/article/view/76

Acharya K and Acharya N (2017). Alternatives to fight against coccidiosis: A review. Nepalese Veterinary Journal, 34: 152-167. Available at: https://www.nepjol.info/index.php/nvj/article/download/22918/19495/

Al-Massad M, Al-Ramamneh D, Al-Sharafat A, Abdelqader A, and Hussain N (2018). Effect of using garlic on the economical and physiological characteristics of broiler chickens. Russian Agricultural Sciences, 44(3): 276-281. Available at: https://link.springer.com/article/10.3103/S10683674180300

96

Alfaro D, Silva A, Borges S, Maiorka F, Vargas S, and Santin E (2007). Use of Yucca schidigera extract in broiler diets and its effects on performance results obtained with different coccidiosis control methods. Journal of Applied Poultry Research, 16(2): 248-254. DOI: https://www.doi.org/10.1093/japr/16.2.248

Allen PC, Lydon J, and Danforth HD (1997). Effects of components of Artemisia annua on coccidia infections in chickens. Poultry Science, 76(8): 1156-1163. DOI: https://www.doi.org/10.1093/ps/76.8.1156

Alnassan AA, Thabet A, Daugschies A, and Bangoura B (2015). *In vitro* efficacy of allicin on chicken *Eimeria tenella* sporozoites. Parasitology Research, 114(10): 3913-3915. Available at: https://link.springer.com/article/10.1007/s00436-015-4637-2

Arina MI and Harisun Y (2019). Effect of extraction temperatures on tannin content and antioxidant activity of *Quercus infectoria* (Manjakani). Biocatalysis and Agricultural Biotechnology, 19: 101104. DOI: https://www.doi.org/10.1016/j.bcab.2019.101104

Brisibe EA, Umoren UE, Brisibe F, Magalhäes PM, Ferreira JF, Luthria D, Wu X, and Prior RL (2009). Nutritional characterisation and antioxidant capacity of different tissues of Artemisia annua L. Food Chemistry, 115(4): 1240-1246. DOI: https://www.doi.org/10.1016/j.foodchem.2009.01.033

Brisibe EA, Umoren UE, Owai PU, and Brisibe F (2008). Dietary inclusion of dried Artemisia annua leaves for management of coccidiosis and growth enhancement in chickens. African Journal of Biotechnology, 7(22): 4083-4092. Available at: https://www.ajol.info/index.php/ajb/article/view/59520

Chung LY (2006). The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. Journal of Medicinal Food, 9(2): 205-213. DOI: https://www.doi.org/10.1089/jmf.2006.9.205

Cobaxin-Cárdenas ME (2018). Natural compounds as an alternative to control farm diseases: Avian coccidiosis. Farm animals diseases, recent omic trends and new strategies of treatment. pp. 135-149. DOI: https://www.doi.org/10.5772/intechopen.72638

de Gussem M (2007). Coccidiosis in poultry: Review on diagnosis, control, prevention and interaction with overall gut health. Proceedings of the 16th European Symposium on Poultry Nutrition. pp. 253 - 261. Available at: https://asset-pdf.scinapse.io/prod/1578533817/1578533817.pdf

del Cacho E, Gallego M, Francesch M, Quílez J, and Sánchez-Acedo C (2010). Effect of artemisinin on oocyst wall formation and sporulation during *Eimeria tenella* infection. Parasitology International, 59(4): 506-511. DOI: https://www.doi.org/10.1016/j.parint.2010.04.001

Dragan L, Gyorke A, Ferreira JF, Pop IA, Dunca I, Dragan M, Mircean V, Dan I, and Cozma V (2014). Effects of Artemisia annua and Foeniculum vulgare on chickens highly infected with *Eimeria tenella* (Phylum Apicomplexa). Acta Veterinaria Scandinavica, 56: 22. Available at: https://actavetscand.biomedcentral.com/articles/10.1186/1751-0147-56-22

Dragan L, Titilincu A, Dan I, Dunca I, Dragan M, and Mircean V (2010). Effects of Artemisia annua and Pimpinella anisum on *Eimeria tenella* (Phylum Apicomplexa) low infection in chickens. Scientia Parasitologica, 11(2): 77-82. Available at: http://www.zooparaz.net/scientia/2010_11_02/sp2010-pp077-082%20-%20Dragan.pdf

Ekiert H, Świątkowska J, Klin P, Rzepiela A, and Szopa A (2021). Artemisia annua–importance in traditional medicine and current state of knowledge on the chemistry, biological activity and possible applications. Planta Medica, 87(08): 584-599. DOI: https://www.doi.org/10.1055/a-1345-9528

Huang Q, Liu X, Zhao G, Hu T, and Wang Y (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Animal Nutrition, 4(2): 137-150. DOI: https://www.doi.org/10.1016/j.aninu.2017.09.004

Haug A, Williams RB, and Larsen S (2006). Counting coccidial oocysts in chicken faeces: A comparative study of a standard McMaster technique and a new rapid method. Veterinary Parasitology, 136(3-4): 233-242. DOI: https://www.doi.org/10.1016/j.vetpar.2005.11.024

Ibrahim D, Ismail TA, Khalifa E, Abd El-Kader SA, Mohamed DI, Mohamed DT, Shahin SE, and Abd El-Hamid M I (2021). Supplementing garlic nanohydrogel optimized growth, gastrointestinal integrity and economics and ameliorated necrotic enteritis in broiler chickens using a clostridium perfringens challenge model. Animals, 11(7): 2027. DOI: https://www.doi.org/10.3390/ani11072027

Idris M, Abbas R, Masood S, Rehman T, Farooq U, Babar W, Hussain R, Raza A, and Riaz U (2017). The potential of antioxidant rich essential oils against avian coccidiosis. World's Poultry Science Journal, 73(1): 89-104. DOI: https://www.doi.org/10.1017/S0043933916000787

Jiao J, Yang Y, Liu M, Li J, Cui Y, Yin S, and Tao J (2018). Artemisinin and Artemisia annua leaves alleviate *Eimeria tenella* infection by facilitating apoptosis of host cells and suppressing inflammatory response. Veterinary Parasitology, 254: 172-177. DOI: https://www.doi.org/10.1016/j.vetpar.2018.03.017

Johnson J and Reid WM (1970). Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Experimental Parasitology, 28(1): 30-36. DOI: https://www.doi.org/10.1016/0014-4894(70)90063-9

Kim WH and Lillehoj HS (2019). Immunity, immunomodulation, and antibiotic alternatives to maximize the genetic potential of poultry for growth and disease response. Animal Feed Science and Technology, 250: 41-50. DOI: https://www.doi.org/10.1016/j.anifeedsci.2018.09.016

Lee K, Lee K, Kim G, Kim J, Yeon J, Cho S, Chang B, and Kim S (2016). Effects of dietary fermented garlic on the growth performance, relative organ weights, intestinal morphology, cecal microflora and serum characteristics of broiler chickens. Brazilian Journal of Poultry Science, 18(3): 511-518. DOI: https://www.doi.org/10.1590/1806-9061-2016-0242

Lillehoj HS and Lillehoj EP (2000). Avian coccidiosis. A review

of acquired intestinal immunity and vaccination strategies. Avian Diseases, 44(2): 408-425. DOI: https://www.doi.org/10.2307/1592556

Masood S, Abbas RZ, Iqbal Z, Mansoor M, Sindhu Z, Zia M, and Khan JA (2013). Role of natural antioxidants for the control of coccidiosis in poultry. Pakistan Veterinary Journal, 33(4): 401-407. Available at: http://pvj.com.pk/pdf-files/33_4/401-407.pdf

Muthamilselvan T, Kuo TF, Wu YC, and Yang WC (2016). Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. Evidence-Based Complementary and Alternative Medicine, 2016: 2657981. DOI: https://www.doi.org/10.1155/2016/2657981

Nagamune K, Beatty WL, and Sibley LD (2007). Artemisinin induces calcium-dependent protein secretion in the protozoan parasite Toxoplasma gondii. Eukaryotic Cell, 6(11): 2147-2156. DOI: https://www.doi.org/10.1128/EC.00262-07

Nahed A, Abd El-Hack ME, Albaqami NM, Khafaga AF, Taha AE, Swelum AA, El-Saadony MT, Salem HM, El-Tahan AM, and AbuQamar SF et al. (2022). Phytochemical control of poultry coccidiosis: A review. Poultry science, 101(1): 101542. DOI: https://www.doi.org/10.1016/j.psj.2021.101542

Navidshad B, Darabighane B, and Malecky M (2018). Garlic: An alternative to antibiotics in poultry production, a review. Iranian Journal of Applied Animal Science, 8(1): 9-17. Available at: https://ijas.rasht.iau.ir/article_538739_10bf7af3d2370e37f6f2a07eb1988470.pdf

Noack S, Chapman HD, and Selzer PM (2019). Anticoccidial drugs of the livestock industry. Parasitology Research, 118(7): 2009-2026. DOI: https://www.doi.org/10.1007/s00436-019-06343-5

Ogbuewu I, Okoro V, Mbajiorgu E, and Mbajiorgu C (2019). Beneficial effects of garlic in livestock and poultry nutrition: A review. Agricultural Research, 8(4): 411-426. DOI: https://www.doi.org/10.1007/s40003-018-0390-y

Peek H and Landman W (2011). Coccidiosis in poultry: Anticoccidial products, vaccines and other prevention strategies. Veterinary Quarterly, 31(3): 143-161. DOI: https://www.doi.org/10.1080/01652176.2011.605247

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

Septembre-Malaterre A, Lalarizo Rakoto M, Marodon C, Bedoui Y, Nakab J, Simon E, Hoarau L, Savriama S, Strasberg D, Guiraud P et al. (2020). Artemisia annua, a traditional plant brought to light. International Journal of Molecular Sciences, 21(14): 4986. DOI: https://www.doi.org/10.3390/ijms21144986

Starčević K, Krstulović L, Brozić D, Maurić M, Stojević Z, Mikulec Ž, Bajić M, and Mašek T (2015). Production performance, meat composition and oxidative susceptibility in broiler chicken fed with different phenolic compounds. Journal of The Science of Food and Agriculture, 95(6): 1172-1178. DOI: https://www.doi.org/10.1002/jsfa.6805

Tewari A and Maharana B (2011). Control of poultry coccidiosis: Changing trends. Journal of Parasitic Diseases, 35(1): 10-17. DOI: https://www.doi.org/10.1007/s12639-011-0034-7

Thangavel G, Mukkalil R, and Chirakkal H (2020). Plant parts and extracts having anticoccidial activity. United States Patents, pp. 1-36. Available at: https://patentimages.storage.googleapis.com/9f/e5/f4/b57b87bd50a764/US10568923.pdf

Tosi G, Massi P, Antongiovanni M, Buccioni A, Minieri S, Marenchino L, and Mele M (2013). Efficacy test of a hydrolysable tannin extract against necrotic enteritis in challenged broiler chickens. Italian Journal of Animal Science, 12(3): e62. DOI: https://www.doi.org/10.4081/ijas.2013.e62

Waqas M, Akhtar R, Akbar H, Lateef M, Rashid I, and Ijaz M (2018). Evaluation of anticoccidial activity of different extraction products of allium sativum (garlic) in broilers. Journal of The Hellenic Veterinary Medical Society, 69(3): 1055-1058. DOI: https://www.doi.org/10.12681/jhvms.1887

Willcox M, Bodeker G, Bourdy G, Dhingra V, Falquet J, Ferreira JF, Graz B, Hirt HM, Hsu E, and de Magalhães PM et al. (2004). Artemisia annua as a traditional herbal antimalarial. Traditional Medicinal Plants and Malaria, 4: 43-59. Available at: https://lavierebelle.org/IMG/pdf/20196_annua_as_a_traditional_herbal_antimalarial.pdf

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 103-111, March 25, 2023

Research Paper, PII: S2322455X2300011-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.11

Processing of *Sargassum binderi* Seaweed for Supplementation in Poultry Diet

Yelsi Listiana Dewi[1], Ahadiyah Yuniza[2], Nuraini[2], Kesuma Sayuti[3], and Maria Endo Mahata[2*]

[1]*Department of Livestock Breeding, Faculty of Military Logistics, Indonesia Defense University, Belu, Indonesia*
[2]*Department of Animal Nutrition and Technology, Faculty of Animal Science Andalas University, Padang, Indonesia*
[3]*Department of Agricultural Technology, Faculty of Agricultural Technology, Andalas University, Padang, Indonesia*

*Corresponding author's Email: maria@ansci.unand.ac.id

Received: 18 November 2022
Accepted: 06 January 2023

ABSTRACT

Sargassum binderi has been potentially used as laying hen feed since it contains bioactive compounds useful for poultry health. In addition, the high alginate content of *S. binderi* has made it inappropriate for the poultry diet. Therefore, the alginate content should be reduced before its use in poultry feed. This study aimed to reduce the alginate of *S. binderi* for use as laying hen feed. The experiment was performed in two phases in a completely randomized design. The first phase included heated *S. binderi* in the autoclave and the second phase entailed the immersion of *S. binderi* in whiting filtrate. The treatments in the physical method contained a control group, and four treatment groups heating for 15, 30, 45, and 60 minutes. The treatments in the chemical method had a control group and four treatment groups with immersion periods of 1, 2, 3, and 4 hours. Each treatment was repeated five times, and the investigated parameters were crude protein, total dry matter, organic matter, ash, and alginate, respectively. The heating durations of *S. binderi* in an autoclave and different immersion periods of *S. binderi* in whiting filtrate did not significantly affect total dry matter, organic matter, ash, alginate, and crude protein. The results of this study showed that physical treatment (heat treatment) and chemical treatments (whiting filtrate immersion) did not have a significant effect on the alginate content, crude protein, ash, dry matter, and organic matter.

Keywords: Alginate, Heating, Laying hen, *Sargassum binderi*, Whiting filtrate

INTRODUCTION

As a tropical region, Indonesian waters have seaweed germplasm resources of 6.42% of the world's total seaweed biodiversity (Nofriya, 2015) with an area of 12.123.383 ha, which is the habitat of seaweed or the largest in the world (Kementerian Kelautan dan Perikanan, 2020). The total seaweed production in Indonesia reached 9.746.946 tons (Fu, 2021). Seaweed is a marine resource that can be developed as a non-conventional feed ingredient for poultry. According to Jacob (2022) and Morais et al. (2020), seaweed can be used as animal feed.

Advantages of seaweed are vitamin, mineral, fiber, and bioactive compound source (Mahata et al., 2015; Reski et al., 2022), sea instead of arable land-based production, and high productivity in terms of biomass produced per unit of surface area (Buschmann et al., 2017). According to Mahata et al. (2015), *S. binderi* seaweed contains 6.93% crude protein, 7.76% crude fiber, 20.89% alginate, 1.07% crude fat, 0.64% Ca, and 0.62% Phosphor. Brown seaweed is reported to contain bioactive compounds, such as alginate (Sanjeewa et al., 2017; Pereira and Cotas, 2020), fucoidan (Thanh et al., 2013; Laeliocattleya et al., 2020; Ponce and Stortz, 2020), fucoxanthin (Muradian et al., 2015; Zhang et al., 2015; Seo et al., 2016; Sulistiyani et al., 2021), and polyunsaturated fatty acids (PUFA; Carrillo et al., 2012; Siahaan et al., 2018). This bioactive substance exhibits stimulant, anti-inflammatory, antiviral, antibiotic, anti-thrombin, anticoagulant, hypocholesterolemic, and anti-thrombin actions (Pal et al., 2014; Hakim and Patel, 2020). Nonetheless, the inclusion of seaweed in animal diets is hampered by the high content of ash and poorly digestible carbohydrates (Sharma et al., 2018), high content of salt (Dewi et al., 2018; Reski et al., 2020), low digestibility (Bikker et al., 2016, 2020), limited shelf life (Paull and Chen, 2008; Stevant et al., 2017), and high cost of

To cite this paper: Dewi YL, Yuniza A, Nuraini, Sayuti K, and Mahata ME (2023). Processing of *Sargassum binderi* Seaweed for Supplementation in Poultry Diet. *J. World Poult. Res.*, 13(1): 103-111. DOI: https://dx.doi.org/10.36380/jwpr.2023.11

production (van den Burg et al., 2013). To address some of these disadvantages, seaweed can be immersed in water to reduce the ash (minerals + sand + salt) content outside the leaves (Dewi et al., 2018)

Alginate in brown seaweed is an abundant polysaccharide that reaches 25-45% dry weight (Rinaudo, 2014). The alginate content in poultry rations is limited because it will bind nutrients and inhibit the absorption of food substances in the digestive tract, interfering with the performance of poultry production. However, the right alginate concentration in the ration will trap fat and cholesterol in the digestive tract, then alginate and fat are excreted with the feces to reduce the fat content in the body and eggs of poultry (Dewi et al., 2023). Mushoilaeni et al. (2015) reported that giving alginate 0.75-1% to rats reduced serum cholesterol by 53%.

Processing *S. binderi* seaweed to reduce its alginate content can be done by physical methods, such as heat treatment. Several researchers have carried out this processing method. Processing of seaweed *Sargassum* spp. and *S. dentifebium* with physical methods using heat treatment have been reported by Al-Harthi et al. (2011), El-Deek et al. (2011), and Al-Harthi and El-Deek (2012). Physical methods affect the decrease in viscosity and depolymerization of alginate. According to Mishra (2019), the decrease in alginate viscosity is influenced by temperature, alginate concentration, and heating time. In addition to using physical methods, alginate can also be derived using chemical methods, such as soaking with the whiting filtrate. Mishra (2019) reported that an alkaline solution could degrade alginate. Furthermore, alginate in an alkaline media would be broken down by an elimination reaction catalyzed by OH- to produce 4,5-unsaturated uronic acid. Heating and soaking treatment with whiting water filtrate is easy to do and inexpensive.

The following is information about how processing *S. binderi* seaweed physically and chemically affects the amount of alginate it contains and how this affects the nutritional value of the seaweed. The main objective of this study was the reduction of the alginate content of *S. binderi* seaweed before being used in the poultry diet.

MATERIALS AND METHODS

Preparation of *Sargassum binderi* seaweed samples

The simple random sampling technique was utilized to obtain *S. binderi* seaweed samples from Sungai Nipah Coast, Pesisir Selatan Regency, West Sumatra, Indonesia, which were then composited into one. All parts of this seaweed were taken (talus, bladder, and holdfast). Furthermore, this seaweed was bathed in flowing water for 15 hours to minimize its salt content, and the seaweed was ready for treatment.

Heating *Sargassum binderi*

The *S. binderi* seaweed was soaked in running water for 15 hours, weighed as much as 200 g for each treatment, and then put into a plastic bag. Afterward, it was steamed in an autoclave with a hot steam pressure of 2 atm and a temperature of 121^{o}C. The time of applying hot steam pressure was adjusted according to the length of treatment time. After the procedure was finished, the seaweed was taken from the autoclave and allowed to cool in the open air for 20 minutes before being dried in a 60°C oven until the moisture content was roughly 14%. The current study used a completely randomized design with five treatments containing a control group and four treatment groups heating for 15, 30, 45, and 60 minutes. Each treatment group has five replicates.

The immersion of *Sargassum binderi*

The *S. binderi* seaweed was soaked in flowing water for 15 hours and weighed as much as 200 g for each treatment. This seaweed was immersed in a 0.07% whiting filtrate with a pH of 12. The seaweed was soaked according to the duration of the immersion treatment. After the soaking process was completed, the seaweed was removed from the area and then dried in an oven at 60°C until the water content was approximately 14%. This experiment was conducted using a completely randomized design with five treatments containing a control group and four treatment groups with immersion periods of 1, 2, 3, and 4 hours. Each treatment group has five replicates.

Sample preparation and analysis

After *S. binderi* seaweed was processed by physical (heat treatment) and chemical methods (soaking in whiting filtrate), the dried seaweed was finely ground into powder. After physical and chemical treatment, the seaweed powder was ready to be analyzed for the content of total dry matter, organic matter, ash, alginate, and crude protein.

Parameters

Alginate was analyzed using the method of Dewi et al. (2019). Dry matter, organic matter, ash, and crude protein were assessed using the AOAC method (1990).

Analysis of alginate content

One gram of dried seaweed was soaked in 10 ml of 0.5% HCl for 30 minutes, followed by immersion in 10 ml of 0.5% NaOH for 30 minutes. Then, the sample was extracted with 10 ml of 7.5% Na2CO3 at 50°C for 2 hours in a water bath. The next step was filtering the sample. Furthermore, the filtrate obtained was precipitated by adding 10 ml of 5% HCl, then 10 ml of NaCl was added to oxidize the seaweed pigments or color carrier groups. After that, the gel formed was vortexed and separated using a centrifuge for 15 minutes at a speed of 3500 rpm. The gel precipitate obtained was dissolved with 10 ml of 5% NaOH to convert alginic acid into alginate salt, after which it was precipitated with 95% isopropanol solution to form alginate salt. The precipitate was dried at 60°C and weighed until a constant weight was obtained. Content analysis of the dry matter, organic matter, ash, and crude protein used proximate analysis (AOAC, 1990).

Data analysis

Data were analyzed by analysis of variance using the WPS Excel-Statistics 2022 software (version 11.2.0.11254) for a completely randomized design. Before analysis, all percentages were subjected to logarithmic transformation log10 x + 1 to normalize data distribution. Mean values for each treatment were further tested by Duncan Multiple Range Test (DMRT), and the significance was declared when $p < 0.05$.

RESULTS AND DISCUSSION

Processing of *Sargassum binderi* seaweed by using heat treatment

The average content of dry matter, organic matter, ash, alginate, and crude protein of *S. binderi* seaweed treated by heat treatment is shown in Table 1.

The results of the analysis of variance showed that the application of heat treatment had a significant effect ($p < 0.05$) on the total dry matter content and a very significant effect ($p < 0.01$) on the content of organic matter, ash, and crude protein. However, it had no significant effect on the alginate content of *S. binderi* seaweed ($p > 0.05$). Based on the results of the DMRT further test, the total content of dry matter, organic matter, ash, and crude protein of *S. binderi* seaweed that was not treated with heat treatment (control) was significantly different ($p < 0.05$) from *S. binderi* seaweed which was not treated. Alginate content of the control and treatment was not significantly different ($p > 0.05$). There was no significant difference among the treatments (15, 30, 45, and 60 minutes of heating) in terms of dry matter, organic matter, ash, alginate, and crude protein ($p > 0.05$).

Table 1. Effects of *Sargassum binderi* seaweed processing by a physical method on total dry matter, organic matter, ash, alginate, and crude protein

Heating treatment (minute)	Total dry matter in fresh weight (%)	Organic matter (%)	Ash (%)	Alginate (%)	Crude protein (%)
Control	11.74[a]	81.52[b]	18.48[a]	41.98	14.49[b]
15	10.49[b]	83.21[a]	16.79[b]	39.80	16.00[a]
30	10.51[b]	83.24[a]	16.76[b]	40.16	15.58[a]
45	10.74[b]	83.66[a]	16.34[b]	41.52	15.70[a]
60	10.73[b]	83.62[a]	16.38[b]	40.86	15.89[a]

[a,b] Different superscript letters in the same column show significantly different ($p < 0.05$)

As can be seen in Table 1, dry matter was lower in heat treatments, compared to the control. The heat treatment led to the loss of some nutrients, such as vitamins, crude fats, dyes (fucoxanthin), and some minerals in seaweed. Similarly, previous studies indicated negative effects of heat treatment, including loss of vitamins (Jakobsen and Knuthsen, 2014; Lee et al., 2018), crude fat (Barszcz et al., 2014; Mosisa, 2017), and ash (Mosisa, 2017). In addition, fucoxanthin in seaweed is also damaged by heating, as described by Oryza (2011) and Susanto et al. (2017). According to Zhang et al. (2015), fucoxanthin is the dominant pigment in brown seaweed, and high temperatures easily damage this pigment. The results reported by Yip et al. (2014) indicated that fucoxanthin was stably stored at 4-25°C, and Oryza (2011) stated that fucoxanthin was relatively stable at 80°C for an hour and was damaged above 100°C.

Seaweed heat treatment for 15, 30, 45, and 60 minutes contained lower ash and higher organic matter than seaweed without treatment (control). It could be influenced by the heat application process, which causes the loss of some of the minerals contained in the seaweed.

The results of this study agree with those of Mosisa (2017), as the ash content for all treatments (dehulled traditional cooking, traditional unhulled cooking, dehulled pressure cooking, and unhulled pressure cooking) on black climbing (*Phaseolus coccineus* L.) generally decreased. This study indicates that minerals such as calcium, phosphorus, iron, and zinc are lost during the heating treatment process. Udensi et al. (2010) reported that heat treatment (autoclaving) on *Mucuna flagellipes* caused the ash content to decrease. The heat application causes a weakening of the bond structure, and the texture hardens (Sharma et al., 2012). This is thought to cause the loss of some minerals during the heating process. The decreased ash content caused the percentage of seaweed organic matter to increase in this treatment.

The heating treatment of seaweed for 15, 30, 45, and 60 minutes caused the crude protein content to be higher than the crude protein of seaweed without heating treatment. The decrease could influence the percentage of seaweed ash content treated with heating, increasing the percentage of organic matter and crude protein. In addition, the increased crude protein content is also influenced by the characteristics of the seaweed crude protein, which is not easily damaged by heating treatment. Deniaud-Bouët et al. (2017) reported that proteins and glycoproteins are a number of additional components of brown algal cell walls. Furthermore, they explained that brown seaweed protein combines with other components, namely alginate, fucoidan, and cellulose. Therefore, the percentage of crude protein in this study increased after being given heating treatment. According to Cascais et al. (2021), heat could alter protein yield in specific species and affect other species differently.

The alginate content of unprocessed seaweed (control) and heat treatment for 15, 30, 45, and 60 minutes was not significantly different ($p > 0.05$). Likewise, the treatment duration of heating for 15, 30, 45, and 60 minutes was not significantly different ($p > 0.05$) concerning the alginate content. Factors influencing alginate degradation are temperature and heating methods (steaming, hot steam pressure, and roasting). For heat stress treatment in the current study, an autoclave with a temperature of 121^{o}C and a pressure of 1 atm were used. It is estimated that this temperature could not degrade alginate, even though the length of heat treatment was extended to 60 minutes. The results found in the current study were in accordance with the results found by Widyastuti (2009) that alginate melted at 121.77-123.11^{o}C. The results of the present study were also in accordance with the study results found by Aida et al. (2010) that alginate can be dissociated into oligosaccharides by heating treatment at a temperature of 180 to 260°C with an alginate and water ratio of 1:25 (w/v). Therefore, the alginate contained in seaweed in this study cannot be dissociated. It is estimated that the heating temperature which was given for each treatment was still below the minimum temperature level to dissociate alginate; thus, this temperature level cannot dissociate alginate. According to Yuliani and Hartati (2011), the effect of temperatures of 150°C for 60 minutes indicates Ca-alginate depolymerization. Sartal et al. (2012) also reported that alginate could not melt during the heating process.

The heating treatments of seaweed for 15, 30, 45, and 60 minutes were not significantly different on dry matter, organic matter, ash, alginate, and crude protein of seaweed ($p > 0.05$). Processing material with hot steam pressure only causes changes in the chemical structure and does not change the chemical composition (Murni et al., 2008). Furthermore, Maehre et al. (2016) stated that heating treatment increased the protein availability of dulse red grass (*Palmaria palmate*) but not brown kelp seaweed (*Alaria esculenta*). Furthermore, it can be stated that heating seaweed causes partial or complete protein denaturation; thereby, this process facilitates the enzyme reaction in the digestive tract and increases protein utilization.

Although this heat treatment did not affect the nutritional content, it improved its quality as a poultry feed ingredient to positively affect the livestock, which was in line with findings reported by Hwang et al. (2012). The positive effects of heating include increasing the availability or quality of nutrients and inhibiting anti-nutrients. According to Maehre et al. (2016), heat treatment increases protein utilization in the red seaweed dulse (*Palmaria palmata*). Similarly, El-Deek et al. (2011) is of the opinion that processing methods, such as autoclaving and boiling, can improve the quality of foodstuffs, such as seaweed, by affecting fiber and nutrient availability. They reported that seaweed processing by this method improved the nutritional value and production performance of chickens compared to raw products. Processing with the autoclave method produces better nutrition, positively affecting livestock production performance (Al-Harthi et al., 2011).

Processing of *Sargassum binderi* seaweed by using the whiting filtrate

Table 2 shows the average content of total dry matter, organic matter, ash, alginate, and crude protein of

S. binderi seaweed treated by soaking seaweed in the whiting filtrate.

The results of the analysis of variance showed that the treatment of soaking seaweed in whiting filtrate had a significant effect on the total dry matter content and crude protein ($p < 0.05$) and an insignificant effect on the organic matter content, ash, and seaweed alginate ($p > 0.05$). Based on the results of the DMRT further test, the total dry matter and crude protein content of seaweed that was not treated with long soaking in the whiting filtrate (control) were significantly different ($p < 0.05$) with the treatment duration of immersion for 1, 2, 3, and 4 hours. However, there were insignificant differences among the groups in terms of organic matter, ash, and seaweed alginate ($p > 0.05$). The treatment duration of immersion in the whiting filtrate solution for 1, 2, 3, and 4 hours was not significantly different in the total dry matter, organic matter, ash, and alginate ($p > 0.05$), but the crude protein was significantly different among the treatments ($p < 0.05$).

Table 2. Effects of processing *Sargassum binderi* seaweed by a chemical method on total dry matter, organic matter, ash, alginate, and crude protein

Soaking time treatment	Total dry matter in fresh weight (%)	Organic matter (%)	Ash (%)	Alginate (%)	Crude protein (%)
Control	11.74[a]	81.48	18.52	41.98	14.49[c]
Group 1	10.19[b]	80.88	19.12	44.63	15.16[b]
Group 2	10.23[b]	81.08	18.92	43.33	15.53[ab]
Group 3	10.34[b]	80.95	19.05	44.62	15.51[ab]
Group 4	10.48[b]	81.82	18.18	44.66	15.93[a]

[a,b] Different superscript letters in the same column show significantly different ($p < 0.05$)

The total dry matter content of *S. binderi* seaweed after being treated for 1, 2, 3, and 4 hours of immersion in whiting filtrate showed a decrease. This decrease in total dry matter is thought to be caused by the dissolution of some inorganic seaweed materials, such as salt, sand, or other impurities that are still attached to the seaweed. In addition, the immersion also causes the dissolution of water-soluble vitamins, namely vitamins B and C. Therefore, the total dry matter content of *S. binderi* seaweed, which is treated by immersion in whiting filtrate, decreases at different soaking times, showing differences in the total dry matter content. Similarly, Martinson et al. (2012), Mack et al. (2014), and Giannakourou and Taoukis (2021) found that immersion treatment affects the loss of water-soluble macro minerals. Furthermore, Longland et al. (2014) stated that hay soaked in water caused the loss of some minerals and vitamins during the soaking process. Salt is an easily soluble compound in water (Nelson and Cox, 2013; Dewi et al., 2018). According to Hastuti et al. (2013), Vitamin C is unstable in alkaline solutions. In the current study, the whiting filtrate is an alkaline solution with a pH of 12, causing damage and dissolution of Vitamin C in the whiting water filtrate so that it impacts reducing the total dry matter of seaweed. The results of the current study were in accordance with the results found by Aregawi et al. (2014), indicating that sesame straw treated with 3% $Ca_2(OH)_2$, which is alkaline, caused the decrease of dry matter content from 89.7% to 76.6%.

The organic matter, ash, and alginate content of *S. binderi* seaweed had no effect after being treated with different immersion times in whiting filtrate. The reason can be that the concentration of whiting (0.07%) used to reach pH 12 is too low, even though the pH of the whiting filtrate has met the requirements as an alkaline solution predicted to decompose alginate. However, the filtrate concentration of 0.07% whiting water is thought to be classified as the weak base and has not been able to loosen alginate bonds or degrade alginate in seaweed. According to Owen et al. (1984), calcium hydroxide or Ca $(OH)_2$ is a weak base compared to NaOH, and the treatment conditions determine its effectiveness. The weak base treatment of Ca $(OH)_2$ to be more effective for degrading crude fiber from rice straw takes a longer and higher reaction time and dose (Trach et al., 2001). According to Anjalani et al. (2013), the effectiveness of the Ca $(OH)_2$ treatment on the nutritional content of palm leaves was due to the short duration and the low dose level.

The crude protein content of *S. binderi* seaweed increased along with the duration of soaking the seaweed in the whiting filtrate. Increasing the crude protein of seaweed after immersion with whiting filtrate is the same as the mechanism of increasing the crude protein of

seaweed after immersion in running water to reduce the salt content of the seaweed.

CONCLUSION

The results of the current study showed that physical treatment (heat treatment) and chemical treatments (whiting filtrate immersion) did not significantly affect the alginate content, crude protein, ash, dry matter, and organic matter. Based on the findings, it is recommended that the temperature level should be increased and the alkaline source should be changed by other strong alkaline sources in future studies.

DECLARATIONS

Acknowledgments

This research was supported and funded by the Ministry of Education, Culture, Research, and Technology Republic of Indonesia funded this study through PMDSU No: 059/SP2H1LT/DRPM/IV 2017. The authors also want to thank the Ministry of Education, Culture, Research, and the Technology Republic of Indonesia, and the Rector of the Universitas Andalas, which have provided the opportunity for us to conduct this study.

Authors' contributions

Dewi conducted paper writing, data collection, and statistical analysis. Yuniza, Nuraini, Sayuti, and Mahata contributed to the study design and development of the research idea. All authors drafted the manuscript and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

This article has been checked by all authors, and ethical issues such as plagiarism, publication consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy were not found.

Availability of data and materials

The prepared data of the present study will be sent by the corresponding author according to the reasonable requests.

REFERENCES

Aida TM, Yamagata T, Watanabe M, and Smith RL (2010). Depolymerization of sodium alginate under hydrothermal conditions. Carbohydrate Polymers, 80(1): 296-302. DOI: https://www.doi.org/10.1016/j.carbpol.2009.11.032

Al-Harthi MA and El-Deek AA (2012). Effect of different dietary concentrations of brown marine algae (*Sargassum dentifebium*) prepared by different methods on plasma and yolk lipid profiles, yolk total carotene and lutein plus zeaxanthin of laying hens. Italian Journal of Animal Science, 11(4): e644. DOI: https://www.doi.org/10.4081/ijas.2012.e64

Al-Harthi MA, El-Deek AA, and Attia YA (2011). Impacts of dried whole eggs on productive performance, quality of fresh and stored eggs, reproductive organs, and lipid metabolism of laying hens. British Poultry Science, 52(3): 333-344. DOI: https://www.doi.org/10.1080/00071668.2011.569009

Anjalani R, Budhi SPS, and Hartadi H (2013). The effects of different levels of calcium hydroxide and water addition for crude palm leaves fermentation on its chemical composition and *in vitro* digestibility**.** Buletin Peternakan, 37(2): 107-113. DOI: https://www.doi.org/10.21059/buletinpeternak.v37i2.2428

Aregawi T, Animut G, Kebede K, and Kassa H (2014). Effect of lime and/or urea treatment of sesame (*Sesamum indicum L.*) straw on feed intake, digestibility, and body weight gain of sheep. Livestock Research for Rural Development, 26(8): 146. Available at: http://www.lrrd.org/lrrd26/8/areg26146.htm

Association of official analytical chemists (AOAC) (1990). Official methods of analysis, 15th Edition. Association of official analytical chemists, Inc., Virginia. USA, pp. 69-84. Available at: https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf

Barszcz M, Tuśnio A, Taciak M, Paradziej-Łukowicz J, Molenda M, and Morawski A (2014). Effect of the composition and autoclave sterilization of diets for laboratory animals on pellet hardness and growth performance of mice. Annals Animal Science, 14(2): 315-328. DOI: https://www.doi.org/10.2478/aoas-2013-0064

Bikker P, Stokvis L, van Krimpen MM, van Wikselaar PG, and Cone JW (2020). Evaluation of seaweeds from marine waters in Northwestern Europe for application in animal nutrition. Animal Feed Science and Technology, 263: e114460. DOI: https://www.doi.org/10.1016/j.anifeedsci.2020.114460

Bikker P, van Krimpen MM, van Wikselaar P, Tan BH, Scaccia N, van Hal JW, Huijgen WJJ, Cone JW, and López-Contreras AM (2016). Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals, and biofuels. Journal of Applied Phycology, 28(6): 3511-3525. DOI: https://www.doi.org/10.1007%2Fs10811-016-0842-3

van den Burg SWK, Stuiver M, Veenstra FA, Bikker P, López-Contreras AM, Palstra AP, Broeze J, Jansen HM, Jak RG, Gerritsen AL et al. (2013). A triple P review of the feasibility of sustainable offshore seaweed production in the North Sea. LEI report. Wageningen UR., Wageningen, Netherlands. Available at: https://research.wur.nl/en/publications/a-triple-p-review-of-the-feasibility-of-sustainable-offshore-seaw

Buschmann AH, Camusa C, Infanteb J, Neoric A, Israele A, Hernández-Gonzáleza MC, Peredaa SV, Gomez-Pinchettic JL, Golbergf A, Niva Tadmor-Shalevd N et al. (2017). Seaweed production: Overview of the global state of

exploitation, farming and emerging research activity. European Journal of Phycology, 52(4): 391-406. DOI: https://www.doi.org/10.1080/09670262.2017.1365175

Carrillo S, Bahena A, Casas M, Carranco ME, Calvo CC, Ávila E, and Pérez-Gi F (2012). The alga *Sargassum* spp. as alternative to reduce egg cholesterol content. Cuban Journal of Agricultural Science, 46(2): 181-186. Available at: https://cjascience.com/index.php/CJAS/article/view/67

Cascais M, Monteiro P, Pacheco D, Cotas J, Pereira L, Marques J C, and Gonçalves AMM (2021). Effects of heat treatment processes: Health benefits and risks to the consumer. Applied Sciences, 11: 8740. DOI: https://www.doi.org/10.3390/app11188740

Deniaud-Bouët E, Hardouin K, Potin P, Kloareg B, and Hervé C (2017). A review about brown algal cell walls and fucose-containing sulfated polysaccharides: Cell wall context, biomedical properties and key research challenges. Carbohydrate Polymers, 175: 395-408. DOI: https://www.doi.org /10.1016/j.carbpol.2017.07.082

Dewi YL, Yuniza A, Nuraini, Sayuti K, and Mahata ME (2023) Efeects of different dietary concentration of fermented brown algae *Sargassum binderi* on plasma lipid profiles, yolk lipid, and cholesterol total of laying hens. Journal of Animal and Plant Sciences, 33(1): first Online. DOI: https://www.doi.org/10.36899/JAPS.2023.1.0588

Dewi YL, Yuniza A, Nuraini, Sayuti K, and Mahata ME (2018). Immersion of *Sargassum binderi* seaweed in river water flow to lower salt content before use as feed for laying hens. International Journal of Poultry Science, 17(1): 22-27. DOI: https://www.doi.org /10.3923/ijps.2018.22.27

Dewi YL, Yuniza A, Nuraini, Sayuti K, and Mahata ME (2019). Fermentation of *Sargassum binderi* seaweed for lowering alginate content of feed in laying hens. Journal World Poultry Research, 9(3): 147-153. DOI: https://www.doi.org /10.36380/jwpr.2019.18

El-Deek AA, Al-Harthi MA, Abdalla AA, and Elbanoby MM (2011). The use of brown algae meal in finisher broiler diets. Egyptian Poultry Science, 31(4): 767-781. Available at: https://scholar.google.com.eg/citations?view_op=view_citation&hl=en&user=jnwzw8EAAAAJ&citation_for_view=jnwzw8EAAAAJ:u-x6o8ySG0sC

Fu H (2021). Lima provinsi dengan jumlah produksi rumput laut terbesar [Five provinces with the largest amount of seaweed production]. Berita Daerah.co.id. Available at: https://www.beritadaerah.co.id/2021/03/15/lima-provinsi-dengan-jumlah-produksi-rumput-laut-terbesar/

Giannakourou MC and Taoukis PS (2021). Effect of alternative preservation steps and storage on vitamin C stability in fruit and vegetable products: Critical review and kinetic modelling approaches. Foods, 10(1): 2630. DOI: https://www.doi.org/ 10.3390/foods10112630

Hakim MM and Patel IC (2020). A review on phytoconstituents of marine brown algae. Future Journal of Pharmaceutical Sciences, 6: 129 DOI: https://www.doi.org/10.1186/s43094-020-00147-6

Hastuti S, Kurnianti YD, and Fakhry M (2013). Produksi manisan rambutan kering denganvariasi konsentrasi larutan kapur dan karakteristik pengeringan [Dry rambutan candy production with variation lime solution concentration and drying characteristics]. Agrointek, 7(1): 38-42. DOI: https://doi.org/10.21107/agrointek.v7i1.2048

Hwang IG, Shin YJ, Lee S, Lee J, and Yoo SM (2012). Effects of different cooking methods on the antioxidant properties of red pepper (*Capsicum annuum* L.). Preventive Nutrition and Food Science, 17: 286-292. DOI: https://www.doi.org/10.3746/pnf.2012.17.4.286

Jacob J (2022). Seaweed in poultry diets. Small and backyard poultry. Available at: https://poultry.extension.org/articles/feeds-and-feeding-of-poultry/feed-ingredients-for-poultry/seaweed-in-poultry-diets/

Jakobsen J and Knuthsen P (2014). Stability of vitamin D in foodstuffs during cooking. Food Chemistry, 148: 170-175. DOI: https://www.doi.org/10.1016/j.foodchem.2013.10.043

Kementeri Kelautan and Perikanan RI (2020). Rencana strategis kementerian kelautan dan perikanan tahun 2020-2024 [Strategic plan of the Ministry of Maritime Affairs and Fisheries for 2020-2024]. peraturan menteri kelautan dan perikanan republik Indonesia Nomor 17/permen-kp/2020. Available at: https://kkp.go.id/artikel/21471-rencana-strategis-kkp-tahun-2020-2024

Laeliocattleya RA, Yunianta, Suloi AF, Gayatri PP, Putri NA, and Anggraeni YC (2020). Fucoidan content from brown seaweed (*Sargassum filipendula*) and its potential as radical scavenger. Journal of Physics: Conference Series, 1430: 012023. Available at: https://iopscience.iop.org/article/10.1088/1742-6596/1430/1/012023/pdf

Lee S, Choi Y, Jeong H S, Lee J, and Sung J (2018). Effect of different cooking methods on the content of vitamins and true retention in selected vegetables. Food Science and Biotechnology, 27(2): 333-342. DOI: https://www.doi.org/10.1007/s10068-017-0281-1

Longland AC, Barfoot C, and Harris PA (2014). Effect of period, water temperature and agitation on loss of water-soluble carbohydrates and protein from grass hay: Implications for equine feeding management. Veterinary Record, 174(3): 68. DOI: https://www.doi.org/10.1136/vr.101820

Mack SJ, Dugdale AH, Argo C, Morgan RA, and McGowan CM (2014). Impact of water-soaking on the nutrient composition of UK hays. Veterinary Record, 174(18): 452. DOI: https://www.doi.org/10.1136/vr.102074

Maehre HK, Edvinsen GK, Eilertsen KE, and Elvevoll EO (2016). Heat treatment increases the protein bioaccessibility in the red seaweed dulse *(Palmaria palmata)* but not in the brown seaweed winged kelp *(Alaria esculenta)*. Journal of Applied Phycology, 28: 581-590. DOI: https://www.doi.org/10.1007/s10811-015-0587-4

Mahata ME, Rizal Y, Dewi YL, Sativa MO, Reski S, Hendro, Zulhaqqi, and Zahara A (2015). The potency of brown seaweed from Sungai Nipah beach as poultry feed. Penelitian Mandiri, Fakultas Peternakan Universitas Andalas, Padang, Indonesia. Available at: http://repo.unand.ac.id/44776/1/Penelitian%20Mandiri%20Prof%20Maria%20et%20al%20%282015%29%20OK.pdf

Martinson KL, Hathaway M, Jung H, and Sheaffer C (2012). The effect of soaking on protein and mineral loss in orchardgrass

and alfalfa hay. Journal of Equine Veterinary Science, 32(12): 776-782. DOI: https://www.doi.org/10.1016/j.jevs.2012.03.007

Mishra M (2019). Encyclopedia of polimer application, 1st Edition. CRC Press., Boca Raton, p. 118. DOI: https://www.doi.org/10.4324/9781351019422

Morais T, Inácio A, Coutinho T, Ministro M, Cotas J, Pereira L, and Bahcevandziev K (2020). Seaweed potential in the animal feed: A review. Journal of Marine Science and Engineering, 8(8): 559. DOI: https://www.doi.org/10.3390/jmse8080559

Mosisa MT (2017). Effect of processing on proximate and mineral composition of black climbing (*P. coccineus* L.) bean flour. African Journal of Food Science, 11(3): 74-78. DOI: https://www.doi.org/10.5897/AJFS2016.1475

Muradian K, Vaiserman A, Min KJ, and Fraifeld VE (2015). Fucoxanthin and lipid metabolism: A minireview. Nutrition, Metabolism, and Cardiovascular Diseases, 25(10): 891-897. DOI: https://www.doi.org/10.1016/j.numecd.2015.05.010

Murni R, Suparjo BL, Akmal A, and Ginting G (2008). Waste treatment methods for animal feed. Buku ajar tekhnologi pemanfaatan limbah untuk pakan. Laboratorium makanan ternak fakultas peternakan Universitas Jambi (Indonesia), 46-47. Available at: http://marjuki.lecture.ub.ac.id/files/2013/04/METODE-PEMANFAATAN-LIMBAH-SEBAGAI-PAKAN.pdf

Mushoilaeni W, Supartini N, and Rusdiana E (2015). Decreasing blood cholesterol levels in rats induced by alginate of *Sargassum duplicatum* and *Turbinaria* sp. derived from Yogyakarta. Asian Journal Agriculture and Food Sciences, 3(4): 321-326. Available at: https://ajouronline.com/index.php/AJAFS/article/view/2863

Nelson DL and Cox MM (2013). Lehninger's principles of biochemistry, 10th Edition. WH Freeman and Company., New York, p. 51.

Nofriya N (2015). Utilization of seaweed genetic resources as an alternative energy source in the future. Jurnal Teknik Lingkungan UNAND, 12(1): 38-47. DOI: https://www.doi.org/10.25077/dampak.12.1.38-47.2015

Oryza (2011). Fucoxanthin. Dietary ingredient for prevention of metabolic syndrome, autoxidation and cosmetics. Oryza Oil and Fat Chemical, Available at: http://www.oryza.co.jp/html/english/pdf/Fucoxanthin2.0M.pdf

Owen E, Klopfenstein T, and Urio NA (1984). Treatment with other chemicals. In: F. Sundstol and E. Owen (Editors). Straw and other fibrous by-products as feed. Elsevier and Science publishers., Amsterdam. pp. 248-273.

Pal A, Kamthania MC, and Kumar A (2014). Bioactive compounds and properties of seaweeds- A review. Open Access Library Journal, 1(4): e752. DOI: https://www.doi.org/10.4236/oalib.1100752

Paull RE and Chen NJ (2008). Postharvest handling and storage of the edible red seaweed *Gracilaria*. Postharvest Biology and Technology, 48(2): 302-308. DOI: https://www.doi.org/10.1016/j.postharvbio.2007.12.001

Pereira L and Cotas J (2020). Alginates - recent uses of this natural polymer. Introductory Chapter: Alginates - A general overview. IntechOpen., Coimbra. pp. 1-16. DOI: https://www.doi.org/10.5772/intechopen.88381

Ponce NMA and Stortz CA (2020). A comprehensive and comparative analysis of the fucoidan compositional data across the phaeophyceae. Frontiers in Plant Science, 11: 556312. DOI: https://www.doi.org/10.3389/fpls.2020.556312

Reski S, Mahata ME, and Rizal Y (2020). Soaking seaweed *Turbinaria murayana* in river water flow before used for poultry feed ingredients. Jurnal Peternakan Indonesia, 22(2): 211-217. DOI: https://www.doi.org/10.25077/jpi.22.2.211-217.2020

Reski S, Mahata ME, and Rusli RK (2022). The impact of dietary fermented seaweed (*Turbinaria murayana*) with fruit Indigenous micro organism's (IMO's) as a starter on broiler performance, carcass yield and giblet percentage. Advances in Animal and Veterinary Sciences, 10(7): 1451-1457. DOI: https://www.doi.org/10.17582/journal.aavs/2022/10.7.1451.1457

Rinaudo M (2014). Biomaterials based on natural polysaccharide: Alginate. TIP Revista Especializada en Ciencias Químico-Biológicas, 17(1): 92-96. DOI: https://www.doi.org/10.1016/S1405-888X(14)70322-5

Sanjeewa KKA, Lee WW, Kim JI, and Jeon YJ (2017). Exploiting biological activities of brown seaweed *Ishige okamurae* Yendo for potential industrial applications: a review. Journal of Applied Phycology, 29: 3109-3119. DOI: https://www.doi.org/10.1007/s10811-017-1213-4

Sartal CG, Alonso MCB, and Barrera PB (2012). Application of seaweed in the food industry. In: SK. Kim (Editor), Handbook of marine macroalgae: Biotechnology and applied phycology, 1st Edition. John Wiley and Son Ltd., West Sussex, p. 10. DOI: https://www.doi.org/10.1002/9781119977087.ch34

Seo MJ, Seo YJ, Pan CH, Lee OH, Kim K J, and Lee BY (2016). Fucoxanthin suppresses lipid accumulation and ROS production during differentiation in 3T3-L1 adipocytes. Phytotherapy Research, 30(11): 1802-1808. DOI: https://www.doi.org/10.1002/ptr.5683

Sharma DK, Andersen SB, Ottosen CO, and Rosenqvist E (2012). Phenotyping of wheat cultivars for heat tolerance using chlorophyll a fluorescence. Functional Plant Biology, 39(11): 936-947. DOI: https://www.doi.org/10.1071/FP12100

Sharma S, Neves L, Funderud J, Mydland LT, Øverland M, and Horn SJ (2018) Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*. Algal Research, 32: 107-112. DOI: https://www.doi.org/10.1016/j.algal.2018.03.012

Siahaan EA, Asaduzzaman AKM, and Pangestuti R (2018). Chemical compositions of two brown seaweeds species from Karimun Jawa, Indonesia. Marine Research in Indonesia, 43(2): 71-78. DOI: https://www.doi.org/10.14203/mri.v43i2.480

Stevant P, Marfaing H, Rustad T, Sandbakken I, Fleurence J, and Chapman A (2017). Nutritional value of the kelps *Alaria esculenta* and *Saccharina latissima* and effects of short-term storage on biomass quality. Journal of Applied Phycology, 29: 2417-2426. DOI: https://www.doi.org/10.1007/s10811-017-1126-2

Sulistiyani Y, Sabdono A, Afiati N, and Haeruddin (2021). Fucoxanthin identification and purification of brown algae commonly found in Lombok Island, Indonesia. Biodiversitas, 22(3): 1527-1534. DOI: https://www.doi.org/10.13057/biodiv/d220358

Susanto E, Fahmi AS, Agustini TW, Rosyadi S, and Wardani AD (2017). Effects of different heat processing on fucoxanthin, antioxidant activity and colour of Indonesian brown seaweeds. IOP Conference Series: Earth Environmental Science, 55: 012063. DOI: https://www.doi.org/10.1088/1755-1315/55/1/012063

Thanh TTT, Tran VTT, Yuguchi Y, Bui LM, and Nguyen TT (2013). Structure of fucoidan from brown seaweed *Turbinaria ornat*e as studied by electrospray ionization mass spectrometry (ESIMS) and small angle X-ray Scattering (SAXS) techniques. Marine Drugs, 11(7): 2431-2443. DOI: https://www.doi.org /10.3390/md11072431

Trach NX, Mo M, and Dan CX (2001). Effects of treatment of rice straw with lime and/or urea on its chemical composition, *in-vitro* gas production, and in-sacco degradation characteristics. Livestock Research for Rural Development, 13(4): 35. Available at: http://lrrd.cipav.org.co/lrrd13/4/trac134a.htm

Udensi EA, Arisa NU, and Ikpa E (2010). Effects of soaking and boiling and autoclaving on the nutritional quality of *Mucuna flagellipes*, ukpo. African Journal of Biochemistry Research, 4(2): 47-50. Available at: https://academicjournals.org/journal/AJBR/article-full-text-pdf/6CD16C811036

Widyastuti S (2009). Alginate content of the seaweeds grown in coastal zone of Lombok extracted by two extraction methods. Jurnal Teknologi Pertanian, 10(3): 144-152. Available at: https://jtp.ub.ac.id/index.php/jtp/article/view/293/360

Yip WH, Joe LS, Mustapha WAW, Maskat MY, and Said M (2014). Characterisation and stability of pigments extracted from *Sargassum binderi* obtained from Semporna, Sabah. Sains Malaysiana, 43(9): 1345-1354. Available at: http://journalarticle.ukm.my/7669/1/08_Wu_Hon_Yip.pdf

Yuliani and Hartati S (2011). Sterilization heat effect to gel base physical properties: Gelling agent cmc-na and ca-alginate case study. The Second International Conference on pharmacy and advanced pharmaceutical sciences, Yogyakarta, Indonesia, pp. 101-105. Available at: http://repository.usd.ac.id/id/eprint/12741

Zhang H, Kong R, Tang Y, Han C, Zhang Y, Zhang S, Liu Z, Qu J, and Wang X (2015). Fucoxanthin: A promising medicinal and nutritional ingredient. Evidence-Based Complementary and Alternative Medicine, 2015: 723515. DOI: https://www.doi.org/10.1155/2015/723515

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 112-119, March 25, 2023

Research Paper, PII: S2322455X2300012-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.12

Clostridium perfringens in Broiler Chickens: Isolation, Identification, Typing, and Antimicrobial Susceptibility

Nashwa M. Eid [1*], Eman F.Ahmed [2], Salama A.S. Shany [1], Al-Hussien M . Dahshan[1], and Ahmed Ali[1]

[1]*Poultry Disease Department- Faculty of Veterinary Medicine-Beni-Suef University, Beni-Suef, Egypt*
[2]*Animal Health Research Institute, Giza, Egypt*

Corresponding author's E-mail: nashwaeid666@gmail.com

Received: 12 December 2022
Accepted: 02 February 2023

ABSTRACT

Necrotic enteritis (NE) is a common worldwide poultry disease caused by the bacterium of *Clostridium perfringens* (*C. perfringens*) which has significant economic losses in the poultry industry as well as the cost of treatment and preventive measures. The current study was conducted to evaluate the incidence of NetB toxin positive in *C. perfringens* on different farms in Egypt. In the years 2020 and 2021, on industrial broiler farms (15-45 days- old), 100 intestinal samples were collected consisting of 30 healthy Ross broiler chickens and 70 unhealthy Ross broiler chickens. Culture and biochemical characterization (Catalase, urease, sugar fermentation, gelatin liquefaction, nitrate reduction, and lecithinase reaction tests) confirmed that *C. perfringens* was isolated at a rate of 10% (3/30) from apparently healthy broiler chickens and 40% from unhealthy broiler chickens. Thirty-one isolates were tested for toxigenicity and typing by ELISA kits and the results showed that 80% of the isolates from unhealthy broiler chickens were *C perfringens* type A alpha-toxin (toxigenic), 20% were non-toxigenic, and 66.7% isolates from apparently normal broiler chickens were toxigenic. The same thirty-one (44%) *C. perfringens* isolates were detected by PCR to investigate the presence of the NetB toxin gene in apparently healthy and unhealthy broilers and subsequently detect the role of NetB toxin in inducing NE. Of the samples, 82% of the isolates from unhealthy chicks were found to incode NetB gene, while none of the isolates from healthy broiler chickens had NetB. *Clostridium perfringens* showed sensitivity to amoxicillin, amoxicillin with clavulanic acid and ampiclox, intermediate for ofloxacin, and high resistance to cephalexin, streptomycin, colistin sulfate, erythromycin, sulfa trimethoprim, gentamycin, and oxytetracycline. The present study revealed the importance of NetB gen in the appearance of clinical signs of NE in broiler chickens.

Keywords: Alpha toxin, Antibiotic sensitivity test, *Clostridium perfringens*, Necrotic enteritis, NetB

INTRODUCTION

Necrotic enteritis (NE), as a major global threat, is an enteric disease caused by *Clostridium perfringens* (*C.perfrengens*) type A and C, contributing to economic losses (Jesudhasan et al., 2021). Several experimental studies investigate the induction of NE occurred mainly by *C. perfringens* which is Net B positive rather than alpha-toxin (Keyburn et al., 2013). NetB is a crucial virulence determinant in a *C. perfringens* strain isolated from NE. The toxin shared only 38% amino acid sequence identity with two other pore-forming toxins, beta-toxin, and *Staphylococcus aureus alpha-toxin* (Mohiuddin et al., 2021).

Clostridium perfringens is a Gram-positive, anaerobic bacilli bacterium, spore-forming found in nature as a normal flora in animals and humans' gastrointestinal tracts (Mora et al., 2020). Alpha, beta, epsilon, and iota are four typing of *C. perfringens* from A to G based on the ability to produce toxins (Sarmah et al., 2021). More than 15 toxins are produced by *C. perfringens*, including collagenase (κ-toxin), *Clostridium perfringens* enterotoxin

To cite this paper: Eid NM, Ahmed EF, Shany SAS, Dahshan AM, and Ali A (2023). *Clostridium perfringens* in Broiler Chickens: Isolation, Identification, Typing, and Antimicrobial Susceptibility. *J. World Poult. Res.*, 13(1): 112-119. DOI: https://dx.doi.org/10.36380/jwpr.2023.12

(CPE), bacteriocin adhesins, proteolytic enzymes, collagenolytic enzymes, and tpel (Uzal et al., 2014). The largest class of bacterial protein toxins is pore-forming toxins, which include perfringolysin O, NetB toxin, beta2 toxin, and enterotoxin (Lee and Lillehoj, 2022). Pore-forming toxins are a common mechanism of cell death as it generates pores to access the enterocytes (Lee and Lillehoj, 2022).

Clinical NE is distinguished in poultry by a significant increase in mortality without warning signs, whereas subclinical NE is distinguished as depressed and having diarrhea with low performance, high feed conversion ratio (FCR), and poor weight gain (Broom, 2017; Tsiouris, 2016). Diarrhea is sometimes associated with acute NE, but not always, although water-to-food ratios may be increased (Calefi et al., 2014). Gross lesions in small intestines were ballooned, friable, and contained brown blood-tinged fluid with a foul odor. Furthermore, the mucosa of infected poultry is covered by a tan-to-yellow pseudo-membrane resembling a "Turkish towel" (Hofacre et al., 2018).

These bacteria require predisposing factors as the most well-known risk infectious factor is coccidiosis which induces mucosal damage to the gut epithelium (Moore, 2016), facilitating *C. perfringens* colonization and proliferation (Kaldhusdal et al., 2021). Concurrently, the damage can cause ruptured epithelial cells to release plasma proteins into the lumen of the gut, which serves as a rich nutrient as it includes more than 11 amino acids that represent growth factors and vitamins for *C. perfringens*. (Mora et al., 2020). Moreover, *Eimeria* infection causes a mucogenic response in the host, resulting in the production of mucous, leading to *C. perfringens* growth (Moore, 2016). In addition to that, poultry infection by coccidia may cause immunological stress, making them more susceptible to *C. perfringens* infection (Boulianne et al., 2020).

Furthermore, as a nutritional factor, the diet was rich in indigestible, water-soluble non-starch polysaccharides (NSP), including rye, wheat, and barley, known to increase intestinal viscosity (Boulianne et al., 2020). Indigestible water-soluble non-starch polysaccharides could also leave undigested nutrients available for microbial proliferation and engage with glycoproteins on the intestinal epithelium to increase mucin production, thus also encouraging *C. perfringens* overgrowth. High protein of animal sources diets, particularly those based on fishmeal, provide an abundance of nutrients, including specific amino acids that *C. perfringens* cannot synthesize, causing an increase in bacterial growth (Yang et al., 2019). Moreover, gizzerozine, a biogenic amine observed in fishmeal, has been linked to broiler chicken alimentary tract erosion (Wu et al., 2014). In addition, adding fishmeal to the diet has an adverse effect as it destabilizes and alters the underlying gut microbial population, which predisposes to NE in broiler chickens (Moore, 2016).

Furthermore, management factors, such as acute diet changes, high-density broiler chickens housing, and extreme environmental temperatures, are also important risk factors for NE (Antonissen et al., 2014).

According to new research on the NetB toxin, immune responses to the toxin can provide some protection against NE (Prescott et al., 2016). NetB toxin is fully accountable for necrotizing tissues, causing perforations in epithelial cell membranes, destruction, and bowel leakage (Adhikari et al., 2020). The present study was conducted to investigate whether NetB is an essential factor for inducing NE in broiler chickens.

MATERIALS AND METHODS

Ethical approval

The animal use protocol in this study was approved by the Institutional Animal Care and Use Committee (022-374).

Sampling methods

From the broiler chicken farms of Egypt that are reared in a deep litter system for meat production, and had a high mortality rate of above 15%, at the age of 2-4 weeks, a hundred intestinal samples were collected (70 from unhealthy Ross broiler chickens, and 30 from apparently healthy Ross broiler chickens). Broiler chickens were clinically examined for observation of clinical signs and gross lesions related to NE under the supervision of the farms` veterinarians. Unhealthy broiler chickens suffered from depression, diarrhea, and a low growth rate. Samples were collected after a post-mortem examination of the intestine and taken by sterilized forceps. Samples were transported to the laboratory in an ice box immediately.

Isolation and identification of *Clostridium perfringens*

Samples were inoculated in cooked meat media and incubated in a Gas pack anaerobic gar at 37°C with anaerobe kits (Oxoid, India) for 24 hours. The cultures were then cultivated onto 10% sheep blood agar supplemented with 200 µg/ml neomycin sulfate and then incubated anaerobically at 37°C for 24 hours. Colonies

with a transparent double zone of hemolysis were presumptive and identified by biochemical methods. Catalase, urease, sugar fermentation, gelatin liquefaction, nitrate reduction, and lecithinase reaction tests were used (Rana et al., 2023).

Typing of *Clostridium perfringens*

Sandwich the enzyme-linked immunosorbent assay (ELISA) kits (Bio-X Diagnostics, Belgium) were used to detect *C. perfringens* typing (Alpha, Beta, and Epsilon toxins) in 31 isolates from culture supernatants (28 isolates from dead broiler chickens and 3 from apparently heavy ones) according to the manufacturer's instructions as a new alternative method for detection typing and toxinogenicity of *C. perfringens*. Specific monoclonal and polyclonal antibodies against *C. perfringens* (Alpha, Beta, and Epsilon toxins), as well as a monoclonal antibody specific for a structural protein of this bacterium, were immobilized. These antibodies could capture specific toxins or bacteria that may be present in the sample culture.

Detection of NetB

Bacterial DNA was extracted from bacterial cultures overnight using the QIAamp DNA extraction Mini Kit (Indian) according to the manufacturer's instructions. Oligonucleotide primers (F: GCTGGTGCTGGAATAAATGC and R: TCGCCATTGAGTAGTTTCCC) (Metabion AG, Germany) targeting the NetB gene of CP were used. The PCR of 20 µl consisted of 10 µl of 2X PCR Mix (Thermo Scientific™, USA.), 1 µl of each forward and reverse primer, 5 µl of template DNA, and 3 µl of PCR grade water. The first denaturation at 95°C for 5 minuts was followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. Amplicons (383bp) were separated on 1.5 percent agarose gel (Sigma, USA) and photographed using an ultraviolet (UV) Tran illuminator (Keyburn et al., 2010).

Antimicrobial susceptibility testing

The disc diffusion technique was used on Muller Hinton agar plate to test commercially prepared, fixed concentration paper antibiotic disc (Ampiclox, amoxicillin with clavulanic acid, amoxicillin, and tetracycline, streptomycin, colistin sulfate, chloramphenicol, sulfa-trimethoprim, gentamycin, ofloxacin, and oxytetracycline Oxoid Corporation, UK). Plates were incubated anaerobically at 37°C in a Gas Park anaerobic jar for 24 hours (Algammal and Elfeil, 2015). The diameter of the zone is closely linked to the sensitivity of the isolate and the rate of drug diffusion through the agar medium, and the zone of the diameter of each drug is interpreted using the clinical laboratory standard NCCLS criteria (Reller et al., 2009) or those included in the United State Food and Drug Administration.

RESULTS

Isolation and identification of *Clostridium perfringens*

Microbiological and molecular methods were used to screen 100 broiler chickens (70 unhealthy broiler chickens and 30 randomly selected from a healthy flock of broilers). Isolation of *C. perfringens* on blood agar forms a double zone of hemolysis (Figure 1).

Figure 1. Cultivation of *Clostridium perfringens* on blood agar

Prevalence of *Clostridium perfringens*

An investigation of 100 intestinal samples (30 from healthy broiler chickens and 70 from freshly dead broiler chickens suffered from severe dehydration, dilatation of the small intestine, and necrosis showed that the prevalence of *C. perfringens* was 10% (3/30) in extremely healthy broiler chicken and 40% (28/70) in dead cases according to culture and biochemical tests as in Table 1.

Typing of *Clostridium perfringens*

According to culture and biochemical identification of *C. perfringens*, 31 isolates were typed by sandwich ELISA kits (28 isolates were from dead broiler chickens and 3 from apparently healthy ones). It was revealed that 22 isolates were positive for *C. perfringens* type A alpha-toxin from dead broiler chickens, while 2 healthy broiler chickens were positive for alpha toxin (Table 2).

Detection of NetB toxin

NetB gene 82% (23/28) isolates from unhealthy broiler chicken had been NetB positive and 3 apparently normal isolates were NetB negative, as shown in figures 2 and 3, respectively, and Table 3. Typing of 31 *C. perfringens* isolates using ELISA test revealed that 80% of isolates from unhealthy broiler chicken were type A (positive for the alpha toxin). Moreover, 66.7% of healthy chickens were positive for alpha toxin type A.

Table 1. Incidence of *Clostridium perfringens* in healthy and unhealthy broiler chickens in Egypt

Groups of broiler chicken	Incidence (Number)	Percent (%)
Unhealthy (70)	28	40
Healthy (30)	3	10
Total (100)	31	31

Table 2. Typing of *Clostridium perfringens* according to the collected samples from the small intestines of broiler chickens in Egypt

Number of *Clostridium perfringens* isolates	ELISA	
	Alpha positive	Percentage (%)
Unhealthy broiler chicken (28)	22	80
Healthy broiler chicken (3)	2	66.7

Table 3. Detection of NetB from broiler chickens in Egypt

Groups	NetB gene	
	Number	Percentage (%)
Unhealthy broiler chicken (28)	23	82
Healthy broiler chicken (3)	0	0

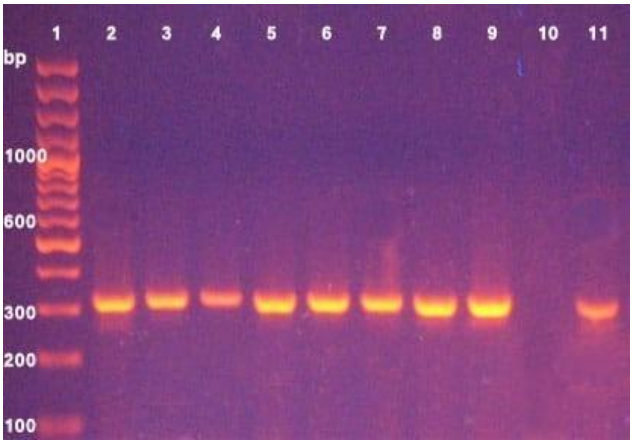


Figure 2. Detection of NetB from unhealthy broiler chickens in Egypt using PCR. Lane 1: 100bp pair DNA ladder, Lane 2 to 9 isolates are *C. perfringens* NetB-positive, Lane 10: control negative, Lane 11: control positive.

Figure 3. Detection of NetB from healthy broiler chickens in Egypt using PCR. Lane 1 to 3 isolates from apparently healthy broiler chicken are *C. perfringens* NetB- negative.

Antimicrobial susceptibility testing

Clostridium perfringens showed sensitivity at 100% to amoxicillin, amoxicillin with clavulanic acid and ampiclox, intermediate at 100% for ofloxacin, and high resistance to cephalexin (82%), streptomycin (100%), colistin sulfate (100%), erythromycin at (100%), sulfa trimethoprim (100%), gentamycin (100%), and oxytetracycline (89%; Oxoid, UK) according to the diameter of zone of inhibition as shown in Table 4.

Table 4. Antibiogram profile of the *Clostridium perfringens* isolates

isolates	Antibiotics and concentarion										
	AML 25 µg	AMC 30 µg	AX 30 µg	OFX 5 µg	CL 30 µg	S 10 µg	CT 10 µg	E 15 µg	SXT 25 µg	CN 30µg	OT 30µg
CP.EGY4	S	S	S	I	S	R	R	R	R	R	R
CP.EGY5	S	S	S	I	S	R	R	R	R	R	I
CP.EGY9	S	S	S	I	I	R	R	R	R	R	R
CP.EGY12	S	S	S	I	R	R	R	R	R	R	R
CP.EGY18	S	S	S	I	R	R	R	R	R	R	R
CP.EGY22	S	S	S	I	R	R	R	R	R	R	R
CP.EGY24	S	S	S	I	R	R	R	R	R	R	R
CP.EGY27	S	S	S	I	R	R	R	R	R	R	R
CP.EGY29	S	S	S	I	R	R	R	R	R	R	I
CP.EGY31	S	S	S	I	R	R	R	R	R	R	R
CP.EGY34	S	S	S	I	R	R	R	R	R	R	R
CP.EGY35	S	S	S	I	R	R	R	R	R	R	R
CP.EGY53	S	S	S	I	R	R	R	R	R	R	R
CP.EGY57	S	S	S	I	R	R	R	R	R	R	R
CP.EGY62	S	S	S	I	R	R	R	R	R	R	R
CP.EGY64	S	S	S	I	R	R	R	R	R	R	R
CP.EGY69	S	S	S	I	R	R	R	R	R	R	R

AML: Amoxicillin, AMC: Amoxicillin with clavulanic acid, AX: Ampiclox, OFX: Ofloxacin, CL: Cephalexin, S: Streptomycin, CT: Colistin sulfate, E: Erythromycin, CN: Erythromycin Gentamycin, OT: Ox tetracycline, SXT: Sulfa trimethoprim. S: Sensitive, I: Intermediate, R: Resistance

DISCUSSION

Necrotic enteritis is an acute enterotoxemia affecting poultry caused by *C. perfringens* and characterized by severe depression followed quickly by an asudden increase in flock mortality. Unhealthy broiler chickens showed ruffled feathers and diarrhea before death. The gross lesions were found in the small intestine, which was ballooned, friable, and contained brown fluid. Furthermore, the mortality rate may reach 50%. Presumptive diagnosis in the current study is based on gross lesions, culture characters, microscopic examination, and biochemical tests. More importantly, *C. perfringens* is responsible for subclinical infections associated with chronic intestinal mucosa damage, resulting in reduced weight gain and low-performance and consequently significant economic losses (Hofacre et al., 2018). The present study confirmed the importance of NetB as a major virulence factor in the appearance of symptoms in NE as the rate of isolation of *C. perfringens* was higher in unhealthy broiler chickens than that of healthy ones (40 percent and 10%, respectively), which agrees with Rizk et al. (2020), indicating a high occurrence 70% of *C. perfringens* in unhealthy broiler chickens of NE but a lower incidence in healthy broiler chickens (22%).

In the present study, detection of the NetB gene using PCR revealed that out of 28 isolates of unhealthy ones, 23 were found to encode the NetB toxin gene 82% meanwhile. None of the isolates from healthy broiler chickens had the NetB toxin gene. These may reflect the importance of the presence of NetB toxin in inducing NE, which may agree with previous studies (Keyburn et al., 2013; Keyburn et al., 2010), indicating that 70% (31/44) of *C. perfringens* isolates from poultry affected by NE had been positive for the NetB gene, suggested that NetB toxin is important in inducting NE. The NetB gene was found in the majority of necrotic enteritis-infected broiler chickens but not in healthy broiler chickens. In the same vein, Mwangi et al. (2019) detected NetB toxin in 81% of unhealthy broiler chickens. However, their finding for healthy broiler chickens where NetB toxin was detected (68%) was too high and did not reflect the role of NetB. The isolation of NetB positive from normal healthy broiler chickens does not change the fact that these isolates are highly pathogenic (Smyth and Martin, 2010). As healthy broiler chickens get a diverse *C. perfringens* population, there may be a limited number of NetB-positive isolates (Abildgaard et al., 2010). However, another study confirmed that *C. perfringens* NetB negative was detected in NE in broiler chickens (Chalmers et al., 2008). These findings could imply that NE is a multifactorial disease (Williams, 2005). Coccidiosis mucosal damage, poor sanitation, unbalanced nutrition, and poor housing are all stressors that contribute to the rapid growth of *C. perfringens* and the massive production of toxins (Jia et al., 2009). This suggests that these virulent strains may require additional predisposing conditions to proliferate to the point where they can cause NE.

The sensitivity of *C. perfringens* strains to penicillin, amoxicillin, and amoxicillin with clavulanic acid are 100%. These findings agree with Gharaibeh et al. (2010) as the combination of amoxicillin and clavulanic acid was effective against *C. perfringens* strains. On the other hand, the isolates were resistant at 100% to streptomycin. Similarly, Silva et al. (2009) found the resistance to streptomycin could be due to the absence of quinones in *C. perfringens*. In addition, the resistance of isolates to colistin sulfate and gentamycin was 100% except for oxytetracycline at 89% which agrees with Park et al. (2010), reporting presence of the tetP gene as the primary cause for resistance to oxytetracycline. Moreover, the resistance of isolates to sulfa-trimethoprim was 100%, and cephalexin was 82%. These results were almost identical to those obtained by Llanco et al. (2012), who detected the resistance to sulfa-trimethoprim and cephalexin. Gad et al. (2011) found that most *C. perfringens* strains in turkey flocks were sensitive to Sulfa trimethoprim, and the tested isolates were resistant to erythromycin (macroloide group). According to Anju et al. (2021), the resistance is due to the presence of the *ermQ* and *ermB* genes, which code for the production of enzymes for the di methylation of the 23S rRNA. This results in the inhibition of antibacterial drug action on bacteria, which is primarily responsible for *C. perfringens* resistance to the Macrolides group (erythromycin). Johansson et al. (2004) reported the isolates of *C. perfringens* were susceptible to erythromycin, meaning that a pattern of increased resistance against antimicrobial agents is commonly used in the control and treatment of NE.

CONCLUSION

Type A is the most predominant type in inducing NE in poultry as NetB is essential, especially with alpha toxins. It may act as a synergistic factor in inducing the NE in poultry. It is recommended to vaccinate broiler chickens with *C. perfringens* strain positive for NetB toxin to protect flocks from NE.

DECLARATIONS

Acknowledgments

The authors are thankful to Animal Health Research Institute in Dokki and to the department of poultry diseases in the faculty of veterinary medicine at Beni-Suef University Beni-Suef, Egypt, for the validation, equipment, and laboratory where this research was performed and completed. There is no funding source for my study.

Authors' contributions

Nashwa Mohamed. Eid contributed to the conceptualization, methodology, data curation, formal analysis, visualization, writing, original draft preparation, review, and editing of the manuscript. Eman Fathy. Ahmed is responsible for data curation, formal analysis, visualization, writing, review, and editing of the manuscript. Salama Abohamra Shany contributed to data curation, formal analysis, visualization, writing, review, and editing of the manuscript. Al-Hussien Momamed Dahshan contributed by writing, reviewing, and editing the manuscript. Ahmed Ali Ahmed contributed through conceptualization, methodology, data curation, formal analysis, visualization, writing, original draft preparation, review and editing of the manuscript, and supervision of the present study. All authors have read and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The authors have no competing interests.

Ethical consideration

All relevant ethical issues have been checked by all the authors.

Availability of data and materials

The authors confirm that the data showing the findings of this study are available within the article.

REFERENCES

Abildgaard L, Sondergaard TE, Engberg RM, Schramm A, and Højberg O (2010). *In vitro* production of necrotic enteritis toxin B, NetB, by netB-positive and netB-negative *Clostridium perfringens* originating from healthy and diseased broiler chickens. Veterinary Microbiology, 144: 231-235. DOI: https://www.doi.org/10.1016/j.vetmic.2009.12.036

Adhikari, P, Kiess A, Adhikari R, and Jha R (2020). An approach to alternative strategies to control avian coccidiosis and necrotic enteritis. Journal of Applied Poultry Research, 29(2): 515-534. DOI: https://www.doi.org/10.1016/j.japr.2019.11.005

Algammal AM and Elfeil WM (2015). PCR based detection of Alpha toxin gene in *Clostridium perfringens* strains isolated from diseased broiler chickens. Benha Veterinary Medical Journal, 29(2): 333-338. DOI: https://www.doi.org/10.21608/bvmj.2015.31744

Anju K, Karthik K, Divya V, Priyadharshini MLM, Sharma RK, and Manoharan S (2021). Toxinotyping and molecular characterization of antimicrobial resistance in *Clostridium perfringens* isolated from different sources of livestock and poultry. Anaerobe, 67: 102298. DOI: https://www.doi.org/10.1016/j.anaerobe.2020.102298

Antonissen G, Van Immerseel F, Pasmans F, Ducatelle R, Haesebrouck F, Timbermont L, Verlinden M, Janssens GP, Eeckhaut V, Eeckhout M et al. (2014). The mycotoxin deoxynivalenol predisposes for the development of *Clostridium perfringens*-induced necrotic enteritis in broiler chickens. PLoS One, 9(9): e108775. DOI: https://www.doi.org/10.1371/journal.pone.0108775

Boulianne M, Uzal FA, and Opengart K (2020). Clostridial diseases. In: Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair V, Suarez DL, de Wit S, Grimes T, Johnson D, Kromm C et al. (Editiors). Diseases of Poultry, pp. 966-994. DOI: https://www.doi.org/10.1002/9781119371199.ch22

Broom LJ (2017). Necrotic enteritis: Current knowledge and diet-related mitigation. World's Poultry Science Journal, 73(2): 281-292. DOI: https://www.doi.org/10.1017/S0043933917000058

Calefi AS, Honda BTB, Costola-de-Souza C, de Siqueira A, Namazu LB, Quinteiro-Filho WM, da Silva Fonseca JG, Aloia TPA, Piantino-Ferreira AJ, and Palermo-Neto J (2014). Effects of long-term heat stress in an experimental model of avian necrotic enteritis. Poultry Science, 93(6): 1344-1353. DOI: https://www.doi.org/10.3382/ps.2013-03829

Chalmers G, Martin S, Hunter D, Prescott J, Weber L, and Boerlin P (2008). Genetic diversity of *Clostridium perfringen*s isolated from healthy broiler chickens at a commercial farm. Veterinary Microbiology, 127(1-2): 116-127. DOI: https://www.doi.org/10.1016/j.vetmic.2007.08.008

Gad W, Hauck R, Krüger M, and Hafez H (2011). Determination of antibiotic sensitivities of *Clostridium perfringens* isolates from commercial turkeys in Germany *in vitro*. Archiv für Geflügelkunde, 75(2): 80-83. Available at: https://www.european-poultry-science.com/Determination-of-antibiotic-sensitivities-of-span-classws-name-Clostridium-perfringensspan-isolates-from-commercial-turkeys-in-Germany-span-classws-name-in-vitrospansupa-,QUlEPTQyMjA2MjgmTUlEPTE2MTAxNA.html

Gharaibeh S, Al Rifai R, and Al-Majali A (2010). Molecular typing and antimicrobial susceptibility of *Clostridium perfringens* from broiler chickens. Anaerobe, 16(6): 586-589. DOI: https://www.doi.org/10.1016/j.anaerobe.2010.10.004

Hofacre CL, Smith JA, and Mathis GF (2018). An optimist's view on limiting necrotic enteritis and maintaining broiler gut health and performance in today's marketing, food safety, and regulatory climate. Poultry Science, 97(6): 1929-1933. DOI: https://doi.org/10.3382/ps/pey082

Jesudhasan PR, Bhatia SS, Sivakumar KK, Praveen C, Genovese KJ, He HL, Droleskey R, McReynolds JL, Byrd JA, Swaggerty CL et al. (2021). Controlling the colonization of *Clostridium perfringens* in broiler chickens by an electron-beam-killed vaccine. Animals, 11(3): 671. DOI: https://www.doi.org/10.3390/ani11030671

Jia W, Slominski BA, Bruce HL, Nyachoti CM, and Jones RO (2009). Enzyme addition facilitates the post-disease compensatory growth of broiler chickens challenged with *Clostridium perfringens*. Canadian Journal of Animal Science, 89(3): 369-381. DOI: https://www.doi.org/10.3382/ps.2008-00204

Johansson A, Greko C, Engström B, and Karlsson M, (2004). Antimicrobial susceptibility of Swedish, Norwegian and Danish isolates of *Clostridium perfringens* from poultry, and distribution of tetracycline resistance genes. Veterinary Microbiology, 99(3-4): 251-257. DOI: https://www.doi.org/10.1016/j.vetmic.2004.01.009

Kaldhusdal M, Skjerve E, Hansen MK, Hamnes IS, David B, Hanssen SA, and Løvland A (2021). The incidence of necrotic enteritis in turkeys is associated with farm, season and faecal Eimeria oocyst counts. BMC Veterinary Research, 17: 292. DOI: https://www.doi.org/10.1186/s12917-021-03003-8

Keyburn AL, Portela RW, Sproat K, Ford ME, Bannam TL, Yan X, Rood JI, and Moore RJ (2013). Vaccination with recombinant NetB toxin partially protects broiler chickens from necrotic enteritis. Veterinary Research, 44: 54. DOI: https://www.doi.org/10.1186/1297-9716-44-54

Keyburn AL, Yan XX, Bannam TL, Van Immerseel F, Rood JI, and Moore RJ (2010). Association between avian necrotic enteritis and *Clostridium perfringens* strains expressing NetB toxin. Veterinary Research, 41(2): 21. DOI: https://www.doi.org/10.1051/vetres/2009069

Lee KW and Lillehoj HS (2022). Role of *Clostridium perfringens* necrotic enteritis B-like toxin in disease pathogenesis. Vaccines, 10(1): 61. DOI: https://www.doi.org/10.3390/vaccines10010061

Llanco L, Ferreira AJP, Nakano V, and Avila-Campos MJ (2012). Toxinotyping and antimicrobial susceptibility of *Clostridium perfringens* isolated from broiler chickens with necrotic enteritis. International Journal of Microbiology, 4(7): 290-294. DOI: http://www.doi.org/10.9735/0975-5276.4.7.290-294

Mohiuddin M, Yuan W, Song Z, Liao S, Qi N, Li J, Lv M, Wu C , Lin X, Hu J et al. (2021). Experimental induction of necrotic enteritis with or without predisposing factors using netB positive *Clostridium perfringens* strains. Gut Pathogens, 13: 68. DOI: https://www.doi.org/10.1186/s13099-021-00463-z

Moore RJ (2016). Necrotic enteritis predisposing factors in broiler chickens. Avian Pathology, 45(3): 275-281. DOI: https://www.doi.org/10.1080/03079457.2016.1150587

Mora ZVdl, Macías-Rodríguez ME, Arratia-Quijada J, Gonzalez-Torres YS, Nuño K, and Villarruel-López A (2020). *Clostridium perfringens* as foodborne pathogen in broiler production: Pathophysiology and potential strategies for controlling necrotic enteritis. Animals, 10(9): 1718. DOI: https://www.doi.org/10.3390/ani10091718

Mwangi S, Timmons J, Fitz-Coy S, and Parveen S (2019). Characterization of *Clostridium perfringens* recovered from broiler chicken affected by necrotic enteritis. Poultry Science, 98(1): 128-135. DOI: https://www.doi.org/10.3382/ps/pey332

Park M, Rooney AP, Hecht DW, Li J, McClane BA, Nayak R, Paine DD, and Rafii F (2010). Phenotypic and genotypic characterization of tetracycline and minocycline resistance in *Clostridium perfringens*. Archives of Microbiology, 192: 803-810. DOI: https://www.doi.org/10.1007/s00203-010-0605-5

Prescott JF, Smyth JA, Shojadoost B, and Vince A (2016). Experimental reproduction of necrotic enteritis in chickens: A review. Avian Pathology, 45(3): 317-322. DOI: https://www.doi.org/10.1080/03079457.2016.1141345

Rana EA, Nizami TA, Islam MS, Barua H, and Islam MZ (2023). Phenotypical identification and toxinotyping of *Clostridium perfringens* isolates from healthy and enteric disease-affected chickens. Veterinary Medicine International, 2023: 2584171. DOI: https://www.doi.org/10.1155/2023/2584171

Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ (2009). Antimicrobial susceptibility testing: A review of general principles and

contemporary practices. Clinical Infectious Diseases, 49(11): 1749-1755. DOI: https://www.doi.org/10.1086/647952

Rizk AM, El-Tawab A, Awad A, El-Bardisy MM, and Mayouf S (2020). Prevalence of *Clostridium perfringens* infection and virulence genes molecular study in broiler chickens. Benha Veterinary Medical Journal, 38: 70-74. DOI: https://www.doi.org/10.21608/BVMJ.2020.23619.1162

Sarmah H, Hazarika R, Tamuly S, Deka P, Manoharan S, and Sharma RK (2021). Evaluation of different antigenic preparations against necrotic enteritis in broiler birds using a novel *Clostridium perfringens* type G strain. Anaerobe, 70: 102377. DOI: https://www.doi.org/10.1016/j.anaerobe.2021.102377

Silva R, Salvarani F, Assis R, Martins N, Pires P, and Lobato F (2009). Antimicrobial susceptibility of *Clostridium perfringens* strains isolated from broiler chickens. Brazilian Journal of Microbiology, 40(2): 262-264. DOI: https://www.doi.org/10.1590/S1517-838220090002000010

Smyth JA and Martin TG (2010). Disease producing capability of netB positive isolates of *C. perfringens* recovered from normal chickens and a cow, and netB positive and negative isolates from chickens with necrotic enteritis. Veterinary Microbiology, 146(1-2): 76-84. DOI: https://www.doi.org/10.1016/j.vetmic.2010.04.022

Tsiouris V (2016). Poultrymanagement: A useflool for the control of necrotic enteritis in poultry. Avian Pathology, 45(3): 323-325. https://www.doi.org/10.1080/03079457.2016.1154502

Uzal FA, Freedman JC, Shrestha A, Theoret JR, Garcia J, Awad MM, Adams V, Moore RJ, Rood JI, and McClane BA (2014). Towards an understanding of the role of *Clostridium perfringens* toxins in human and animal disease. Future Microbiology, 9(3): 361-377. DOI: https://www.doi.org/10.2217/fmb.13.168

Williams R (2005). Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathology, 34(3): 159-180. DOI: https://www.doi.org/10.1080/03079450500112195

Wu SB, Stanley D, Rodgers N, Swick RA, and Moore RJ (2014). Two necrotic enteritis predisposing factors, dietary fishmeal and Eimeria infection, induce large changes in the caecal microbiota of broiler chickens. Veterinary Microbiology, 169(3-4): 188-197. DOI: https://www.doi.org/10.1016/j.vetmic.2014.01.007

Yang WY, Lee YJ, Lu HY, Branton SL, Chou CH, and Wang C (2019). The netB-positive *Clostridium perfringens* in the experimental induction of necrotic enteritis with or without predisposing factors. Poultry Science, 98(11): 5297-5306. DOI: https://www.doi.org/10.3382/ps/pez311

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 120-126, March 25, 2023

Research Paper, PII: S2322455X2300013-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.13

Effects of Dietary Supplementation of Vitamin E on Growth Performance and Immune System of Broiler Chickens

Rozi Khan Sadiq*[1], Mohammad Azim Abrahimkhil [1], Najibullah Rahimi [1], Sayed Ziaulhaq Banuree [1], and Sayed Attaul Haq Banuree[2]

[1]*Department of Animal Production, Nangarhar University, Nangarhar Province, Afghanistan*
[2]*Department of PreClinic Nangarhar University, Nangarhar Province, Afghanistan*

Corresponding author's E-mail: rozisadiq2012@gmail.com

Received: 09 January 2023
Accepted: 25 February 2023

ABSTRACT

As a potent antioxidant, Vitamin E may lessen the potentially harmful consequences of such oxidative stress to protect broilers against immune-pathological damage. Broiler chicken growth and viability are enhanced by Vitamin E supplementation. The present study aimed to investigate the effects of Vitamin E dietary supplementation on broiler chickens' growth performance and health status. A total of 48 one-day-old Ross chicks were randomly divided into two groups of control and treatment (supplementation of Vitamin E at a dose of 300 mg/kg diet) with three replicates per group. The study included an equal number of Ross breed chicks and Vitamin E dosage in two trials on two different dates (January and March, 2022). In both trials, the obtained results indicated no significant changes in weight gain in the control and treatment groups. In both trials, there were no significant differences in the spleen weight of the control and treated groups; however, from day 1 to 28 of the second trial, the bursa of Fabricius was heavier in the treated group than in the control group. Additionally, Vitamin E had no significant effects on the mitogenic responses to phytohemagglutinin (PHA) and Concanavalin A (Con A). Dosages of 20 and 10 µl for both PHA and Con A did not significantly affect the rate of pure lymphocyte proliferation in chicks fed 300 mg Vitamin E /kg feed. Cell-mediated immunity did not differ significantly between the two trials. The percentages of CD4, CD8, Bu1, and MHCII molecules in the spleen and cecal tonsil of the chicks that received Vitamin E 300 mg/kg feed did not change significantly. The antibody titers against infectious bronchitis and infectious bursal disease vaccines showed no significant differences. On day 42, there was a trend toward an increase in antibody titer in the case of the Newcastle disease vaccine. In conclusion, 300 mg/kg of Vitamin E added to the diet did not improve growth performance and immunity in broiler chicks.

Keywords: Broiler chicken, Growth performance, Immune system, Vitamin E

INTRODUCTION

The Food and Agriculture Organization (FAO) of the United Nations has recently shown significant growth in the global production of poultry meat (FAO, 2017). To stop lipid oxidation processes in the meat and improve meat quality, Vitamin E should be added to broiler chickens' diet (Vieira et al., 2021). This can be attributed to broiler chickens' ongoing stress from infections, high development rates, and the constantly changing environmental conditions in their housing facilities (Lohakare et al., 2005). A profound change in the productivity of the broiler chicken industry has been achieved via intentional genetic selection through traditional quantitative techniques (Hunton, 2006). Enzymes that eliminate free radicals generated during regular metabolic activity depend on antioxidants to operate appropriately (Niu et al., 2009). Systemic risks caused by free radical damage can be prevented or avoided by antioxidant enzymes, which convert them into

comparatively stable molecules (Niu et al., 2009). Antioxidant dietary supplements increase plasma glutathione peroxidase activity and reduce lipid peroxidation, which is a defensive mechanism affecting liver cells under heat stress (Calik et al., 2022). Vitamin E functions as a cellular enzymatic activity regulator (Schneider, 2005) and shields the cell membranes and tissues from lipoperoxidation damage by free radicals (Gao et al., 2010, Traber and Stevens, 2011). In order to avoid lipid oxidation in the meat and improve meat quality, broiler chicken diets must be supplemented with Vitamin E (Vieira et al., 2021).

Broiler chickens are more susceptible to pathogen infection due to their fast growth rate and the environmental problems associated with modern chicken production methods (McCorkle and Glick, 1980). Tocopherols and tocotrienols, which are fat-soluble and have antioxidant capabilities, are both included in Vitamin E (Packer et al., 2001). Vitamin E is an important fat-soluble nutrient. Its roles in domestic animal production are indispensable since animals cannot synthesize Vitamin E (Zhao et al., 2021). Vitamin E is an excellent biological chain-breaking antioxidant that protects cells and tissue from lipoperoxidation damage by free radicals (Sahin et al., 2006). Dietary supplementation of Vitamin E reduces AST and ALT activity (Amevor et al., 2022), decreases feed consumption, and improves growth parameters in broiler chickens (Dalia, 2018). Various doses of Vitamin E dietary supplementation may enhance the performance of broiler chicks (Calik et al., 2022).

Regarding broiler chickens' growth performance and relative organ weights, different Vitamin E sources (lipid soluble and water soluble) and their inclusion amounts from 22.11 to 67 mg/kg in diets show minimal effects (Pitargue et al., 2019). In broiler chickens, oxidative stress causes biological damage that negatively affects growth performance and health (Estevez, 2015). Vitamin E is mainly found close to the membrane interface in the hydrocarbon section of the membrane's lipid bilayer near the oxidase enzymes that initiate the creation of free radicals (Packer, 1991). Additionally, it has been demonstrated that Vitamin E antioxidants can enhance health by increasing humoral and cell-mediated immunity in broiler chickens (Leshchinsky and Klasing, 2001). It has been observed that Vitamin E enhances the survival, proliferation, and functionality of lymphocytes, macrophages, and plasma cells by defending them against oxidative damage (Maggini et al., 2007). Therefore, dietary supplementation of Vitamin E strengthens the immune system under stress conditions (Attia et al., 2016).

The recommended dosage of Vitamin E is 6.7-53.6 mg Vitamin E /Kg of broiler chickens' diet (NRC, 1994), depending on their growth stage (Aviagen, 2014). However, additional antioxidants, such as Vitamin C and Selenium, the kind and quantity of fats consumed, and environmental conditions all impact the necessary dietary amounts (NRC, 1994). Consequently, there is ongoing discussion regarding the appropriate levels of Vitamin E inclusion in broiler diets (Kuttappan et al., 2012).

MagginiGiven the possible advantages of Vitamin E on the health of fast-growing broiler chicks, the immunomodulatory effects of Vitamin E on broiler chickens need to be further investigated. This study aimed to assess how dietary Vitamin E affected the health and growth performance of broiler chickens.

MATERIALS AND METHODS

Ethical approval

This study was performed at Animal Production Department's Poultry Research farm. All experimental procedures involving animals were conducted in accordance with the Institutional Care guidelines of Nangarhar University Nangarhar, Afghanistan. Chickens were cared for using husbandry guidelines and standard operating procedures of broiler chickens and approved by the ethics committee of Nangarhar University, Afghanistan (12.07.2021-06).

Study design

The research was conducted in two trials of 1 and 2 in January and March 2022, respectively. A total of 48 one-day-old mixed Ross strain broiler chicks (50% male) with an average body weight of 45 g were purchased from a local hatchery in Jalalabad, Nangarhar Province, Afghanistan. The chickens were randomly divided into two groups (24 chickens in each control and the Vitamin E supplemented group). Each group had three replicates, and each replicate included eight broiler chicks. The study included an equal number of Ross breed chicks and Vitamin E dosage in two trials on two different dates The cage for each replicate was designed with a 65 cm length, 60 cm width, and 30 cm height. Total mortality for the 6 weeks was 8%. Weekly management of cage temperature and humidity included 33°C and 40% relative humidity for the first week, 30°C and 50% relative humidity for the second week, 27°C and 50% relative humidity for the third week, and 24°C and 50% relative humidity for the fourth

week, and 21°C and 60% relative humidity for the Fifth and sixth weeks. The cage was fully lit during the first 24 hours, then there was one hour of darkness on the following day, and after they reached 100-150-g body weight, the light was turned off for six hours until the experiment's completion. Lighting density was controlled (20-40 Lux).

Feed preparation

Accordingly, starter feed (0-21 days) and grower feed (22-42 days) were produced in mash form, and during both experiment trials, feed and water were always freely available. The basal diet was supplied to meet the nutrient requirements of Ross broiler chicks' as a recommendation of the National Research Council (NRC, 1994). Accordingly, starter feed (0-21 days) and grower feed (22-42 days). Feed was produced in mash form, and during both trials, feed, and water were always in free availability to the chicks (Cinar et al., 2014; Table 1).

Vaccination

The use of particular vaccinations for the specific antibody titer measurement was considered in accordance with the experiment's protocol. In Afghanistan, the production of broilers is affected by the viral illnesses Newcastle disease (ND), infectious bronchitis (IB), and infectious bursal disease (IBD). As a result, IBD, ND, and IB vaccines were administered to chickens (Lin et al., 2005). The first dose of the NB (ND+IB) vaccine (NISSEIKEN company, Japan) was administered to each chick on day 6 as an eye drop (0.03 ml). On days 18 and 32, the vaccine was administered again through the water.

On days 12 and 24, each chicken received a single dosage (0.015 ml/kg) of the IBD vaccine (NISSEIKEN company, Japan) directly using an eye dropper. All vaccinations were preserved at a temperature of 2-5°C.

Immunoproliferation assay

For the immunoproliferation assay, 15 samples of spleen from the control and 15 samples from the treatment groups were randomly obtained under sterilized conditions. The samples were then mixed with about 5 ml of phosphate-buffered saline (PBS). The suspension was prepared following the guideline methodology and centrifuged (Germany) at 300 x g for 5 minutes at room temperature (27°C).

Concanavalin (Con A) was used in this test in doses of 20 µl (6.25 µg) and 10 µl (3.125 µg) for T-cell activation, and 20 µl (37.5 µg) and 10 µl (18.75 µg) for B-cell activation. T-cell activation also utilized phytohemagglutinin (PHA), which was also used at 20 and 10 µl. The specific microplate containing the samples was incubated for 72 hours at 40°C before being thoroughly examined (Tay et al., 2020).

Flow cytometry assay

In the case of the flow cytometry assessment (McKinnon, 2018), 15 spleen and 15 cecal tonsil samples from the control and treatment groups, respectively, were randomly collected under sterile conditions. The samples were added to specific wells, such as CD4, CD8, and Bu1, and washed twice. Each well received 100 µl of PBS, including the control, and was centrifuged (Germany) at 300 x g for 5 minutes at room temperature (27°C). The PBS was discarded after centrifuging, and 100 µl of the antibody solution (1-time antibody and 99-times PBS) was added to each well except for the control. Centrifuging was repeated for 3 minutes, and the mixture was incubated for an hour. Each well received a 100 µl 10 x solution of propidium iodide (PI) after the plates were cleaned three times (one time PI and nine times PBS). In the next step, 100µl of each well content was transferred to relevant tubes for flow cytometry analysis (BD Facs canto II, United State). All of the CD4 (CT-4), CD8 (CT-8), BU1 (L22), and MHC class II (2Gll) antibodies utilized in this investigation were from Southern Biotech in Birmingham, Alabama, USA. A flow cytometer was used to evaluate the samples. About 1-2 ml of blood was drawn from the wing vein for a particular antibody titer. No heparinized blood collection tubes were used. The blood was centrifuged at 120 x 100 rpm for 20 minutes. The resultant serum was then placed in a special tube and maintained at -20°C for further analysis (Kaspers et al. 1993).

Statistical analysis

All data were analyzed by R statistical software version 2.14.2. T-test was used to compare the means of the Vitamin E supplemented group with those of the control group. Means and standard errors were used to represent the values for the observed results. The results were considered significantly different at $p < 0.05$.

Table 1. The ingredients and nutrient levels of the broiler chicken basal diet for a period of 42 days

Ingredients (%)	Starter (Day 1-21)	Grower (Day 22-42)
Corn seed	64.60	69.94
Soybean meal, *CP 48%	30.25	24.19
Soybean oil	0.00	0.50
Fish meal	1.00	1.00
Dicalcium phosphate	1.60	1.57
Calcium carbonate	1.36	1.30
Salt	0.46	0.40
Mineral-vitamin premix[a]	0.50	0.50
DL-methionine	0.17	0.15
HCL-lysine	0.00	0.44
Nutrients composition		
Metabolizable energy (kcal/kg)	2,988	3,083
Crude protein (%)	(23)	(20)
Calcium (%)	1.01	0.96
Phosphorus (%)	0.50	0.48
Vitamin E (mg/kg)	(6.7)	(6.7)
Selenium (mg/kg)	(0.15)	(0.15)

Source: NRC (1994), *CP: Crude protein; [a] Mineral-vitamin premix provided the following per kilogram of diet: vitamin A, 5,500 IU; vitamin D3, 1,100 IU; vitamin E, 10 IU; riboflavin, 4.4 mg; vitamin B12, 12 mg; nicotinic acid, 44 mg; menadione, 1.1 mg; biotin, 0.11 mg; thiamine, 2.2 mg; and ethoxyquin, 125 mg, Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.17 mg; I, 0.46 mg; and Ca, minimum:150 mg, maximum: 180 mg

RESULTS

Growth performance

The findings showed that adding Vitamin E at 300 mg/kg to the feed had no significant effect on the growth performance of broiler chicks from 1 to 42 days of age ($p > 0.05$, Table 2).

The obtained results indicated no significant difference in spleen weight between the control and Vitamin E -supplemented groups ($p > 0.05$, Table 3). However, there was a significant difference in the bursa of Fabricius between the control and treatment groups in the first trial ($p < 0.05$).

Immunoproliferation assay

Dietary supplementation of 300 mg Vitamin E /kg feed did not suppress mitogenic responses significantly to PHA and Con A ($p > 0.05$). Pure lymphocyte proliferation rates were not significantly different between control, and Vitamin E supplemented groups ($p > 0.05$), where the PHA was used at 20 and 10 µl as well as Con A at 20 and 10 µl (Table 4).

Flow cytometry

After 4 and 6 weeks of age, there was no significant difference between control and Vitamin E supplemented groups in both trials in terms of immune molecules percentage ($p > 0.05$). Dietary supplementation of Vitamin E at 300 mg/kg feed in Ross broiler chicks had no significant effect on the percentages of CD4, CD8, Bu1, and MHC II molecules in the spleen and cecal tonsil ($p > 0.05$).

Specific antibody titers

In the Vitamin E 300 mg/kg supplemented group, total antibody titers for IBD and IB after days 14, 28, and 42 did not change significantly ($p > 0.05$). After days 14 and 28, there was no significant difference between the control and Vitamin E supplemented groups in both trials for Newcastle disease ($p > 0.05$); however, there was an increase after day 42 ($p > 0.05$).

Table 2. Feed intake, feed efficiency, and mean body weight differences in broiler chickens

Group	Trial 1			
	BWG (g/chick)	BWG (g/chick)	Feed intake (g/chick)	FCE
	day 14	days 14-28	days 14-28	
Control	363 ± 25	634 ± 53	982 ± 50	1.59 ± 0.12
Treatment	309 ± 27	565 ± 44	824 ± 45	1.48 ± 0.09
	day 42	days 28-42	days 28-42	
Control	1536 ± 76	902 ± 121	1354 ± 21	1.62 ± 0.21
Treatment	1476 ± 118	910 ± 99	1478 ± 43	1.71 ± 0.20
	Trial 2			
	day 14	days 14-28	days 14-28	
Control	261 ± 4	568 ± 46	867 ± 42	1.63 ± 0.12
Treatment	269 ± 13	650 ± 51	1091 ± 0.5	1.75 ± 0.14
	day 42	days 28-42	days 28-42	
Control	1259 ± 119	691 ± 161	1530 ± 62	3 ± 0.32
Treatment	1421 ± 85	771 ± 116	1561 ± 15	2.5 ± 0.44

FCE: Feed conversion efficiency, BWG: Body weight gain, Trial 1: Experimental exploratory-1 conducted on January 2022, Trial 2: Experimental exploratory-2 conducted on March 2022.

Table 3. Effects of Vitamin E dietary supplementation on some internal organs of broiler chickens

Organ (g/100gw) body	Age (day)	Trial 1		Trial 2	
		Control	Treatment	Control	Treatment
Spleen	28	0.136 ± 0.02	0.139 ± 0.02	0.117 ± 0.01	0.105 ± 0.01
	42	0.224 ± 0.05	0.329 ± 0.04	0.146 ± 0.03	0.100 ± 0.02
Bursa of Fabricius	28	0.193 ± 0.04[a]	0.311 ± 0.02[b]	0.219 ± 0.03[a]	0.205 ± 0.01[a]
	42	0.206 ± 0.02	0.182 ± 0.04	0.147 ± 0.01	0.172 ± 0.01

[a, b] different values in the same row are significantly different ($p < 0.05$). Trial 1: Experimental exploratory-1 conducted on January 2022, Trial 2: Experimental exploratory-2 conducted on March 2022.

Table 4. Effect of dietary Vitamin E and mitogen concentration on the proliferation of mononuclear cells

Vitamin E (mg/kg)	Number of chickens	Age	Con A		PHA	
			6.25 (µg/ml)	3.12 (µg/ml)	37.5 (µg/ml)	18.75 (µg/ml)
0	4	Second week	0.56 ± 0.04	1.54 ± 0.24	1.95 ± 0.17	1.17 ± 0.07
300	4		0.78 ± 0.09	1.22 ± 0.12	1.64 ± 0.12	0.94 ± 0.06
0	4	Fourth week	1.09 ± 0.08	1.06 ± 0.03	1.02 ± 0.04	0.96 ± 0.03
300	4		0.9 ± 0.23	1.17 ± 0.04	1.42 ± 0.24	1.02 ± 0.04
0	4	Sixth week	1.09 ± 0.03	1.055 ± 0.03	1.23 ± 0.04	0.96 ± 0.03
300	4		1.25 ± 0.06	0.999 ± 0.02	0.97 ± 0.03	0.96 ± 0.05

Con A: Concanavalin A, PHA: Phytohemagglutinin A

DISCUSSION

Numerous dietary components, such as vitamins A and C, help animals incite the best immunological responses. Vitamin E is well known as an efficient chain-breaking antioxidant preventing oxidative damage to body tissues (Voljc et al., 2011). Moreover, it was demonstrated that the antioxidant properties of Vitamin E can improve animal health by improving cell-mediated and humoral immunity in broiler chickens (Leshchinsky and Klasing, 2001). The findings of the current study indicated that 300 mg/kg of Vitamin E as a dietary supplement did not affect broiler chickens' performance.

The inclusion of Vitamin E did not significantly affect broiler chicken development and relative organ weights when comparing 22.11 to 67 mg/kg feed (Pitargue et al., 2019).

According to the findings of the current investigation, broiler chicks' live body weight was not improved by a 300 mg/kg Vitamin E -supplemented diet. According to Dalia (2018), the addition of Vitamin E at 100 mg/kg feed considerably reduced feed consumption and enhanced growth parameters, which is contrary to the findings of the current study.

The results of the present study supported those of Goni et al. (2007), who found that broiler chicks' average daily gain, average daily feed intake, and feed conversion ratio were unaffected by Vitamin E supplementation up to 400 mg/kg. The current study findings are opposed to those of Swain et al. (2000), indicating that dietary supplementation of α-tocopherol acetate at 300 mg/kg feed could considerably increase average daily gain and significantly decrease average daily feed intake. At the dose of 300 mg/kg, Vitamin E supplemented diet revealed a significant increase in the relative weight of the bursa of Fabricius ($p < 0.05$). However, the dietary supplementation of Vitamin E had no impact on the relative weights of the spleen.

Dietary supplementation of 300 mg Vitamin E /kg feed did not suppress mitogenic responses significantly to PHA and Con A ($p > 0.05$). Pure lymphocyte proliferation rates were not significantly different where the PHA was used at 20 and 10 µl as well as Con A at 20 and 10 µl. The results were in contrast to those of Leshchinsky and Klasing (2001), who found that 16.75-33.5 mg Vitamin E /kg feed of broiler chicks significantly changed mitogenic response to PHA and Con A.

After 4 and 6 weeks of age, there were no significant differences between control and Vitamin E supplemented groups in both trials in the current study regarding immune molecules percentage ($p > 0.05$). Vitamin E at a dosage of 300 g/Kg had no significant effect on the proportions of CD4, CD8, Bu1, and MHCII molecules in the spleen or in the cecal tonsil ($p > 0.05$).

On days 14, 28, and 42 after IBD and IB vaccinations, there were no significant differences between the control and treatments in terms of humoral antibody titer ($p > 0.05$). On days 14 and 28 of the

experiment, the antibody titer of the chicks supplemented with 300 mg Vitamin E /kg feed did not increase significantly for the ND vaccine ($p > 0.05$); meanwhile, on day 42 of the experiment, the results showed that there was the tendency for titer rising for ND vaccine ($p < 0.05$). Overall, the results of the present study are in contrast to those of Niu et al. (2009), who demonstrated that broiler raised under heat stress produced considerably more immunoglobulin G (IgG) and immunoglobulin M (IgM) in response to 200 mg/kg dietary Vitamin E.

In the current study, there was no increase in antibody titer against the IB virus in the treated group supplemented with 300mg Vitamin E /kg feed, which was in contrast to the findings of Leshchinsky and Klasing (2001), indicating that supplementation of Vitamin E between 0-16.75 mg/kg feed could increase antibody production to attenuated IB vaccine.

Immunocompetence is not considered in the NRC's suggested Vitamin E level (NRC, 1994), despite the fact that this level (0.67mg/kg feed) is sufficient to stop oxidative damage. However, few research studies outline the exact requirements of Vitamin E for a certain immunological function.

CONCLUSION

The current study indicated that dietary supplementation of 300 mg Vitamin E /kg feed did not improve broiler chicks' live body weight, feed intake, and feed consumption ratio throughout 6 weeks. The study also revealed that Vitamin E at 300 mg/kg feed did not raise immunological responses of broiler chicks. In conclusion, a high-level dose (300 mg/kg) intake of dietary Vitamin E alone is not advised as a growth promoter and immunological enhancer. As a result, it is difficult to recommend the ideal dietary Vitamin E concentration for growth improvement and immunocompetence, which calls for more research in the area.

DECLARATION

Acknowledgments

This research was not funded by any governmental or non-governmental organization. All financial expenses have been provided by the researchers. The authors would like to thank the authorities f Nangarhar University's Veterinary Faculty and the staff of the faculty's animal farm and laboratory for their tireless efforts to carry out the research.

Authors' contributions

Rozi Khan, Mohammad Azim, and Najibullah created the idea and designed the study. Rozi Khan and Sayed Ziaul Haq collected data. Sayed Attaul Haq performed the statistical analysis and wrote the paper. All authors checked and confirmed the final analysis data and the last revised manuscript before publication in the journal.

Competing interests

The authors declare that there is no conflict of interest in this research work.

Ethical consideration

Before submitting, the authors verified for ethical concerns such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, multiple publishing and/or submission, and redundancy.

Availability of data and materials

The presented data of this study will be sent by the authors upon to a reasonable request.

REFERENCES

Amevor FK, Cui Z, Ning Z, Shu G, Du X, Jin N, Deng X, Xu D, Tian Y, Zhang Y et al. (2022). Dietary quercetin and vitamin E supplementation modulates the reproductive performance and antioxidant capacity of aged male breeder chickens, Poultry Science, 101(6): 101851. DOI: https://www.doi.org/10.1016/j.psj.2022.101851

Attia YA, Abd El-Hamid AE-HE, Abedalla AA, Berika MA, Al-Harthi MA, Kucuk O, Sahin K, and Abou-Shehema BM (2016). Laying performance, digestibility, and plasma hormones in laying hens exposed to chronic heat stress as affected by betaine, vitamin C, and/or vitamin E supplementation. SpringerPlus, 5: 1619. DOI: https://www.doi.org/10.1186/s40064-016-3304-0

Aviagen (2014). Ross PM3 broiler: Nutrition specifications. Aviagen Limited., Newbridge, Midlothian, Scotland. Available at: http://en.aviagen.com/assets/Uploads/RossPM3BroilerNutritionSpecs2014-EN.pdf

Calik A, Emami NK, White MB, Walsh MC, Romero LF, and Dalloul RA (2022). Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, part I: Growth performance, body composition, and intestinal nutrient transporters. Poultry Science, 101(6): 101857. DOI: https://www.doi.org/10.1016/j.psj.2022.101857

Cinar M, Yildirim E, Yigit AA, Yalcinkaya I, Duru O, Kisa U, and Atmaca N (2014). Effects of dietary supplementation with vitamin C and vitamin E and their combination on growth performance, some biochemical parameters, and oxidative stress induced by copper toxicity in broilers. Biological Trace Element Research Journal, 158: 186-196. DOI: https://www.doi.org/10.1007/s12011-014-9926-6

Dalia AM, Loh TC, Sazili AQ, Jahromi MF,and Samsudin AA (2018). Effects of vitamin E, inorganic selenium, bacterial organic selenium, and their combinations on immunity response in broiler

chickens. BMC Veterinary Research, 14: 249. DOI: https://www.doi.org/10.1186/s12917-018-1578-x

Estevez M (2015). Oxidative damage to poultry: From farm to fork. Poultry Science, 94(6): 1368-1378. DOI: https://www.doi.org/10.3382/ps/pev094

Food and agriculture organization of the united nation (FAO) (2017). Food outlook biannual report on global food markets. Available at: https://www.fao.org/3/i7343e/i7343e.pdf

Gao J, Lin H, Wang X, Song Z, and Jiao H (2010). Vitamin E supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. Poultry. Science, 89(2): 318-327. DOI: https://www.doi.org/10.3382/ps.2009-00216

Goni I, Brenes A, Centeno C, Viveros A, Saura-Calixto F, Rebol´e A, Arija I, and Estevez R (2007). Effect of dietary grape pomace and Vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. Poultry Science Journal, 86(3): 508-516. DOI: https://www.doi.org/10.1093/ps/86.3.508

Hunton P (2006). 100 years of poultry genetics. World's Poultry Science Journal, 62(3): 417-428. DOI: https://ww.doi.org/10.1017/S0043933906001048

Kaspers B, Lillehoj HS, and Lillehoj EP (1993). Chicken macrophages and thrombocytes share a common cell surface antigen defined by a monoclonal antibody. Vetetrinary Immunology and Immunopathology, 36(4): 333-346. DOI: https://www.doi.org/10.1016/0165-2427(93)90029-4

Kuttappan VA, Goodgame SD, Bradley CD, Mauromoustakos A, Hargis BM, Waldroup PW, and Owens CM (2012). Effect of different levels of dietary Vitamin E (DL–tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets. Poultry Science, 91(12): 3230-3235. DOI: https://www.doi.org/10.3382/ps.2012-02397

Leshchinsky TV and Klasing KC (2001). Relationship between the level of dietary Vitamin E and the immune response of broiler chickens. Poultry Science, 80(11): 1590-1599. DOI: https://www.doi.org/10.1093/ps/80.11.1590

Lin YF, Chang SJ, Yang JR, Lee YP, and Hsu AL (2005). Effects of supplemental vitamin E during the mature period on the reproduction performance of Taiwan native chicken cockerels. British Poultry Science, 46(3): 366-373. DOI: https://www.doi.org/10.1080/00071660500098186

Lohakare J, Ryu M, Hahn T W, Lee J, and Chae B (2005). Effects of supplemental ascorbic acid on the performance and immunity of commercial broilers. Journal of Applied Poultry Research, 14(1): 10-19. DOI: https://www.doi.org/10.1093/japr/14.1.10

Maggini S, Wintergerst ES, Beveridge S, and Hornig DH (2007). Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. British Journal of Nutrition, 98(S1): 29-35. DOI: https://www.doi.org/10.1017/S0007114507832971

McCorkle FRED and Glick B (1980). The effect of aging on immune competence in the chicken: Antibody-mediated immunity. Poultry Science, 59(3): 669-72. DOI: https://www.doi.org/10.3382/ps.0590669

McKinnon KM (2018). Flow cytometry: An overview. Current Protocols Immunology, 120(1): 1-5. DOI: https://www.doi.org/10.1002/cpim.40

National Research Council (NRC) (1994). Nutrient Requirements of Poultry, 9th Edition. National Academies Press., Washington, D.C. DOI: https://www.doi.org/10.17226/2114

Niu ZY, Liu FZ, Yan QL, and Li WC (2009). Effects of different levels of Vitamin E on growth performance and immune response of broilers under heat stress. Poultry Science, 88(10): 2101-2107. DOI: https://www.doi.org/10.3382/ps.2009-00220

Packer L (1991). Protective role of Vitamin E in biological systems. The American Journal of Clinical Nutrition, 53(4): 1050S-1055S. DOI: https://www.doi.org/10.1093/ajcn/53.4.1050S

Packer L, Weber SU, and Rimbach G (2001). Molecular aspects of α-Tocotrienol antioxidant action and cell signaling. The Journal of Nutrition, 131(2): 369S-373S. DOI: https://www.doi.org/10.1093/jn/131.2.369S

Pitargue FM, Kim JH, Goo D, Delos Ryes J B, and Kil DY (2019). Effect of Vitamin E sources and inclusion levels in diets on growth performance, meat quality, alpha-tocopherol retention, and intestinal inflammatory cytokine expression in broiler chickens. Poultry Science, 98(10): 4584-4594. DOI: http://www.doi.org/10.3382/ps/pez149

Sahin N, Sahin K, Onderci M, Karatepe M, Smith M, and Kucuk O (2006). Effects of dietary lycopene and vitamin E on egg production, antioxidant status and cholesterol levels in Japanese quail. Asian-Australasian Journal of Animal Sciences, 19: 224. DOI: https://www.doi.org/10.5713/ajas.2006.224

Schneider C (2005). Chemistry and biology of vitamin E. Molecular Nutrition Food Research, 49(1): 7-30. DOI: https://www.doi.org/10.1002/mnfr.200400049

Swain BK, Johri TS, and Majumdar S (2000). Effect of supplementation of Vitamin E , selenium and their different combinations on the performance and immune response of broilers. British Poultry Science, 41(3): 287-292. DOI: https://www.doi.org/10.1080/713654938

Tay C, Kanellakis P, Hosseini H, Cao A, Toh BH, Bobik A, and Kyaw T (2020). B Cell and CD4 T Cell interactions promote development of atherosclerosis. Frontiers in Immunology. 10: 3046. DOI: https://www.doi.org/10.3389/fimmu.2019.03046

Traber MG and Stevens JF (2011). Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radical Biology Medicine, 51(5): 1000-1013. DOI: https://www.doi.org/10.1016/j.freeradbiomed.2011.05.017

Vieira V, Marx FO, Bassi LS, Santos MC, Oba A, de Oliveira SG, and Maiorka A (2021). Effect of age and different doses of dietary vitamin E on breast meat qualitative characteristics of finishing broilers. Animal Nutrition, 7(1): 163-167. DOI: https://www.doi.org/10.1016/j.aninu.2020.08.004

Voljc M, Frankic T, Levart A, Nemec M, and Salobir J (2011). Evaluation of different vitamin E recommendations and bioactivity of *α*-tocopherol isomers in broiler nutrition by measuring oxidative stress *in vivo* and the oxidative stability of meat. Poultry Science, 90(7): 1478-1488. DOI: https://www.doi.org/10.3382/ps.2010-01223

Zhao H, Chen Y, Wang S, Wen C, and Zhou Y (2021). Effects of dietary natural vitamin E supplementation on laying performance, egg quality, serum biochemical indices, tocopherol deposition and antioxidant capacity of laying hens. Italian Journal of Animal Science, 20(1): 2254-2262. DOI: https://www.doi.org/10.1080/1828051X.2021.2002733

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 127-135, March 25, 2023

Research Paper, PII: S2322455X2300014-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.14

Effects of *Lactobacillus*-Fermented Feed on Production Performance and Carcass Quality of Broiler Chickens

Rizki Palupi*, Fitri Nova Liya Lubis, Anggriawan Naidilah Tetra Pratama, and Muhakka

Department of Technology and Livestock Industry, Faculty of Agricultural, Sriwijaya University, Indralaya, South Sumatera, Indonesia

*Corresponding author's Email: palupiarda@yahoo.com

Received: 21 December 2022
Accepted: 13 February 2023

ABSTRACT

The quality of broiler chicken carcasses is greatly influenced by feed management and the number of nutrients digested in the digestive tract that will be utilized for optimal meat production. The study aimed to determine the effect of feeding fermented feed at different times on the production performance and quality of broiler chicken carcasses. The number of day-old chicks used in this study was 180 broiler chickens strain Cobb. This study was carried out experimentally using a complete randomized design consisting of four treatments and five replications. Each treatment carried out in this experiment consisted of a different length of time, namely feeding for 2, 3, 4, and 5 weeks. Experimental parameters included feed consumption, weight gain, and ration conversion. In addition, the carcass quality was investigated as live weight, carcass percentage, and percentage of broiler chicken abdominal fat. The results showed that the longer the time of fermentation feed significantly increased feed consumption by 40.07% and increased 13.77% weight gain, as well as decreased ration conversion by 25.33%. Furthermore, the same results were also obtained regarding live weight by 17.80% and increased percentage of the carcass by 8.84%, while the percentage of abdominal fat decreased by 12.90%. It can be concluded that the provision of fermented feed for 5 weeks can improve the production performance and carcass quality of broiler chickens.

Keywords: Broiler chicken, Carcass quality, Fermented feed, Performance

INTRODUCTION

Broiler chickens have a role as a source of animal protein that is in demand by consumers. This demand is because broiler chicken carcasses can be produced faster than other livestock. However, the quality of broiler carcasses is greatly influenced by feed management and the number of nutrients digested in the digestive tract that will be utilized for optimal meat production (Baéza et al., 2022). Various attempts have been made to increase the amount of digested nutrients and increase digestibility by processing feed before consumption. Feed processing that can be pursued is fermenting the feed. One of the feed processing that can be done is by fermenting the feed. The fermentation process can increase feed digestibility and crude protein content and reduce crude fiber in feed (Khempaka et al., 2014). The fermented feed provides several benefits, including improving nutritional properties (reducing fiber and increasing protein content) and intestinal health. It will accelerate broiler chicken's growth (Sugiharto, 2019).

The fermentation process requires an inoculant to speed up the breakdown of nutrients in the feed (Romero et al., 2017). *Lactobacillus* are lactic acid bacteria often used in fermentation (Romero et al., 2017; Singracha et al., 2017; Nair et al., 2019). Lactic acid bacteria will grow and develop on the substrate during fermentation, so the feed contains probiotics utilized by livestock to help the food digestion process. Astuti et al. (2015) found that probiotics could be given at a concentration of 0.6 v/w in broiler up to 28 days of age. The higher the level of the probiotic *Lactobacillus* species in feed, the better the effect on the growth (Pradikta et al., 2018). Previously, Mcnaught and MacFie (2001) stated that many probiotics could attach firmly to intestinal cells, including several types of lactic acid bacteria, such as *Lactobacillus*

casei, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and a large number of *Bifidobacteria*. The ability to stick to the digestive tract will cause probiotic microbes to develop appropriately, and pathogenic microbes such as *Escherichia coli* and *Salmonella Typhimurium* in the digestive tract will be reduced from the animal host cells (McNaught and MacFie, 2001).

The feeding duration of specific feeds will allow probiotics to stick to poultry's digestive tract for a more extended period, affecting livestock production (Al-Khalaifah, 2018; Al-Khalaifa et al., 2019). The results of the research by Zulfan and Zulfikar (2020) and Naji et al. (2015) indicated that fermented feed ingredients could be given in commercial rations without disturbing the growth and increasing the income over feed costs of broiler. Furthermore, feeding in the early phase of growth or during the brooding period can affect the growth of broiler day-old chicks (DOC; Al-Khalaifa et al., 2019). Cell multiplication or hyperplasia occurs when chicks are 1 to 14 days old. The multiplication of these cells includes the development of the digestive tract, respiratory tract, and immune system. Body cells will increase in number by way of cell division. The hyperplasia process will affect further growth in the form of hypertropia growth, cells will increase in size or cell maturation (Fatmaningsih and Nova, 2016). Based on the description above, the current study aimed to determine the effect of fermented feed at different times on the production performance and carcass quality of broiler chickens.

MATERIALS AND METHODS

Ethical approval

An animal feeding experiment was conducted at the experimental station, Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya, South Sumatera, Indonesia. The animals were cared for according to the Indonesian Institute of Sciences Animal Welfare Guidelines. The approval of the experiment was granted from Universitas Sriwijaya with approval number KPPHP-2021-1.

Methods and sampling preparation

This study used 180 DOC of Cobb strain broilers obtained from Charoen Pokphand, Indonesia, with an average weight of 38 g and placed in 20 postal cages with a size of 100 cm × 100 cm. The temperature of the cage environment when conducting the study ranged from 32.2-33°C, with humidity ranged 68.1-85.7. Nine broilers were placed in each cage. The cages had feed dishes, water containers, and 60-watt incandescent lamps for lighting and warmth while the chicks were in them. Water and feed were prepared *ad libitum*. The feed used during the starter period was HI Pro (Charoen Pokphand, Indonesia), the feed is given to DOC up to the age of 14 days, and at the time, the finisher was MR1-P (Cheiljedang, Indonesia), the finisher feed given at the age of 3 to 5 weeks of age. The composition of the nutrients in the feed used during the study is shown in Table 1.

Table 1. Nutrient composition of feed given at the starter and finisher period in broiler chickens strain cobb

Nutrient	Starter feed (1-21 days of age, HI Pro)*	Finisher feed (22-35 days of age, MR1 – P)**
Water content (%)	13	13
Crude protein (%)	22.00-23.00	21.50-23.00
Crude fibre (%)	5.00	4.00
Fat (%)	5.00	8.00
Ash (%)	7.00	6.50
Calcium (%)	0.90	0.90-1.20
Phosphor (%)	0.60	0.70-1.00
Metabolizable energy (kcal/kg)	3,020-3,120	2,750-2,768
Methionine	0.61	0.56
Methionine plus Cysteine	0.78	0.75
Lysine	1.28	1.28
Vitamin C (IU)	300	300
Selenium (ppm)	0.1	0.5

* Incorporated Company Charoen Pokphand, Indonesia, produces the feed. ** Incorporated Company Cheil Jedang Super Feed, Lampung produces the feed.

Before the feed was given to the experimental animals, the feed was fermented for 7 days using Super lacto, a product of the Central Proteina Prima Tbk company, Indonesia, containing the *Lactobacillus burlgarius* bacteria of $8.9x10^{8.}$ $CFUmL^{-1}$. Based on the product label, the fermentation process was carried out by diluting Super lacto at a concentration of 15%. Afterward, feed inoculation process was performed by spraying the inoculant evenly (4% w/v) on the feed. The feed was stirred evenly so that the fermentation process ran optimally. After evenly packing, it was stored for 7 days. Then, 10% of the fermented feed was used in the broiler ration (Sun et al., 2022) based on the length of time defined for the treatments.

As for the measurement of carcass quality in research carried out at the final stage of the study. Samples were taken randomly as many as 2 in each treatment and replicated. Then the chicken samples were fasted for 8 hours before being slaughtered.

Experimental design

This research was conducted using a completely randomized design consisting of four treatments and five replications for each treatment. Each treatment carried out in this experiment consisted of a different length of time, namely feeding for 2 (P1), 3 (P2), 4 (P3), and 5 weeks (P4).

Observed variables

All parameters for calculating the value of observations made in this study are based on research conducted by Palupi et al. (2022).

Feed consumption

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

Body weight gain

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

Feed conversion ratio

Conversion of rations was measured based on the ratio between weight gain and feed consumption.

Live weight

The quality of the broiler carcass included the measurement of live weight, which was measured based on the results of weighing at the end of the study (g).

Carcass percentage

Percentage of the carcass was calculated based on the percentage of weight comparison of broilers without blood, feathers, head, legs, and digestive organs (g) divided by live weight (g). Carcass quality parameters were measured at the end of the study. Slaughtering of chickens is done by as many as two chickens every replication.

Percentage of abdominal fat

Percentage of abdominal fat (%), which is calculated based on the percentage of the comparison between the weight of abdominal fat contained in the abdominal cavity and fat attached to the digestive organs (g) with the live weight of broilers (g).

Statistical analysis

The data will be processed using SPSS software (version 20), based on the design used. Analysis of Variance (ANOVA) analyzes data from observation during the study. If there is a significant difference, a further test is carried out using Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Production performance

The average performance of broiler production observed during the research was feed consumption, body weight gain, and feed conversion. The data are presented in Table 2.

Table 2. Production performance at the starter and finisher period in broiler chicken

Treatment	Feed consumption (g/head/day)	Weight gain (g/head/day)	Feed conversion ratio
P1	78.17 ± 3.45^{a}	51.83 ± 6.24^{a}	1.50 ± 0.22^{a}
P2	86.71 ± 4.12^{b}	55.55 ± 4.88^{a}	1.56 ± 1.45^{a}
P3	96.43 ± 3.41^{c}	60.28 ± 5.61^{b}	1.60 ± 1.03^{a}
P4	118.24 ± 2.66^{d}	65.80 ± 3.87^{b}	1.80 ± 1.56^{b}

^{abc} Different superscript letters in the same column showed a significant difference ($p < 0.05$). P1: Fermented feed for 2 weeks, P2: Fermented feed for 3 weeks. P3: Fermented feed for 4 weeks, P4: Fermented feed for 5 weeks.

Feed consumption

The analysis of variance revealed that the duration of feeding fermented with *Lactobacillus* had a significant effect on the consumption of broiler chicken ration ($p < 0.05$). The average consumption of broiler rations during the study ranged from 78.17 to 118.24 (g/head/day). The most extended fermented feed would affect the feed consumption; this was assumed because adding feed fermented with *Lactobacillus* has better palatability, so broilers prefer it. Widodo et al. (2013) reported that fermented feed could be given to a level of 15% in the ration because fermented feed with *Lactobacillus* provides aromas, flavors, and shapes that broilers prefer, thereby increasing feed consumption.

Further test results showed that the consumption ratio of P1 was significantly different from P2 ($p < 0.05$). It was hypothesized that the increased consumption at each treatment in this study was due to the duration of the

feed fermented with *Lactobacillus* supplementation, which could lead to improved feed quality. Widodo et al. (2013) found that fermented feed had improved quality compared to feed that had not undergone the fermentation process. This improvement in feed quality is because the feed contains *Lactobacillus*, which helps speed up the digestive process, thereby improving the digestibility of the feed. Getachew (2016) revealed that lactic acid bacteria could live and grow in the intestine and produce enzymes, such as proteases and amylases, which could help improve digestion and produce short-chain fatty acids with antibacterial properties to protect nutritive feed. Therefore, feed intake can be improved by adding feed fermented with lactic acid bacteria.

Feed consumption in P2 was significantly different from P3, suggesting that the increased consumption was due to the palatability of the *Lactobacillus*-fermented feed, which was preferred by livestock ($p < 0.05$). Furthermore, the lactic acid produced by lactobacilli in the gut plays a tremendous role in eliminating pathogenic bacteria and maximizing the digestibility of the feed by ensuring that there is no competition for the utilization of the nutrients ingested. This statement is believed to be due to its impact on consumption (Wikanatsiri et al., 2012).

The consumption of rations at P3 was significantly different from that in P4 ($p < 0.05$); it was suspected that feed fermented with *Lactobacillus* could improve palatability. Furthermore, throughout these 4-5 weeks of treatment, the amount of *Lactobacillus* in the intestine could be increased so that the more extended feeding fermented with *Lactobacillus* caused the more significant population of *Lactobacillus* in the digestive tract of broilers, which caused the ratio consumption and feed digestibility increased. Elbaz (2021) reported that broiler rations supplemented with *Lactobacillus* could increase palatability, feed consumption, broiler immune system (health), productivity, efficiency, and feed consumption. According to Hardiningsih et al. (2006), the benefits of providing *Lactobacillus* in mixed feed to broilers included increased palatability, maintaining microflora, helping increase digestive enzyme activity, reducing bacterial enzyme activity and ammonia production, increased feed consumption and digestion, neutralizing both enterotoxins and toxins, and also stimulates the immune system.

Body weight gain

The analysis of variance showed that the feed fermented with *Lactobacillus* had a significant effect ($p < 0.05$) on the increase in the body weight of broilers. It is suspected that feed fermented with *Lactobacillus* can increase feed digestibility in the digestive tract. As mentioned by Astuti et al. (2015), increasing the number of *Lactobacillus* in the intestine will positively affect broilers' growth because *Lactobacillus* bacteria can break down simple carbohydrates into lactic acid. *Lactobacillus* bacteria can maintain the balance of other beneficial bacteria populations in the small intestine so that feed digestibility improves, affecting the increase in broilers' body weight.

The results of further tests showed that the body weight gain of broilers in treatment P1 was not significantly different ($p > 0.05$) with treatment P2 but significantly different ($p < 0.05$) with treatment P3 and P4. This result is thought to be due to the number of *Lactobacillus* in the digestive tract of broiler chickens because the P1 treatment is the same as the P2 treatment; this could be due to various factors. Uzer et al. (2013) *Lactobacilli* maintain beneficial microflora in the gastrointestinal tract, conversely, suppress the growth of pathogenic bacteria, increase digestive enzyme activity, decrease bacterial enzyme activity and ammonia production, increase food intake and digestion, and enterotoxins and neutralize immunity. A stimulating system that does not compete in digesting food.

The body weight gain of broilers in P2 significantly differed from that in P3 ($p < 0.05$), it was suspected that feed fermented with *Lactobacillus* could protect the digestive tract from pathogenic bacteria and streamline feed consumption. According to Astuti et al. (2015), increasing the number of *Lactobacillus* in the intestine will positively affect the growth of chickens which could break down simple carbohydrates into lactic acid. *Lactobacillus*, in addition to maintaining the digestive tract and improving the nutritional value of feed. Body weight gain is closely related to feeding in terms of quantity related to feeding consumption. If feed consumption is disturbed, it will interfere with growth and vice versa; if feed consumption is sufficient, it will increase broiler body weight. The growth of various types of broiler chickens is highly dependent on the feed consumed and is also determined by the development of the digestive tract (Fanatico et al., 2008; Torrey et al., 2021; Singh et al., 2021).

The weight gains of broilers in the P1 and P2 treatments significantly differed from that in P3 and P4 treatments ($p < 0.05$). It is suspected that feeding fermented feed with *Lactobacillus* can increase body weight gain due to its ability to suppress pathogenic

bacteria. Furthermore, it can also increase feed digestibility, which has the effect of increasing the digestibility of protein, where feed fermented with *Lactobacillus* produces proteolytic enzymes in the digestive tract, which then can help digest protein, thereby increasing body weight (Muck et al., 2018). A fermented feed with *Lactobacillus,* suppressing pathogenic bacteria, can also improve the digestive organs, stimulating bile and pancreatic juice so that it affects the body weight gain of broiler chickens (Uguru et al., 2022). Nurhayati et al. (2015) indicated that the use of fermented feed with *Lactobacillus* in broiler chickens could increase the functionality of the digestive organs of broilers, namely stimulating the gallbladder wall to secrete bile and stimulating the secretion of pancreatic juice, which contains amylase, lipase, and protease enzymes to improve the digestion of feed ingredients such as carbohydrates, fats, and proteins. Thus, the more extended feeding of fermented feed with *Lactobacillus* resulted in better body weight gain.

Feed conversion ratio

The analysis of variance showed that the addition of fermented feed containing *Lactobacillus* with 5 weeks curing time (P4) had a significant effect ($p < 0.05$) on the conversion of broiler rations. It was reported that the role of fermented *Lactobacillus* in feed is essential because the feed provided is efficient in increasing the body weight of broilers *Lactobacillus* is a gram-positive microorganism found in milk, fruits, and soil. These lactobacilli can maintain the natural balance of the chicken intestine, so they can function as natural antibiotics (Chen et al., 2005; Chen et al., 2017). Kiha et al. (2012) indicated that *Lactobacillus* in the digestive tract could suppress pathogenic bacteria, so they do not compete in digesting nutrients and maximize nutrient absorption in the digestive tract. The longer addition of feed fermented with *Lactobacillus* can increase the body weight gain of broiler chickens; in addition, the increase in body weight of broiler chickens can be influenced by the consumption of feed and the nutritional content contained in the feed or the consumption of nutrients in the feed. The longer the feeding time fermented with *Lactobacillus*, the greater the body weight gain.

Carcass quality

The average carcass quality of broilers during the study observed was live weight, carcass percentage, and abdominal fat percentage. The data are presented in Table 3.

Table 3. Average carcass quality of broiler chickens

Treatment	Live body weight (g)	Carcass (%)	Abdomen fat (%)
P1	1699.50 ± 13.44^{a}	72.02 ± 6.43^{a}	$1.24 \pm 0.12_{a}$
P2	1797.50 ± 21.06^{a}	75.02 ± 11.03^{a}	1.32 ± 0.11^{b}
P3	1900.75 ± 20.11^{b}	77.65 ± 5.44^{b}	1.40 ± 0.80^{b}
P4	2002.00 ± 16.37^{c}	78.39 ± 7.18^{b}	1.34 ± 0.32^{b}

^{abc} Different superscript letters in the same column showed a significant difference ($p < 0.05$). P1: fermented feed for 2 weeks, P2: fermented feed for 3 weeks. P3: fermented feed for 4 weeks, P4: fermented feed for 5 weeks.

Live body weight

The average live weight obtained in this study ranged 1699.50-2002.00 gr. The results of the analysis of diversity on the final weight of broiler chickens showed the addition of fermented feed containing *Lactobacillus* with 5 weeks curing time (P4) had a significant effect ($p < 0.05$) on the live weight of broiler chickens. It is suspected that *Lactobacillus* can produce lactic acid in the digestive tract so that it can lower the pH in the digestive tract. Low pH conditions will multiply beneficial bacteria, allowing for faster nutrient absorption and increased growth, affecting broiler chickens' live weight (Akhadiarto, 2010).

On the other hand, the results revealed that the live weight of broiler chickens at P1 was not significantly different ($p > 0.05$) from P2 but was significantly different ($p < 0.05$) from P3 and P4 regarding the live weight of broilers. This result indicated that the effect of feeding time fermented with *Lactobacillus* for 2-3 weeks could lead to the same live weight because *lactobacillus* activity on the duration of feeding fermented at P1 and P2 gave the same effect. However, feeding fermented with *Lactobacillus* for 4 and 5 weeks could increase live weight higher than P1 and P2. This result is presumably because of the length of time *Lactobacillus* in the digestive tract can increase the intestinal villi's surface areas, so that nutrient absorption is better, reducing the growth of pathogenic microorganisms. In line with the research results of Ignatova et al. (2009), it was shown that giving a probiotic supplement (*Lactobacillus*) for 5 weeks positively increases the live weight of broilers and livestock products that are safe for consumption.

Feeding fermented with *Lactobacillus* at P2 was significantly different ($p < 0.05$) compared to P3 and P4 on the results of the weight of broiler chickens. It is suspected that the duration of feeding fermented with *Lactobacillus* for 3 weeks is still in average conditions to produce a live weight. Moreover, because the feed fermented with *Lactobacillus* is given only up to 3 weeks,

after more than 3 weeks, the lactic acid bacteria produced from the *Lactobacillus* fermented feed will be less so that the acidic conditions in the digestive tract are normal. According to Rodríguez-Lecompte et al. (2010), adding *Lactobacillus* to the feed will reduce pH and increase the number of microorganisms in the digestive tract, accelerating the growth of digestive organs and allowing them to develop optimally. Feeding fermented with *Lactobacillus* at 4 and 5 weeks resulted in high body weight gain. It can be assumed that the more extended feeding fermented with *Lactobacillus* will result in greater body weight.

Feeding fermented feed with *Lactobacillus* for 4 weeks was significantly different ($p < 0.05$), compared to feeding fermented feed for 5 weeks. Presumably, the longer the feeding is fermented with *Lactobacillus*, the more lactic acid bacteria are produced, which lower the digestive tracts pH, facilitating the metabolic process and producing a high body weight. This case follows the opinion of Elbaz (2021), which indicated that using *Lactobacillus* in feed aims to balance the microflora in the digestive tract to increase the absorption of nutrients to produce ideal body weight.

Carcass percentage

The average percentage of broiler carcass for each treatment during the study can be seen in Table 3. The average percentage of broiler carcasses obtained in this study ranged from 72.02 to 78.39%. The results of the analysis of variance on broiler carcasses showed the addition of fermented feed containing *Lactobacillus* with 5 weeks curing time (P4) had a significant effect ($p < 0.05$) on the percentage carcasses of broilers. This result was because the duration of feeding fermented with *Lactobacillus* can lower the pH of the digestive tract and facilitate the work of the pepsin enzyme so that protein absorption increases. Baéza et al. (2022) stated that the average percentage of broiler carcasses is around 65-78% of the final body weight.

The results of further tests on the percentage carcasses of broiler carcasses showed that the percentage of carcasses in P1 was not significantly different ($p > 0.05$) with P2 but significantly different ($p < 0.05$) with P3 and P4. This result indicated that the effect of feeding fermented feed with *Lactobacillus* for 2 weeks and three weeks resulted in a carcass percentage of 72.02-75.02% lower than P3 and P4. The reason is that feeding fermented with *Lactobacillus* for 2 and 3 weeks has the potential for the growth of pathogenic bacteria, such as *salmonella* bacteria. The fermented feed can minimize the growth of *salmonella* pathogenic bacteria so that the protein digestion process assisted by enzymes will be slower compared to P3 and P4, while the duration of feeding fermented with *Lactobacillus* for 4-5 weeks resulted in a higher carcass percentage than P1 and P2, ranging from 77.64% to 78.39%. *Lactobacillus* acts as a growth promoter which can increase pepsin enzyme in the digestive tract so that the absorption of nutrients in the intestines and the resulting metabolic products can be utilized by the livestock body to form and add new tissues such as meat formation (Ignatova et al., 2009).

Feeding fermented with *Lactobacillus* in treatment P2 significantly differed when compared to P3 and P4 regarding carcass percentage ($p < 0.05$). This reason is that the length of time of fermented feed with *Lactobacillus* will produce a great percentage of the carcass. After all, *Lactobacillus* will produce lactic acid bacteria, which facilitate the work of the digestive tract, resulting in a high percentage of the carcass. Maunatin and Khanifa (2012) stated that lactic acid bacteria in the digestive system could neutralize toxins produced by pathogenic bacteria, affecting enzyme activity in the small intestine so that the blood will circulate nutrients throughout the body to form meat.

Feeding fermented feed with *Lactobacillus* at P3 was not significantly different ($p > 0.05$) with P4. This case was because the 4-week fermented feed had the same number of *Lactobacillus* bacteria as the fermented feed treatment for 5 weeks, so the enzyme performance produced was the same in the P3 and P4 treatments. One of the increased enzyme performances due to the increase in the microbial population is the pepsin enzyme, to break down protein. Then, feed nutrients will be absorbed throughout the body to form meat deposition. Similarly, Mountzouris et al. (2010) found that the use of probiotics in feed can work optimally in the digestive tract by increasing the number of microbial populations, thereby balancing the microflora in the digestive tract, protecting the digestive system, improving intestinal health and increasing livestock productivity.

Abdominal fat percentage

The average percentage of abdominal fat weight ranged from 1.24 to 1.40%. It can be assumed that feeding fermented with *Lactobacillus* facilitates the hydrolysis of carbohydrates in the digestive tract, facilitating the absorption of glucose and monosaccharides. Meanwhile, feed containing easily digestible carbohydrates will result in increased abdominal fat. Following the view of Jha and Mishra (2021), poultry-fed carbohydrate-based diets have

a higher abdominal fat content than fibrous; easily digestible carbohydrates tend to be converted into energy reserves in the form of fat. According to Hidayat (2015), fat formation occurs due to excess energy consumed. Excess energy in broilers will be stored in the form of abdominal fat.

Further test results revealed that the percentage of abdominal fat given fermented feed with *Lactobacillus* at P1 was significantly different ($p < 0.05$) from P2, P3, and P4 in terms of abdominal fat percentage. It is suspected that the feeding duration fermented with *Lactobacillus* at P1 was still in normal condition resulting in a lower percentage of abdominal fat. In contrast, at P2, P3, and P4, the length of feeding fermented with *Lactobacillus* could reduce the activity of the lipase enzyme that plays a role in the rate of acid synthesis. Fouad and El-Senousey (2014) reported that the decrease in abdominal fat deposition with a decrease in the energy content of the ration was due to the reduced activity of lipase enzymes associated with lipogenic processes in the liver. Lipogenesis is a fat deposition process that includes fatty acid synthesis and triglyceride synthesis that occurs in the liver in the mitochondria, cytoplasm, and adipose tissue. Fat in the body originates from feed and is produced from the synthesis process in the liver (Jensen-Urstad and Semenkovich, 2012). Feeding fermented feed with *Lactobacillus* at P2 and P3 was not significantly different ($p > 0.05$) but significantly different from P4. In this case, presumably because feeding fermented with *Lactobacillus* can optimize the absorption of the digestive tract so that the nutritional content of the feed is more directed at the formation of meat and bones than fat in each treatment. Fouad and El-Senousey (2014) stated that nutrition affects the deposition of abdominal fat in the body of broiler chickens. Reduction of body fat deposits in broilers, including abdominal fat, occurs due to a reduction in fatty acid synthesis in the liver and a decrease in lipase enzyme secretion, thereby reducing fat absorption. Zhang et al. (1999) stated that fatty acids reduce the amount of body fat deposition in broilers by suppressing the activity of the lipase enzyme in plasma.

CONCLUSION

According to the present study's findings, adding fermented *Lactobacillus* feed for 5 weeks could increase body weight gain by 16.30%, decrease feed conversion by 11.10% and improve the carcass quality of broiler chickens. Recommendations for further research are the increased application of fermented feed by *Lactobacillus* as a feed mixture to the number of pathogenic and non-pathogenic microbial populations and the characteristics of the digestive organs in broiler chickens.

DECLARATIONS

Acknowledgments

The authors would like to thank the Incorporated Company Satwa Utama Integrasi as a funding source; and Apriadi, Epanria, and Rafi Megansyah for their assistance during the research.

Authors' contributions

All authors developed the theory and supervised the research. Rizki Palupi contributed to the sample collection and analysis calculations. All authors read 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 reviewed the manuscripts for ethical concerns, such as plagiarism, consent to publish, misconduct, data fabrication and falsification, double publishing and submission, and redundancy.

Availability data and materials

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

REFERENCES

Akhadiarto S (2010). Pengaruh pemberian probiotik temban, biovet dan biolacta terhadap persentase karkas, bobot lemak abdomen dan organ dalam ayam broiler [The effect of addition of probiotics product into drinking water on percentage of carcass, body fat and edible offal]. Jurnal Sains Dan Teknologi Indonesia, 12(1): 53-59. DOI: https://www.doi.org/10.29122/jsti.v12i1.851

Al-Khalaifa H, Al-Nasser A, Al-Surayee T, Al-Kandari S, Al-Enzi N, Al-Sharrah T, Ragheb G, Al-Qalaf S, and Mohammed A (2019). Effect of dietary probiotics and prebiotics on the performance of broiler chickens. Poultry Science, 98(10): 4465-4479. DOI: https://www.doi.org/10.3382/ps/pez282

Al-Khalaifah HS (2018). Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. Poultry Science, 97(11): 3807-3815. DOI: https://www.doi.org/10.3382/ps/pey160

Astuti FK, Busono W, and Sjofjan O (2015). The effect of liquid probiotics in broiler feed for broiler production performance. EnviIndonesian Journal of Evironment and Sustainable Development, 6(2): 99-104.

Baéza E, Guillier L, and Petracci M (2022). Review: Production

factors affecting poultry carcass and meat quality attributes. Animal, 16(1): 100331. DOI: https://www.doi.org/10.1016/j.animal.2021.100331

Chen CY, Chen SW, and Wang HT (2017). Effect of supplementation of yeast with bacteriocin and *Lactobacillus* culture on growth performance, cecal fermentation, microbiota composition, and blood characteristics in broiler chickens. Asian Australia Journal of Animal Sciences, 30: 211-220. DOI: https://www.doi.org/10.5713/ajas.16.0203

Chen YS, Yanagida F, and Shinohara T (2005). Isolation and identification of lactic acid bacteria from soil using an enrichment procedure. Letters in Applied Microbiology, 40(3): 195-200. DOI: https://www.doi.org/10.1111/j.1472-765X.2005.01653.x

Elbaz AM (2021). Effects of diet containing fermented canola meal on performance, blood parameters, and gut health of broiler chickens. Journal of World's Poultry Research, 11(1): 1-7. DOI: https://www.doi.org/10.36380/jwpr.2021.1

Fanatico AC, Pillai PB, Hester PY, Falcone C, Mench JA, Owens CM, and Emmert JL (2008). Performance, livability, and carcass yield of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poultry Science, 87(6): 1012-1021. DOI: https://www.doi.org/10.3382/ps.2006-00424

Fatmaningsih R and Nova K (2016). Broiler performance in conventional brooding system and thermos. Jurnal Ilmiah Peternakan Terpadu, 4(3): 222-229. DOI: http://www.doi.org/10.23960/jipt.v4i3.p%25p

Fouad AM and El-Senousey HK (2014). Nutritional factors affecting abdominal fat deposition in poultry: A review. Asian-Australasian Journal of Animal Sciences, 27(7): 1057-1068. DOI: https://www.doi.org/10.5713/ajas.2013.13702

Getachew T (2016). A review on effects of probiotic supplementation in poultry performance and cholesterol levels of egg and meat. Journal of World's Poultry Research, 6(61): 31-36. Available at: https://jwpr.science-line.com/attachments/article/35/J%20World%20Poult%20Res%206(1)%2031-36,%20March%202016.pdf

Hardiningsih R, Napitupulu RNR, and Yulinery T (2006). Isolation and resistance test of several isolates of *Lactobacillus* in low pH. Biodiversitas, 7(1): 15-17. DOI: https://www.doi.org/10.13057/biodiv/d070105

Hidayat C (2015). Reducing abdominal fat deposition in broiler through feeding management. Indonesian Bulletin of Animal and Veterinary Sciences, 25(3): 125-134. DOI: https://www.doi.org/10.14334/wartazoa.v25i3.1157

Ignatova M, Sredkova V, and Marasheva V (2009). Effect of dietary inclusion of probiotic on chickens performance and some blood indices. Biotechnology in Animal Husbandry, 25(6): 1079-1085. Available at: https://www.cabdirect.org/cabdirect/abstract/20103274351

Jensen-Urstad APL and Semenkovich CF (2012). Fatty acid synthase and liver triglyceride metabolism: Housekeeper or messenger?. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids, 1821(5): 747-753. DOI: https://www.doi.org/10.1016/j.bbalip.2011.09.017

Jha R and Mishra P (2021). Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: A review. Journal of Animal Science and Biotechnology, 12: 51. DOI: https://www.doi.org/10.1186/s40104-021-00576-0

Khempaka S, Thongkratok R, Okrathok S, and Molee W (2014). An evaluation of cassava pulp feedstuff fermented with A. oryzae, on growth performance, nutrient digestibility and carcass quality of broilers. The Journal of Poultry Science, 51(1): 71-79. DOI: https://www.doi.org/10.2141/jpsa.0130022

Kiha AF, Murningsih W, and Tristiarti (2012). Pengaruh pemeraman ransum dengan sari daun pepaya terhadap kecernaan lemak dan energi metabolis ayam broiler [The effect of ripening feed with papaya leaf essence ot fat digestibility and metabolic energy in broilers]. Animal Agricultural Journal, 1(1): 265-276. Available at: https://ejournal3.undip.ac.id/index.php/aaj/article/viewFile/207/214

Maunatin A and Khanifa K (2012). Uji potensi probiotik *Lactobacillus* plantarium secara *in-vitro* [*In-Vitro* probiotic test of *Lactobacillus* plantarum]. Alchemy, 2(1): 26-34. DOI: https://www.doi.org/10.18860/al.v0i0.2298

McNaught CE and MacFie J (2001). Probiotics in clinical practice: A critical review of the evidence. Nutrition Research, 21(1-2): 343-353. DOI: https://www.doi.org/10.1016/S0271-5317(00)00286-4

Mountzouris KC, Tsitrsikos P, Palamidi I, Arvaniti A, Mohnl M, Schatzmayr G, and Fegeros K (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poultry Science, 89(1): 58-67. DOI: https://www.doi.org/10.3382/ps.2009-00308

Muck RE, Nadeau EMG, McAllister TA, Contreras-Govea FE, Santos MC, and Kung L (2018). Silage review: Recent advances and future uses of silage additives. Journal of Dairy Science, 101(5): 3980-4000. DOI: https://www.doi.org/10.3168/jds.2017-13839

Nair J, Xu S, Smiley B, Yang HE, McAllister TA, and Wang Y (2019). Effects of inoculation of corn silage with *Lactobacillus* spp. or *Saccharomyces cerevisiae* alone or in combination on silage fermentation characteristics, nutrient digestibility, and growth performance of growing beef cattle. Journal of Animal Science, 97(12): 4974-4986. DOI: https://www.doi.org/10.1093/jas/skz333

Naji SAH, Al-Gharawi JKM, and Al-Zamili IFB (2015). The effect of starting age of feeding wetting fermented feed on the intestinal flora, humoral and cellular immunity of broiler chicks. International Journal of Advanced Research, 3(1): 41-49. Available at: https://www.journalijar.com/uploads/851_IJAR-4590.pdf

Nurhayati, Wirawati CU, and Putri DD (2015). Penggunaan produk fermentasi dan kunyit dalam pakan terhadap performan ayam pedaging dan income over feed and chick cost [The utilization of fermentation product and curcuma in ration on broiler performance, and income over feed and chick cost]. Zootec, 35(2): 379. DOI: https://www.doi.org/10.35792/zot.35.2.2015.9457

Palupi R, Lubis FNL, and Pratama ANT (2022). The effect of the use of cassava tuber (Manihot esculenta) and indigofera zollingeriana leaf flour combination as a source of energy supplemented with citric acid in ration on broiler small intestine characteristics and productivity. Journal of Advanced Veterinary and Animal Research, 9(3): 471-480. DOI: https://www.doi.org/10.5455/javar.2022.i616

Pradikta RW, Sjofjan O, and Djunaidi IH (2018). Evaluasi penambahan probiotik (*Lactobacillus* sp.) cair dan padat dalam pakan terhadap penampilan produksi ayam petelur [Evaluation additional of liquid and powder probiotic (*Lactobacillus* sp.) in feed on production performance of laying Rona]. Jurnal Ilmu-Ilmu Peternakan, 28(3): 203-212. DOI: https://www.doi.org/10.21776/ub.jiip.2018.028.03.03

Rodríguez-Lecompte JC, Brady J, Camelo-Jaimes G, Sharif S, Crow G, Ramirez-Yanez G, Guenter W, and House JD (2010). Intestinal characterization of avian defensins and cytokines after the early administration of probiotic with organic acids in broilers. Avian Immunology Research Group, Budapest, Hungary.

Romero JJ, Zhao Y, Balseca-Paredes MA, Tiezzi F, Gutierrez-Rodriguez E, and Castillo MS (2017). Laboratory silo type and inoculation effects on nutritional composition, fermentation, and bacterial and fungal communities of oat silage. Journal of Dairy Science, 100(3): 1812-1828. DOI: https://www.doi.org/10.3168/jds.2016-11642

Singh M, Lim AJ, Muir WI, and Groves PJ (2021). Comparison of performance and carcass composition of a novel slow-growing crossbred broiler with fast-growing broiler for chicken meat in Australia. Poultry Science, 100(3): 100966. DOI: https://www.doi.org/10.1016/j.psj.2020.12.063

Singracha P, Niamsiri N, Visessanguan W, Lertsiri S, and Assavanig A (2017). Application of lactic acid bacteria and yeasts as starter cultures for reduced-salt soy sauce (moromi) fermentation. LWT, 78: 181-188. DOI: https://www.doi.org/10.1016/j.lwt.2016.12.019

Steel RGD and Torrie JH (1980). Principles and procedures of statistics. McGraw-Hill Inc., New York.

Sugiharto S (2019). A review on fungal fermented cassava pulp as a cheap alternative feedstuff in poultry ration. Journal of World's Poultry Research, 9(1): 1-6. DOI: https://www.doi.org/10.36380/jwpr.2019.0

Sun H, Chen D, Cai H, Chang W, Wang Z, Liu G, Deng X, and Chen Z (2022). Effects of fermenting the plant fraction of a complete feed on the growth performance, nutrient utilization, antioxidant functions, meat quality, and intestinal microbiota of broilers. Animals, 12(20): 2870. DOI: https://www.doi.org/10.3390/ani12202870

Torrey S, Mohammadigheisar M, Santos M, Rothschild D, Dawson LC, Liu Z, Kiarie EG, Edwards AM, Mandell I, Karrow N et al. (2021). In pursuit of a better broiler: Growth, efficiency, and mortality of 16 strains of broiler chickens. Poultry Science, 100(3): 100955. DOI: https://www.doi.org/10.1016/j.psj.2020.12.052

Uguru JO, Amah UJ, Umoren EP, Unigwe CR, Obinna-Echem PC, Onainor ER, Torhemen M, and Ucheji CC (2022). Carcass traits, nutrient composition and sensory properties of broiler chickens fed diets containing graded levels of fermented cassava. Nigerian Journal of Animal Production, 49(1): 268-283. Available at: https://njap.org.ng/index.php/njap/article/download/3426/2647

Uzer F, Iriyanti N, and Roesdiyanto R (2013). The use of functional feed in rations on consumtion and body weight gain broiler. Jurnal Ilmiah Peternakan, 1(1): 282-288.

Widodo AR, Setiawan H, Sudiyono, Sudibya, and Indreswari R (2013). Kecernaan nutrien dan performan puyuh (*Coturnix coturnix japonica*) jantan yang diberi ampas tahu fermentasi dalam ransum [Digestibility of nutrient and performance of male quail (*Coturnix coturnix japonica*) were given tofu waste fermented in the diet]. Tropical Animal Husbandry, 2(1): 51-57. Available at: https://peternakan.fp.uns.ac.id/wp-content/uploads/2019/08/8-Andrik-et-al-51-57-.pdf

Wikanatsiri, Utama CS, and Suyanto A (2012). Aplikasi proses fermentasi kulit singkong menggunakan stater asal limbah kubis dan sawi pada pembuatan pakan ternak berpotensi probiotik [Application of cassava skin sensitive process using a starter from cabbage and mustard greens in the manufacture of probiotic potential livestock feed]. Seminar Hasil Penelitian LPPM Universitas Muhammadiyah, Semarang, Indonesia, pp. 281-288. Available at: https://jurnal.unimus.ac.id/index.php/psn12012010/article/viewFile/521/570

Zhang X, Roland DA, Mcdaniel GR, and Rao SK (1999). Effect of natuphos® phytase supplementation to feed on performance and ileal digestibility of protein and amino acids of broilers. Poultry Science, 78(11): 1567-1572. DOI: https://www.doi.org/10.1093/ps/78.11.1567

Zulfan Z and Zulfikar Z (2020). Performances of broilers fed the commercial diet partly substituted by fermented feed based onthe mixing of corn, rice bran, and leubim fish (*Canthidermis maculata*) processing by-product meal. Jurnal Ilmiah Ilmu-Ilmu Peternakan, 23(2): 92-103. DOI: https://www.doi.org/10.22437/jiiip.v23i2.10723

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 136-142, March 25, 2023

Research Paper, PII: S2322455X2300015-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.15

Effects of Sex and Rearing Season on Body Weight Gain and Growth Curve Parameters of Local Chickens in Niger

Adamou Guisso Taffa [1*], Bachir Hamani[1], Nassim Moula[2,3], Salissou Issa[4], Chaibou Mahamadou[1], and Johann Detilleux[5]

[1]*Department of Animal Production, Agronomy Faculty, Abdou Moumouni University of Niamey, Niamey, Niger*
[2]*Department of Veterinary Management of Animal Resources, Faculty of Veterinary Medicine, University of Liege, 4000 Liege, Belgium*
[3]*GIGA—Animal Facilities—ULiege—B 34, 4000 Liege, Belgium*
[4]*Department of Animal Production, National Institute of Agronomic Research of Niger, Niamey, Niger*
[5]*Department of equine clinical sciences, Faculty of Veterinary Medicine, University of Liege, 4000 Liege, Belgium*

*Corresponding author's Email: guisso373@gmail.com

Received: 18 January 2023
Accepted: 08 March 2023

ABSTRACT

Local chicken breeding is widespread in Niger, a country with harsh environmental conditions. This study aimed to investigate the effects of sex, temperature, and hygrometry variations on the body weight gain and growth curve of local Nigerien chickens. Two groups of local chickens were followed from hatching to 20 weeks of age. The first and second groups consisted of 96 and 124 chickens, respectively. Three seasons were identified based on continuously recording ambient temperature and humidity over a year. The dry and warm seasons (February, March, April, and May), the wet and warm seasons (June, July, August, and September), and the dry and cold seasons (October, November, December, and January). The average hatch weight was about 24 g, and monthly body weight gains ranged from 100 to 360 g. Asymptotic weights were 2214.02 ± 69.94 g and 1776.93 ± 63.57 g for roosters and 1380.25 ± 25.96 g and 1433.08 ± 71.24 g for hens. The sexual maturity rates indicate that hens are more precocious than roosters. Sex and season had significant impacts on the growth performance of the chickens. In conclusion, the results of the present study indicated that the optimal time to raise local chickens in rural Niger is from June to January, and males are better candidates for meat production.

Keywords: Growth curve, Hygrometry, Local chicken, Temperature, Weight gain

INTRODUCTION

Local chicken represents 55% of the Nigerien poultry population (RGAC, 2007). The production of local chickens contributes greatly to food and nutritional security, especially in rural areas (Ousseini et al., 2018). In addition, Niger is one of the countries in sub-Saharan Africa with the harshest agroecological conditions, mainly located halfway between the Sahara and the Sahel. This location results in a very marked seasonality of the climate, characterized by significant temperature and hygrometry differences between the seasons (CNEDD, 2000).

Traditional chickens seem to be acclimatized to all of Niger's agroecological zones because *gallinacea* is present throughout the country (FAO, 2009). However, due to their physiology, chickens are generally sensitive to significant variations in environmental conditions, such as humidity and temperature. Chickens are homeotherms but cannot eliminate heat sweating due to the presence of feathers (Geraert, 1991). In addition, the energy required to activate the physiological mechanisms of adaptation to high temperatures is important. It comes essentially from the diet or, in case of failure, from body reserves in the form of fatty tissue. This dietary energy is preferentially used to satisfy homeostatic needs rather than production needs (Tattersall et al., 2016). Therefore, in hot weather, food consumption increases without a parallel increase in production. This corresponds to poor feed efficiency, and thus, to an increase in production costs. (Geraert, 1991).

The study of local chickens in Niger is relatively new. A few studies have been conducted to characterize their production systems and their morphometry in rural

areas (NECSD, 2000). In an experimental setting, the aim was to assess their growth performance (Hamani et al., 2022a) and their butchery ability (Hamani et al., 2022b) through the use of non-conventional protein sources (housefly larvae) in their feed. Currently, there have been no references to address the effect of temperature and humidity variations on the growth of local chickens in Niger.

In the same environment of sanitary and food monitoring, the variations of temperature and hygrometry could impact the zootechnical performances of chickens (Ranjan et al., 2019). The objective of the present study was to identify the effects of seasonal variations in temperature and humidity on weight gain and growth curve parameters of local chickens in Niamey, Niger.

MATERIALS AND METHODS

Ethics approval

The data collection was carried out in compliance with the animal welfare and biosecurity established by the Department of Animal Production of the Faculty of Agronomy of the University of Niamey, Niger (AFARNi/FA/DPA-002).

Biological material

The chickens studied were obtained by artificial incubation of eggs from local hens. These local hens were raised at the poultry station of the Faculty of Agronomy of the University of Niamey, Niamey, Niger. The physical and chemical characteristics of the eggs of these parent hens were described based on a study by Guisso Taffa et al. (2022). Both groups in this study were from the same parents. The first group had 96 chicks reared from January to May 2021. The second group entailed 124 chicks that were reared from July to November 2021.

Rearing conditions

Both groups were reared in the same building, with the same feed composition and energy density (Table 1). The feed was in the form of crumbs and distributed *ad libitum*. The rearing building was 11 m long, 5 m wide, and 4.5 m high. Figure 1 shows the interior of the rearing building. For the starter phase (first day to 2 weeks), the chicks were kept under artificial lighting and heating until the second week of age. During this starter phase, lighting was maintained from 8:00 a.m. to 10:00 p.m., and heating was only at night (from 10:00 p.m. to 8:00 a.m.). Figure 2 shows the starter boxes (a) and the chicks during the starter phase (b). During the growth phase, the chickens were reared under natural lighting, and the ambient temperature and humidity of the building were not manipulated. They were recorded daily with a thermo-hygrometer indicating daily maximum and minimum temperatures. The chickens were weighed weekly using a digital dial scale with a precision of one gram. All animals were vaccinated against the two main avian diseases of Newcastle and Gumboro endemic in Niger. Table 2 shows the vaccination protocol that was followed in the current study.

Figure 1. The Interior of the breeding building shows the distribution of the chickens in the cages, with 10 chickens per cage

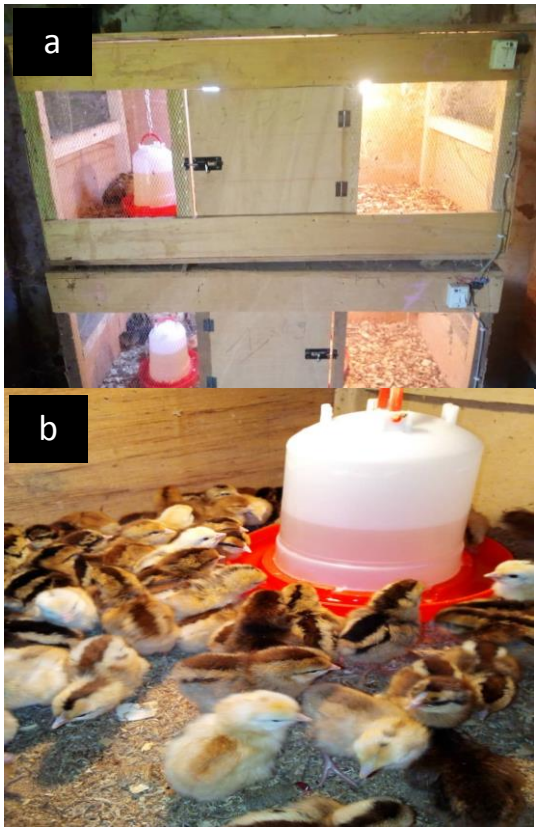


Figure 2. Starter boxes (a) and chicks during the starter phase (b) with lighting and heating system

Table 1. Physical and chemical composition of feeds used for local chickens of Niamey, Niger

Ingredients	Starter (1-14 days)	Grower (15-140 days)
Centesimal composition (%)		
Commercial concentrate *	30	30
Corn	20	20
Wheat Bran	50	50
Analytical composition		
Metabolizable energy (Kcal/kg)	2910.68	2738.70
Dry matter (g/kg)	929	935.9
Fat (g/kg)	65.55	69.56
Mineral matter (g/kg)	73.30	127.47
Ash (g/kg)	10.55	15.49
Crude protein (g/kg)	229.82	172.77
Non-nitrogenous extractives (g/kg)	620.78	614.70

*: An imported commercial feed (Animal Care Services Konsult, Ogere, Nigeria), According to the manufacturer, the essential mineral content of concentrates included a starter (4% Calcium, 4%assimilable phosphorus), and Grower (2.20% Calcium, 1.2% available phosphorus).

Table 2. Vaccination schedule followed during the breeding of the two groups of Niamey's local chickens

Period	Disease	Vaccine	Route
Day 1	Newcastle (NC)	LaSota (Zoetis INC, US)	Oral
Day 5	Gumboro (GB)	Gumboro VAC (Elanco, Netherlands)	Oral
Day 8	Immunization reminder NC	LasSota (Zoetis INC, US	Oral
Day 14	Immunization reminder GB	Avipro Gumboro VAC (Elanco, Netherlands)	Oral
Week 8	Immunization reminder NC	ITA-New (Laprovet, Hungary)	Sub-dermal
Week 16	Immunization reminder NC	ITA-New (Laprovet, Hungary)	Intra-muscular

Each vaccination was accompanied by 3 days of antistress (Introvit A+ or AnimTotal) in the drinking water.

Data analysis and processing

The processing of all data was performed by the free software R project, version 4.1.1 (R project, 2021). The descriptive statistics of the data were performed with the pastecs package, version 1.3.21 (Grosjean et al., 2018).

Analysis of the physical data

Different months were grouped to correctly integrate the temperature and hygrometry data into the multiple linear regression, a grouping of was performed. The aim was to identify seasons with different characteristics regarding temperature and humidity. To do this, the Elbow method, based on the minimization of the sum of squares of deviations (SSwithin), was used to identify the optimal number of groups (DellaData, 2020).

Parameters of the individual growth curve

Non-linear regression using the Gompertz equation was used to determine the parameters of the growth curve of the chickens (Mignon-Grasteau and Beaumont, 2000). These parameters were determined individually for each subject with the package easynls version 5.0 (Arnhold, 2017). The Gompertz equation is as follows:

Yt = Ae^ (-Be^ [-kt])

Yt means weight at time t, A denotes Asymptotic weight, B signifies integration constant, and k is maturation rate.

Multiple regression model

The lm function of the native stats package of the R software was used to evaluate the effects of sex, birth season, and their interaction on hatch weight, monthly weight gains, and growth curve parameters.

The following linear fixed-effects model was used:

$Y_{ijk} = \mu + a_j + b_k + ab_{jk} + \varepsilon_{ijk}$,

Where, Yijk is the hatch weight or monthly body weight gain or one of the growth curve parameters of the ith animal with sex j born in season k; μ denotes the overall mean, aj is the sex fixed effect (j = [1,2]), bk signifies the season fixed effect (k = [season1, season2, season3]), abjk is the sex-season interaction, and εijk accounts for the random residual error.

For each of the models, the significance of the effects of gender and season was identified by comparing the factorial variances to the residual variances using the Fischer test at the significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

Parameters of the growth curves

The parameters of the Gompertz curve were calculated with the weights measured every week. These weights were recorded as P0, P1, P2 up to P20, corresponding to the ages of 1 day (hatching), week 1, week 2, until week 20. Therefore, the estimation is only possible for the two rearing groups and not for the climate

clusters. Thus, for both groups, the males had higher parameters than the females (Table 3). In both groups, the asymptotic weight (A), corresponding to the maximum theoretical weight that could be reached, was higher in males, while the maturation rate of females was higher than that of males.

Previous studies have reported results similar to the present study. In the study by Yapi-Gnaore et al. (2011), where chickens were followed from hatching to 22 weeks of age by weighing them every 2 weeks, males of the Forest and Savannah ecotypes had asymptotic weights of 2220 and 2160 g, respectively, compared to 1570 and 1501 g for females of these same ecotypes. In contrast, at the age of 22 weeks for both sexes, the maturation rates of the 'Forest' and Savanna ecotypes were 0.0189 and 0.0200 g/day for males and 0.0199 and 0.0205 g/day for females, respectively.

The higher maturation rate of females than males indicates that females of the local Niamey hen would reach sexual maturity faster than males. This conclusion was also reached in the study of Moula et al. (2009) and N'Dri et al. (2018), who indicated that traditional chicken strains were up to 20 weeks of age for both studies.

Table 3. Growth curves parameters of Niamey's local chickens by sex and group in Niger

Parameters	Female		Male	
	Group1	Group 2	Group 1	Group 2
A (g)	1380.25 ± 25.96	1433.08 ± 71.24	2214.02 ± 69.94	1776.93 ± 63.57
B	3.998 ± 0.048	3.741 ± 0.056	4.359 ± 0.040	3.879 ± 0.043
K (g /day)	0.0191 ± 0.0004	0.0203 ± 0.0006	0.0179 ± 0.0004	0.0199 ± 0.0007

Values are reported as mean ± standard deviation A: Asymptotic weight; B: Integration constant, K: Maturation rate, Group 1: 96 chicken, Group 2: 124 chickens.

Monthly weight gain

Table 4 tabulates the values of hatching weights and monthly weight gains of the local chicken groups in Niamey. The weight at the hatching of the chicks was about 24 g. From week 4, the chicks gain 200 g of weight every month.

The average hatching weight of chicks obtained in this study is similar to those reported by Youssao et al. (2012) and Binda et al. (2012), who studied traditional chickens without sex distinction for chicks. However, those reported by Akouango et al. (2010) and N'Dri et al. (2018) were higher than the present study. Hatching weight is a parameter related to egg weight, which depends on the hen's age and size. Older and heavier hens lay larger eggs (Nys et al., 2018; Travel et al., 2010). Thus, the low weight of the chicks could be explained by the low weight of the local hens.

In relation to monthly weight gains, Binda et al. (2012) simultaneously studied three traditional strains and two commercial broiler chicken strains (Hybro and Hubbar). The traditional strains in their work showed similar monthly weight gains at 4 and 8 weeks as in the present study. However, the monthly weight gains they recorded for the exotic strains were about three times those of the present study. Therefore, it can be deduced that the low growth rate of the local chickens is mainly related to their genetics. In the present study, the chickens were fed *ad libitum* with high nutritional quality and well-balanced feed accompanied by adequate sanitary monitoring and prophylaxis. Thus, the only limiting factor would be the genetic potential of these animals.

Grouping of the months into seasons

The grouping performed allowed us to divide the months into three categories based on ambient temperature and humidity (Table 5).

This division of months into three categories does not correspond perfectly to the natural climate officially recognized in Niger. Indeed, it is reported that in Niger, the rainy season is from June to September and is characterized by heavy rainfall, wind, and a relative drop in temperature. The rest of the year is dry and sunny. The coldest months are from December to February. On the contrary, the months from March to May are the warmest, reaching 45 degrees in the shade with very moderate decreases at night (FAO, 2005). However, the grouping made in this study assigns the month of February to the category of hot and dry months. This difference could be explained by the fact that the temperature and humidity data used to establish this categorization come from a single environment (the experimental site).

Table 4. Descriptive statistics of hatching weight and monthly weight gain of male and female chickens of Niamey

Parameters	Groups	Sex	N	Min	Max	Mean	SEM
P0	1	Male	41	20	30	24.76	0.52
		Female	55	20	30	25	0.49
	2	Male	66	13	30	23.73	0.38
		Female	58	17	31	23.17	0.39
GP_0-4	1	Male	41	70	195	139.07	4.38
		Female	55	12	160	105.31	4.09
	2	Male	66	75	269	184.86	4.71
		Female	58	90	231	172.24	3.57
GP_4-8	1	Male	41	193	338	272.22	5.53
		Female	55	153	278	210.47	4.10
	2	Male	66	102	401	267.39	7.79
		Female	58	110	368	215.95	6.87
GP_8-12	1	Male	41	258	507	391.85	9.93
		Female	55	160	383	286.27	5.87
	2	Male	66	134	470	313.42	7.51
		Female	58	171	571	262.78	9.74
GP_12-16	1	Male	41	170	473	364.05	9.07
		Female	55	108	313	210.98	5.99
	2	Male	66	81	497	302.30	9.89
		Female	58	116	346	205.55	6.70
GP_16-20	1	Male	41	195	475	332.29	10.95
		Female	55	120	344	212.71	6.98
	2	Male	66	133	469	234.94	8.07
		Female	58	112	551	197.21	10.80

N: Number, Min: Minimum; Max: Maximum; SEM: Standard error mean; P0: Weight at hatching; GP_0-4: Weight gain from hatching to week 4; GP_4-8: Weight gain from week 4 to week 8; GP_8-12: Weight gain from week 8 to week 12; GP_12-16: Weight gain from week 12 to week 16; GP_16-20: Weight gain from week 16 to 20

Table 5. Characteristics of the identified seasons

Seasons	T-min (°C)	T-max (°C)	H-min (%)	H-max (%)	Characteristics
1	24.13	41.40	20.14	31.64	Hot and dry
2	27.23	30.40	20.79	49.17	Hot and humid
3	17.24	36.93	19.89	33.07	Cold and dry

T-min: Minimum temperature; T-max: Maximum temperature; H-min: Minimum humidity; H-max: Maximum humidity. Season 1: February, March, April, and May. Season 2: June, July, August, and September Season 3: October, November, December, and January

Table 6. Effects of sex, season, and their interaction on monthly body weight gain and growth curve parameters of Niamey's local chickens

Parameters	Mean ± Standard error	p-values			Adjusted R^2
		Sex	Season	Sex*Season	
P0 (g)	24.17 ± 0.44	0.891 ^{NS}	0.001 ***	0.368 ^{NS}	0.038
GP_0-4 (g)	105.31 ± 4.29	7.5×10^{-10} ****	2.2×10^{-16} ****	0.016 *	0.496
GP_4-8 (g)	210.47 ± 6.59	3.5×10^{-15} ****	0.928 ^{NS}	0.446 ^{NS}	0.241
GP_8-12 (g)	286.27 ± 8.31	8.9×10^{-15} ****	1.6×10^{-8} ****	0.001 **	0.337
GP_12-16 (g)	210.98 ± 8.25	2.2×10^{-16} ****	0.00016 ***	0.00087 ***	0.507
GP_16-20	212.71 ± 9.20	4.5×10^{-12} ****	2.3×10^{-8} ****	1.9×10^{-5} ****	0.321
A (g)	1380.20 ± 60.95	4.2×10^{-16} ****	0.0042 **	0.0001 ***	0.310
B	3.998 ± 0.048	7.1×10^{-5} ****	3.6×10^{-12} ****	0.0246 *	0.250
K (g/day)	0.0191 ± 0.0005	0.365 ^{NS}	0.00694 **	0.512 ^{NS}	0.025

Sex*Season: interaction between sex and season; P0: Weight at hatching, GP_0-4: Weight gain from hatching to week 4, GP_4-8: Weight gain between weeks 4 and 8, GP_8-12: Weight gain between weeks 8 and 12, GP_12-16: Weight gain between weeks 12 and 16, GP_16-20: Weight gain between weeks 16 and 20; A: Asymptotic weight, B: Integration constant, K: Maturation rate; Significance levels (NS: Not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$)

Effects of sex and season on growth parameters

The effects of sex, season, and their interaction on monthly body weight gain and growth curve are summarized in Table 6. Except for hatch weight and maturation rate, sex significantly affected all other parameters. The effect of season was non-significant only for body weight gain between weeks 4 and 8 ($p > 0.05$). These results indicated that similar to sex, the rearing period also impacts the growth performance of the local Niamey chicken.

The effect of sex is attributable to the sexual dimorphism in this species. Remeš and Székely (2010) investigated a set of 139 breeds of domestic chickens and reported that, on average, males were 21.5% heavier than females, this difference was even more remarkable (68%) in the red jungle fowl (wild species of chicken), where the male was 68.8% heavier than the female. Furthermore, this dimorphism is under hormonal control, as Johnson (1988) had shown that, in chickens, after one week of age, the concentration of growth hormones in the blood plasma of males was significantly higher than that of females.

The effect of seasons on performance was thought to be a consequence of temperature and humidity variations. Chickens are particularly sensitive to high temperatures, which impacts their feeding behavior and efficiency. The impact on feeding behavior was reflected in decreased feed intake and increased water consumption (Scanes et al., 1984). The body preferentially redirects food energy in the process of homeostasis (Tattersall et al., 2016; Ranjan et al., 2019).

CONCLUSION

The results of this study showed that the growth of local chickens in Niamey is affected by sex and variations in temperature and humidity. Weight gain in males is significantly higher than in females at all ages. The effect of temperature and humidity variation is also present at all ages except from the 4 to 8-week period. In a rural context, the optimal period for rearing these chickens would be from June to January. In experimental conditions, maintaining the temperature between 27-36°C and the ambient humidity between 20-49% would be beneficial to the growth of these chickens in Niger. It would be interesting to complete this study by investigating the reproductive aspects (fertility and hatchability of eggs) in both experimental and rural environments.

DECLARATIONS

Funding

This research was funded by ARES (Academy of Research and Higher Education of Belgium, Liege, Belgium) in the framework of Research project for the development AFARNi (Improvement of the poultry sector in the Niamey region, Niamey, Niger).

Authors' contributions

Adamou Guisso Taffa contributed to the conceptualization, investigation, data curation, writing, review, and editing of the manuscript.

Bachir Hamani contributed to the conceptualization, investigation, writing, and review of the manuscript.

Salissou Issa and Nassim Moula contributed to conceptualizing and reviewing the manuscript.

Chaibou Mahamadou and Johann Detilleux were responsible for the conceptualization, resource provision, supervision, and project administration.

All authors have checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

There are no competing interests during this study. The funders were not involved in the study design, data collection, and analysis, nor in the writing of the manuscript.

Ethical considerations

All relevant ethical issues have been checked by all the authors. The authors declared that they presented all data related to this original study in the current manuscript, and the final draft of the manuscript is submitted only to the present journal.

Availability of data and materials

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

REFERENCES

Akouango F, Bandtaba P, and Ngokaka C (2010). Weight growth and productivity of the local chicken *Gallus domesticus* on a farm in Congo. Animal Genetic Resources, 46: 61‑65. DOI: https://www.doi.org/10.1017/S2078633610000706

Arnhold E (2017). Easy nonlinear model. Fit and plot some nonlinear models, Version (5.0). pp. 1-9. Available at: https://www.CRAN.R-project.org/package=easynls

Binda BD, Yousif IA, Elamin KM, and Eltayeb HE (2012). A comparison of performance among exotic meat strains and local chicken ecotypes under Sudan conditions. International Journal of Poultry Science, 11(8): 500‑504. DOI: https://www.doi.org/10.3923/ijps.2012.500.504

DellaData (2020). The kmeans method. Available at: https://www.delladata.fr/kmeans/

Food and agricultural organization (FAO) (2005). Irrigation in Africa in numbers - Survey Aquastat. p. 12. Available at: https://www.fao.org/3/a0232f/a0232f.pdf

Food and agricultural organization (FAO) (2009). Review of the poultry sector in Niger (Expert report). FAO, animal production and health division, Niger, p. 69. Available at: http://www.fao.org/3/ak770f/ak770f00.pdf

Geraert PA (1991). Energy metabolism of broilers in hot climates. INRAE Productions Animales, 4(3): 257-267. DOI: https://www.doi.org/10.20870/productions-animales.1991.4.3.4340

Grosjean P, Ibanez F, and Etienne M (2018). Pastecs: Package for analysis of space-time ecological series. Regularisation, decomposition and analysis of space-time series, version (1.3.21). pp. 3-72. Available at: https://www.CRAN.R-project.org/package=pastecs

Guisso Taffa A, Issa S, Maman-Bachir SA, Detilleux J, Mahamadou C, and Moula N (2022). Production and physico-chemical characteristics of the eggs of the local hen of Niamey (Niger). Tropicultura, 40(3-4): 2144. DOI: https://www.doi.org/10.25518/2295-8010.2144

Hamani B, Guisso Taffa A, Issa S, Mahamadou C, Detilleux J, and Moula N (2022a). Effects of feeding housefly (*Musca domestica*) larvae on the butchery skills and meat sensory characteristics of local chickens in Niger. Veterinary Sciences, 9(10): 553. DOI: https://www.doi.org/10.3390/vetsci9100553

Hamani B, Moula N, Guisso Taffa A, Leyo IH, Mahamadou C, Detilleux J, and Van QCD (2022b). Effect of housefly (*Musca domestica*) larvae on the growth performance and carcass characteristics of local chickens in Niger. Veterinary World, 15(7): 1738‑1748. DOI: https://www.doi.org/10.14202/vetworld.2022.1738-1748

Johnson RJ (1988). Diminution of pulsatile growth hormone secretion in the domestic fowl (*Gallus domesticus*): Evidence of sexual dimorphism. Journal of Endocrinology, 119(1): 101‑109. DOI: https://www.doi.org/10.1677/joe.0.1190101

Mignon-Grasteau S and Beaumont C (2000). Growth charts in birds. INRAE Productions Animals, 13(5): 337‑348. DOI: https://www.doi.org/10.20870/productions-animales.2000.13.5.3802

Moula N, Antoine-Moussiaux N, Farnir F, Detilleux J, and Leroy P (2009). Socio-economic rehabilitation of an endangered local chicken: The Kabyle chicken (*Thayazit lekvayel*). Annale de Médecine Vétérinaire, 153: 178‑186. Available at: http://www.facmv.ulg.ac.be/amv/articles/2009_153_3_05.pdf

N'Dri A, Koua B, Ahouchi V, and Adepo-Gourene A (2018). Body weight and growths curve parameters evaluation of three chicken genotypes (*Gallus gallus domesticus*) reared in claustration. Journal of Advanced Veterinary and Animal Research, 5(2): 188-195. DOI: https://www.doi.org/10.5455/javar.2018.e265

National environment council for sustainable development (CNEDD) (2000). National action program to combat desertification and manage natural resource Management (PAN-LCD/GRN). Office of the Prime Minister, Niger, p. 80. Available at: https://duddal.org/s/bibnum-promap/item/8535

Nys Y, Jondreville C, Chemaly M, and Roudaut B (2018). Qualités des œufs de consommation [Quality of table eggs]. In: V. Berthelot (Editor), Feeding animals and the quality of their products. Tec & Doc Lavoisier., France, chapter 9, p. 315-338.

Ousseini MH, Keambou TC, Hima K, Issa S, Joseline MS, and Bakasso Y (2018). Indigenous chicken production in Niger. Veterinary and Animal Science, 7: 100040. DOI: https://www.doi.org/10.1016/j.vas.2018.11.001

R project (2021). The R project for statistical computing. Version 4.1.1. Available at: https://www.r-project.org/

Ranjan A, Sinha R, Devi I, Rahim A, and Tiwari S (2019). Effect of heat stress on poultry production and their managemental approaches. International Journal of Current Microbiology and Applied Sciences, 8(2): 1548‑1555. DOI: https://www.doi.org/10.20546/ijcmas.2019.802.181

Remeš V and Székely T (2010). Domestic chickens defy Rensch's rule: Sexual size dimorphism in chicken breeds. Journal of Evolutionary Biology, 23(12): 2754‑2759. DOI: https://www.doi.org/10.1111/j.1420-9101.2010.02126.x

General census of agriculture and livestock (RGAC) (2007). Project GCP/NER/041/EC. Ministry of Agricultural Development and Ministry of Animal Resources. p. 118. Available at: https://duddal.org/s/bibnum-promap/item/7827

Scanes CG, Harvey S, Marsh JA, and King DB (1984). Hormones and growth in poultry. Poultry Science, 63(10): 2062‑2074. DOI: https://www.doi.org/10.3382/ps.0632062

Tattersall GJ, Roussel D, Voituron Y, and Teulier L (2016). Novel energy-saving strategies to multiple stressors in birds: The ultradian regulation of body temperature. Proceedings of the Royal Society B, 283: 20161551. DOI: https://www.doi.org/10.1098/rspb.2016.1551

Travel A, Nys Y, and Lopes E (2010). Physiological and environmental factors influencing egg production and quality. INRAE Productions Animales, 23(2): 155-166. DOI: https://www.doi.org/10.20870/productions-animales.2010.23.2.3297

Yapi-Gnaore VC, Loukou EN, Kayang B, and Rognon X (2011). Poids vif et parametres de la courbe De croissance des poulets de race locale (*Gallus domesticus*) en côte d'ivoire. [Live weights and parameters of the growth curve of local chickens (*Gallus domesticus*) in Côte D'Ivoire]. Agronomie Africaine, 23(3): 273‑281. Available at: https://www.ajol.info/index.php/aga/article/view/77824

Youssao IAK, Alkoiret IT, Dahouda M, Assogba MN, Idrissou ND, Kayang BB, Yapi-Gnaore V, Assogba HM, Houinsou AS, Ahounou SG et al. (2012). Comparison of growth performance, carcass characteristics and meat quality of benin indigenous chickens and Label Rouge (T55A-SA51). African Journal of Biotechnology, 11(89): 15569‑15579. DOI: https://www.doi.org/10.5897/AJB11.1747

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 143-148, March 25, 2023

Research Paper, PII: S2322455X2300016-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.16

Effects of Storage Time on Ostrich Egg Quality

John Cassius Moreki[1]*, Denise Florence Mosarwa[1], Joshua Makore[2], and Nicholas Mosweu[1]

[1]*Department of Animal Sciences, Faculty of Animal and Veterinary Sciences, Botswana University of Agriculture and Natural Resources, P/Bag 0027, Gaborone, Botswana*
[2]*Department of Biometry and Mathematics, Faculty of Sciences, Botswana University of Agriculture and Natural Resources, P/Bag 0027, Gaborone, Botswana*

*Corresponding author's E-mail: jmoreki@buan.ac.bw

Received: 19 January 2023
Accepted: 08 March 2023

ABSTRACT

Egg quality is considered as both internal egg quality that focuses on the egg content and external egg quality that focuses on the eggshell. This study investigated the effect of storage time on ostrich egg quality. A total of 15 ostrich eggs were obtained from Dibete Ostrich Multiplication Unit and subjected to five storage periods (0, 3, 6, 9, and 12 days) at room temperature (18-25^{o}C). The measured parameters were egg weight, egg length, egg width, yolk weight, albumen weight, albumen height, yolk height, shell weight, egg specific gravity (ESG), egg surface area, Haugh Unit (HU), egg shape index, albumen ratio, shell ratio, yolk ratio and weight of egg contents. Results showed that storage time did not influence egg weight for eggs stored for 0, 3, and 6 days. On the other hand, storage time significantly affected egg weight for eggs stored at 9 and 12 days. The albumin ratio for egg storage duration had no significant impact on eggs held for 0, 3, or 6 days. However, the albumen ratios of eggs held for 9 and 12 days were impacted by the storage period compared to those stored for 0, 3, and 6 days. The HU for the eggs stored for 0, 3, and 6 days was not affected by storage time. On the contrary, the HU for eggs stored for 9 and 12 days was significantly impacted by storage time as the HU decreased with the prolonged storage time, compared to those stored for 0, 3, and 6 days. The results of this study suggest that ostrich eggs should not be stored for more than 6 days at ambient temperature to avoid egg quality degradation.

Keywords: Cuticle, Egg quality, Ostrich eggs, Storage time

INTRODUCTION

An ostrich (*Struthio camelus*) is the largest and longest bird that belongs to the ratite family, *Struthionidae*. Ostrich eggs are the largest eggs measuring 15.4 cm long and 12.7 cm wide (Cooper et al., 2009), with a thick eggshell of about 1.8 cm that is very resistant to breakage (Ceylan et al., 2006). Similarly, Di Meo et al. (2003) reported the average ostrich egg length and width of 15.4 cm and 12.9 cm, respectively. However, Al-Obaidi et al. (2012) in Iraq found the ostrich egg length and width to be 16.9 cm and 12.6 cm, respectively. The weight and shell of the egg are the main external and internal quality indicators of an egg (van Niekerk, 2014). The internal egg quality focuses on the yolk and the egg white, and the external quality on the soundness of the shell. The amount of time that ostrich eggs are stored significantly affects their quality. Factors such as water activity, temperature, the gaseous environment, and environmental pollutants have an impact on storage (Ceylan et al., 2006).

Eggshells are enclosed in a cuticle, a protective layer that regulates the exchange of gases across the shell and acts as a first line of defense against microbial penetration across the eggshell (Samiullah and Roberts, 2014). Therefore, the washing of eggs is not recommended, especially for eggs from older hens with thinner shells, as it might result in damage to the cuticle leading to bacteria entering into the egg, thus risking the consumers' lives. According to Hassan et al. (2005), storage time affects egg weight loss; hence ostrich eggs should be stored at 16^{o}C for at least 7 days. The study by Sahan et al. (2003) showed that ostrich eggs must be stored at 21^{o}C or less to

maximize hatchability, whereas Archer and Cartwright (2017) recommend storage temperatures of 13 to 18°C for no more than 10 days. Ograk and Altinel (2014) mentioned that ostrich eggs should not be stored for over 10 days while Moreki et al. (2016) stated that ostrich eggs must be stored for at least 11 days to avoid degrading the egg quality characteristics. According to Wilson et al. (1997), the date the egg is laid is a factor that determines how long ostrich eggs can be stored.

Many available published sources give estimates of the maximum storage time for ostrich eggs; however, it is difficult to find up-to-date information specifically suitable for Botswana's climate and biological conditions. Therefore, the objective of this study was to investigate the effects of five storage periods (i.e., 0, 3, 6, 9, and 12 days) on the ostrich egg quality characteristics at room temperature.

MATERIALS AND METHODS

Study site

The experiment was carried out in May 2021 in the Meat Science Laboratory at the Botswana University of Agriculture and Natural Resources (BUAN), Gaborone, Botswana.

Sample preparation

The ostriches at Dibete Ostrich Multiplication Unit (DOMU), a government facility located about 100 km northeast of Gaborone, the capital city of Botswana, were sourced from South Africa and Zimbabwe at different ages. The DOMU is the only operational ostrich facility in the country from which we obtained the eggs for this study, as other facilities have collapsed due to myriad challenges, including the high cost of feeds, the frequent closures of the sole ostrich abattoir, lack of access to credit facilities and lack of technical extension support (Moreki et al., 2012). The ostriches found at DOMU are crosses of South African black and Zimbabwean blue. A total of 15 eggs used in this study came from multiple-age parents. DOMU is located on the periphery of Dibete village of Central District and lies within latitude 22° south and 24° south, north of Gaborone, and longitude 26° west and 28° west (Moreki et al., 2012).

Eggs were bought according to the dates they were laid on the farm and were stored at five different storage periods, including 0, 3, 6, 9, and 12 days at room temperature (20-25°C). Eggs were placed in wooden trays with their sharp ends pointing downwards and stored at room temperature before data collection in accordance with Hassan et al. (2005). In this study, eggs were not covered during storage. The windows for the room in which eggs were stored were opened during the day and closed at night, indicating that the room was naturally ventilated. At 3-day intervals, three eggs were opened and analyzed for egg quality.

Data collection

Prior to breaking eggs for egg quality determination, eggs were individually weighed using an electronic scale sensitive to 0.01 g and their weight was recorded. Egg width and length were measured using Vernier calipers sensitive to 0.01 mm. Thereafter, eggs were carefully opened using a saw to avoid mixing the internal contents, including albumen and the yolk. The contents were removed from the shell and poured into containers. Following the emptying of the shell contents into a container, the shell with membranes was washed with clean water and wiped with a paper towel. Thereafter, shell thickness and shell weight were measured using a Vernier caliper and electronic scale, respectively (Nonga et al., 2010). Shell thickness was measured only at the equator of the egg. The weight of egg contents was obtained by subtracting shell weight from egg weight.

The albumen and yolk were separated by pushing them into separate containers and weighed separately. After separating the yolk from the albumen, the height and the length of the yolk and albumen were measured using a Vernier caliper. The Haugh unit (HU), shell ratio, albumen ratio egg ratio, egg surface area, shell ratio, and egg specific gravity (ESG) were evaluated according to El-Safty and Mahrose (2009) using the formulae below.

$$\text{Egg specific gravity (g/cm}^2\text{)} = \text{Egg weight/Egg volume}$$
$$\text{Egg shape index} = \text{Egg width/Egg length} * 100$$
$$\text{Shell ratio (\%)} = \text{Shell weight/Egg weight} * 100$$
$$\text{Albumen ratio (\%)} = \text{Albumen weight/Egg weight} * 100$$
$$\text{Yolk ratio (\%)} = \text{Yolk weight/Egg weight} * 100$$
$$\text{Yolk index} = \text{Yolk weight/Yolk diameter} * 100$$
$$\text{Haugh unit} = 100 \log (H - 1.7W0.37 + 7.6)$$

Where, H is albumen height and W denotes the egg weight

$$\text{Egg surface area} = 3.9782W^{0.7056}$$

Where, W is the egg weight

Statistical analysis

Data on egg quality traits were subjected to analysis of variance (ANOVA) and analyzed using Statistical Analysis System (SAS) Software (version 9.4, 2012). Duncan's Multiple Range test was used to find significant differences among the means ($p < 0.05$).

RESULTS AND DISCUSSION

Egg quality characteristics

The effects of storage time on egg quality characteristics are presented in Table 1. The egg weight for eggs stored for 0, 3, and 6 days was not affected by storage time, compared to 9 and 12 days ($p > 0.05$). However, compared to 0, 3, and 6 days, storage time had a significant impact on egg weight at 9 and 12 days ($p < 0.05$). Furthermore, the weight of eggs stored for 0 and 9 days was similar to those stored for 3, 6, and 12 days ($p > 0.05$). Eggs that were kept for 9 and 12 days had similar weights. This finding agrees with Khan et al. (2014), who reported a negative effect of storing eggs for more than 3 days on egg weight, hatchability, embryonic development, and hatchling weight in Fayoumi chickens. Similarly, Samli et al. (2005) observed that egg weight decreased with an increased storage time of up to 10 days.

No statistical difference was observed for the egg shape index (ESI) of eggs stored for 0, 3, and 6 days, compared to eggs stored at 9 and 12 days ($p > 0.05$). The present results are consistent with Nedomova and Buchar (2013), who reported that ostrich ESI ranges between 74.48% and 89.72%. The ESI for eggs stored for 9 and 12 days in the present study were significantly affected by storage time, compared to those stored for 0, 3, and 6 days ($p < 0.05$). Eggs stored for 9 and 12 days had greater ESI compared to eggs stored for 0, 3, and 6 days which was in agreement with Tabidi (2011). The ESI values in this study increased with prolonged storage. On the contrary, Tilki and Saatci (2004) found that the ESI of partridge eggs was not affected by storage from 0 to 35 days. The differences in the results of this study and that of Tilki and Saatci (2004) could be attributable to species differences.

According to Table 1, eggs stored for 3 and 6 days had greater shell weight compared to other treatments ($p < 0.05$). Shell weight for eggs stored at 0, 9, and 12 days was not statistically different but differed from the shell weight of eggs stored for 3 and 6 days ($p > 0.05$). The shell weight of eggs stored for days 0, 3, and 6 significantly differed from those stored for days 9 and 12 ($p < 0.05$). These results are inconsistent with Juergens and Bessei (2016), who observed that prolonged storage time of up to 28 days decreases shell weight. The average shell weight in the present study was 0.223 kg which is higher than the result reported by Moreki et al. (2016) who found the shell weight of ostrich eggs from multiple aged ostriches to range from 0.1750 to 0.1707 kg. The fluctuation in shell weight across the storage periods in this study may be attributable to the quality of water at DOMU in Botswana. Water supplied to birds at DOMU came from different sources due to water shortage at the farm. Balnave and Yoselewitz (1987) stated that eggshell quality could be negatively impacted over time by the water with high electrolyte concentrations (saline drinking water). This is because elevated levels of sodium interfere with calcium utilization, an important mineral in shell and bone formation.

The surface area (ESA) of the eggs stored for 0, 3, and 6 days was not affected by storage time, whereas the ESA of the eggs stored at 9 and 12 days was significantly affected by storage time (Table 1). The average ESA in the present study was 606.36 cm^2, which was higher than the value (506.41 ± 2.36) reported by Brassó and Komlósi (2021). The difference in the results may be due to the ostrich sub-species and nutrition.

The storage time had no effect on the HU for the eggs held for 0, 3, and 6 days, compared to eggs stored for 9 and 12 days ($p < 0.05$). In contrast, the storage period had a significant effect on the HU for eggs stored for 9 and 12 days as opposed to those stored for 0, 3, and 6 days because the HU decreased with increased storage time ($p > 0.05$). The mean HU value in this study was 118.20, which is less than the value of 131.16 reported by Moreki et al. (2016) in ostrich eggs. According to Pleti et al. (2009), the HU above 100 shows a good quality ostrich egg. Khan et al. (2014) explained that HU decreases with increased storage time due to the loss of water from the egg.

The albumen ratio for eggs stored for 0, 3, and 6 days was not ($p > 0.05$) affected by storage time and was higher ($p < 0.05$) compared to eggs stored for 9 and 12 days (Table 1). This is because prolonged storage results in more water moving from the albumen to the yolk. The current result agrees with Lapão et al. (1999) who observed that the albumen ratio in the ostrich egg decreases with storage time. In the study by Demirel and Kirikci (2009), it was observed that the HU, albumen index, and yolk index decreased with increased storage time due to the loss of water from the egg. Khan et al. (2014) also attributed the decrease in the albumen ratio to the movement of water from the albumen into the egg yolk. According to Lapão et al. (1999), the decrease in the albumen ratio may be due to an increase in the albumen pH, which can be solved by providing the ostrich with low-pH drinking water. In addition, Balnave et al. (2000) stated that the albumen ratio is negatively affected by increased protein dietary and amino acids content. It is further argued by Ekweozor et al. (2002) that the ingestion of crude oil has a negative effect on the albumen ratio.

The yolk ratio was not affected by storage time ($p > 0.05$). This finding is inconsistent with Khan et al. (2013; 2014), who found that albumen index, albumen weight, yolk index, yolk weight, and HU all decreased after extended storage values in Fayoumi and Rhode Island Red eggs. Similarly, Tabidi (2011) reported that the yolk index decreases with storage. However, this result contradicts the finding of Khan et al. (2014), who reported that the yolk ratio is directly proportional to the storage time and that the yolk ratio increases with increased storage time due to egg water loss.

Table 1 The effects of storage time on ostrich egg quality characteristics

Trait	Storage time (days)					Grand mean	Standard error of the mean
	0	3	6	9	12		
EWT, (g)	1284.00^{ab}	1346.00^{a}	1346.00^{a}	1089.67^{bc}	1050.00^{c}	1223.13	51.73
SI, (%)	74.60^{c}	74.40^{c}	74.10^{c}	78.00^{b}	81.30^{a}	76.30	0.98
SW, (g)	214.67^{bc}	234.67^{a}	238.00^{a}	208.00^{c}	224.00^{bc}	223.87	7.29
ECwt, (g)	0.170^{c}	0.173^{bc}	0.177^{bc}	0.190^{ab}	0.197^{a}	0.181	0.0063
ESA, (cm^2)	620.98^{ab}	641.92^{a}	641.89^{a}	552.98^{c}	574.01^{bc}	606.36	18.08
HU	129.02^{a}	131.11^{a}	131.09^{a}	100.0^{b}	99.79^{b}	118.20	0.79
ARE	55.33^{b}	54.63^{B}	53.94^{b}	44.93^{a}	42.57^{a}	50.28	1.67
YKR	23.96^{a}	29.25^{a}	30.40^{a}	29.75^{a}	28.51^{a}	28.37	3.04
ESG, (g/cm^3)	1.31^{ab}	1.29^{ab}	1.28^{b}	1.39^{a}	1.32^{ab}	1.32	0.32
SR	16.71^{b}	17.44^{bc}	17.68^{abc}	19.20^{ab}	19.55^{a}	18.11	0.59

^{abc} Means within a row that do not share common superscripts differ significantly (P<0.05). EWT: Egg weight; SI: Egg shape index; SW: Shell weight; ESA: Egg surface area; HU: Haugh unit; SR: Shell ratio; ESG: Egg specific gravity; AR: Albumen ratio; YKR: Yolk ratio; ECWT: Weight of egg contents.

Storage time did not affect the ESG of ostrich eggs stored for 0, 3, and 6 days. However, the storage time significantly affected the eggs that had been kept for 9 and 12 days since ESG increased with longer storage (P<0.05). The average ESG value of 1.32 g/cm^3 in the current study is consistent with the values obtained by Koutinhouin et al. (2014) and Moreki et al. (2016), who reported the ESG values of 1.13±0.006 g/cm^3 and 1.16±0.007 g/cm^3, respectively for ostrich eggs. However, Keffen and Jarvis (1984) found that the average ESG of ostrich eggs was 2.0 ± 0.84 g/cm^3. The differences in ESG values in these studies may be due to the age of birds, nutrition, and climatic conditions.

The shell ratio increased ($p < 0.05$) with prolonged storage (Table 1). The shell ratio for eggs stored for 12 days was significantly ($p < 0.05$) higher than those of eggs stored for 0 and 3 days. However, the shell ratios for storage periods 6, 9, and 12 were similar ($p > 0.05$). In addition, the shell ratio for eggs stored for 0, 3, 6, and 9 was not significantly different ($p > 0.05$) from each other. The average shell ratio in this study was 18.11%. This result is closer to the average shell ratio of 19.8% obtained by Hoffman and McMillin (2009) in ostrich eggs. The difference in shell ratios could be attributable to the age of the hen, genotype, calcium deposition, and strain of the bird (Ketta and Tůmová, 2016).

CONCLUSION

Based on the Haugh unit values, which indicate the freshness of the egg, ostrich eggs can only be stored for up to six days at room temperature without significantly losing quality.

DECLARATIONS

Acknowledgments

We wish to thank the Department of Animal Science, Botswana University of Agriculture and Natural Resources, for granting us permission to use the laboratory to carry out this study.

Funding

The Department of Tertiary Education of the Ministry of Education and Skills Development in Botswana provided financial assistance to this study.

Authors' contributions

This study was planned by John Cassius Moreki, who also conducted a review of the relevant literature, wrote and amended the paper, edited it, and then submitted it to the journal for consideration. Denise Florence Mosarwa collected data, surveyed the literature, and drafted the manuscript. Joshua Makore assisted with statistical design and data analysis, while Nicholas Mosweu participated in the practical part of the experiment. All authors checked and confirmed the final draft of the manuscript.

Competing interests

The authors confirm no conflict of interest.

Ethical considerations

The authors have examined ethical issues, including redundancy, plagiarism, misconduct, consent to publish, data fabrication, and double publication.

Availability of data and materials

The dataset of this study is available by the relevant authors upon request.

REFERENCES

Al-Obaidi FA, Al-Shadeedi SM, and Mousa AS (2012). Egg morphology, quality and chemical characteristics of ostrich *Struthio camelus camelus*. Al-Anbar Journal of Veterinary Science, 5(1): 162-167. Available at: https://www.iasj.net/iasj/download/2d42967bc2e4439e

Archer GS and Cartwright AL (2017). Incubating and hatching eggs. Texas A & M AgriLife Extension, pp. 1-13. Available at: https://alec.unl.edu/documents/cde/2017/livestock-management/incubating-and-hatching-eggs-2017.pdf

Balnave D, Gill RJ, Li X, and Bryden WL (2000). Responses of Isa Brown laying hens to a pre-layer diet containing additional calcium and to dietary protein and lysine concentrations during lay. Australian Journal of Agricultural Research, 51(6): 779-784. DOI: https://www.doi.org/10.1071/AR99179

Balnave D and Yoselewitz I (1987). The relation between sodium chloride concentration in drinking water and eggshell damage. British Journal of Nutrition, 58(3): 503-509. DOI: https://www.doi.org/10.1079/bjn19870118

Brassó LD and Komlósi I (2021). Evaluation of egg quality parameters of two Hungarian ostrich populations. Acta Agraria Debreceniensis, 1: 51-57. DOI: https://www.doi.org/10.34101/ACTAAGRAR/1/8523

Ceylan N, Ciftçi I, Mizrak C, Kahraman Z, and Efil H (2006). Influence of different dietary oil sources on performance and fatty acid profile of egg yolk in laying hens. Journal of Animal and Feed Sciences, 20(1): 71-83. DOI: https://www.doi.org/10.22358/jafs/66159/2011

Cooper RG, Lukaszewicz M, and Horbanczuk JO (2009). The ostrich (*Struthio camelus*) egg- a safety seat in the time vehicle. Turkish Journal of Veterinary and Animal Science, 33(1): 77-80. DOI: https://www.doi.org/10.3906/vet-0802-13

Demirel S and Kirikci K (2009). Effect of different egg storage times on some egg quality characteristics and hatchability of pheasants (*Phasianus colchicus*). Poultry Science, 88(2): 440-444. DOI: https://www.doi.org/10.3382/ps.2008-00131

Di Meo C, Stanco G, Cutrignelli MI, Castaldo S, and Nizza A (2003). Physical and chemical quality of ostrich eggs during the laying season. British Poultry Science, 44(3): 386-390. DOI: https://www.doi.org/10.1080/0007166031000085580

Ekweozor IKE, Granville AW, Nkanga EE, and Ogbalu OK (2002). The effects of crude oil contaminated feeds on the yield and quality of eggs of poultry birds (*Gallus domesticus*). Journal of Agriculture in the Tropics and Subtropics, 103(1): 89-97. Available at: https://www.jarts.info/index.php/jats/article/view/1426

El-Safty SA and Mahrose KM (2009). Evaluation of some phenotypic, physiological and egg quality traits of African black neck ostrich under arid desert conditions of Libya. International Journal of Poultry Science, 8(6): 553-558. DOI: http://www.doi.org/10.3923/ijps.2009.553.558

Hassan SM, Siam AA, Mady ME, and Cartwright AL (2005). Egg storage period and weight effects on hatchability of ostrich (*Struthio camelus*) eggs. Poultry Science, 84(12): 1908-1912. Available at: https://www.doi.org/10.1093/ps/84.12.1908

Hoffman LC and McMillin KW (2009). Improving the meat quality of venison and other exotic game. Improving the sensory and nutritional quality of fresh meat, pp. 447-475. DOI: https://www.doi.org/10.1533/9781845695439.3.447

Juergens A and Bessei W (2016). Effects of storage conditions on egg quality. Lohmann Information, 50(1): 22-27. Available at: https://lohmann-breeders.com/media/2020/08/VOL49-GRASHORN-Storage.pdf

Keffen RH and Jarvis MJF (1984). Some measurements relating to ostrich eggs. Ostrich, 55(4): 182-187. DOI: https://www.doi.org/10.1080/00306525.1984.9634485

Ketta M and Tůmová E (2016). Eggshell structure, measurements, and quality-affecting factors in laying hens: A review. Czech Journal of Animal Science, 61(7): 299-309. DOI: https://www.doi.org/10.17221/46/2015-CJAS

Khan MJA, Khan SH, Bukhsh A, and Amin M (2014). The effect of storage time on egg quality and hatchability characteristics of Rhode Island Red (RIR) hens. Veterinarski Arhiv, 84(3): 291-303. Available at: http://wwwi.vef.hr/vetarhiv/papers/2014-84-3-8.pdf

Khan MJA, Khan SH, Bukhsh A, Abbass MI, and Muhammad J (2013). Effect of different storage periods on egg weight, internal egg quality and hatchability characteristics of Fayumi eggs. Italian Journal of Animal Science, 12(2): e51. DOI: https://www.doi.org/10.4081/ijas.2013.e51

Koutinhouin GB, Tougan UP, Boko C, Baba L, Fanou L, Chitou IB, Everaert N, and Thewis A (2014). Egg physical quality and hatchability in captive African ostrich (*Struthio camelus camelus*, Linnaeus 1758) reared in Benin: Effect of season and relationships. International Journal of Advanced Research, 2(6): 510-516. Available at: https://www.journalijar.com/uploads/99_IJAR-3456.pdf

Lapão C, Gama LT, and Chaveiro SM (1999). Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. Poultry Science, 78(5): 640-645. DOI: https://www.doi.org/10.1093/ps/78.5.640

Moreki JC, Kebonye NM, and Tiroesele B (2012). Commercial ostrich farming in Botswana: A case study of dibete ostrich multiplication unit. Journal of Life Science and Biomedicine, 2(5): 192-195. Available at: https://jlsb.science-line.com/attachments/article/17/J.%20Life%20Sci.%20Biomed.%202(5)%20192-195,%202012,%20B37.pdf

Moreki JC, Majuta GK, and Machete JB (2016). External and internal characteristics of ostrich eggs from dibete ostrich farm. International Journal of Advanced Research, 4(9): 1397-1404. DOI: https://www.doi.org/10.21474/IJAR01/1740

Nedomova Š and Buchar J (2013). Ostrich eggs geometry. Acta Universitatis Agriculturae et Silviculturae Brunensis, 61(3): 735-742. DOI: http://www.doi.org/10.11118/actaun201361030735

Nonga HE, Simon C, Karimuribo ED, and Mdegela RH (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from smallholder poultry keepers in Morogoro municipality, Tanzania. Zoonoses and Public Health, 57(5): 339-344. DOI: https://www.doi.org/10.1111/j.1863-2378.2008.01226.x

Ograk YZ and Altinel A (2014). Effects of storage length on the hatchability of ostrich (*Struthio camelus*) eggs. Eurasian Journal of Veterinary Science, 30(1): 30-34. Available at:

https://dergipark.org.tr/en/pub/eurasianjvetsci/issue/24804/262058

Pleti AK, de Lima JJ, and Candido LMB (2009). Internal quality of ostrich eggs after storage in ambient temperature and in cooling condition storage. Ciencia Rural, 39(6): 1864-1868.

Sahan U, Ipek A, and Yilmaz B (2003). The effects of storage temperature and position on embryonic mortality of ostrich (*Struthio camelus*). South African Journal of Animal Science, 33(1): 38-42. Available at: https://www.sasas.co.za/wp-content/uploads/2012/09/sahanvol33no1_0.pdf

Samli HE, Agma A, and Senkoylu N (2005). Effects of storage time and temperature on egg quality in old laying hens. Journal of Applied Poultry Research, 14(3): 548-553. DOI: https://www.doi.org/10.1093/japr/14.3.548

Samiullah S and Roberts J (2014). The eggshell cuticle of the laying hen. World's Poultry Science Journal, 70(4): 693-708. DOI: https://www.doi.org/10.1017/S0043933914000786

Tabidi MH (2011). Impact of storage period and quality on the composition of table egg. Advances in Environmental Biology, 5(5): 856-861. Available at: http://www.aensiweb.com/old/aeb/2011/856-861.pdf

Tilki M and Saatci M (2004). Effects of storage time on external and internal partridge (*Alectoris graceca*) eggs. Revue de Medicine Veterinaire, 155(11): 561-564. Available at: https://avesis.ktu.edu.tr/yayin/fd36c132-1ffc-4d34-8c40-b52d903283c0/effects-of-storage-time-on-external-and-internal-characteristics-in-partridge-alectoris-graeca-eggs

van Niekerk T (2014). Egg quality, Low input breeds. Technical note, pp. 1-4. Available at: https://www.lowinputbreeds.org/fileadmin/documents_organicresearch/lowinputbreeds/tn-4-4-niekerk-2014-egg-quality.pdf

Wilson HR, Eldred AR, and Wilcox CJ (1997). Storage time and ostrich egg hatchability. Journal of Applied Poultry Research, 6(2): 216-220. Available at: https://www.doi.org/10.1093/japr/6.2.216

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 149-160, March 25, 2023

Research Paper, PII: S2322455X2300017-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.17

Isolation and Characterization of Fowl Adenoviruses Associated with Hydro-pericardium Syndrome from Broiler Chickens in Egypt

Eid Hussein[1], Nessreen Fouad Anwar[1], Hossam Shabaan Elsebaey[1], Marwa Ali Abdelmagid[2], Mohammed Abo Elkhair[3], and Osama Mahana[2]*

[1]*Reference Laboratory for Veterinary Quality Control on Poultry Production, Damanhour Branch, Animal Health Research Institute, Agricultural Research Center, Damanhour, Egypt*
[2]*Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Giza, 12618, Egypt*
[3]*Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, Menofia, 32958, Egypt*

*Corresponding author's E-mail: osamamahana2006@gmail.com

Received: 07 January 2023
Accepted: 23 February 2023

ABSTRACT

One of the most prominent viral diseases affecting the poultry industry is hydropericardium syndrome caused by fowl adenoviruses. Hydropericardium syndrome has recently led to significant economic losses in the Egyptian poultry sector. Many outbreaks of hydropericardium syndrome have been documented across the country in the last few years. This research examined the epidemiology and molecular characterization of fowl adenoviruses in broiler chickens in Egypt. Samples were taken from 26 outbreaks of commercial broiler chicken farms in the Beheira and Menofia governorates, Egypt, from January 2021 to March 2022. Adenoviruses genomes were detected in cloacal swabs of 10 flocks using polymerase chain reaction. Clinically, infected broiler chickens (Cobb, Ross, Indian River, Modified-Avian, and Arbor Acres) showed depression, ruffled feathers, retarded growth, and ascites, with mortality rates of 10-28%. The most common postmortem lesions were hydropericardium, yellowish enlarged liver with ecchymotic hemorrhages, pancreatitis, and enteritis. Histopathologically, intranuclear inclusion bodies, commonly basophilic type, were scattered in the hepatocyte, proventriculus, duodenum, kidney, pancreas, and spleen. In addition, depletion of lymphocytes in the bursa of Fabricius and the thymus was observed. Seven samples were selected for gene sequencing of the loop 1 region of the hexon gene. The sequence analysis revealed that all samples were identical and similar to fowl adenoviruses species D serotype 2/11, suggesting that this serotype was the predominant fowl adenoviruses circulating in the study location in the last two years. Further studies are required to address the pathogenicity of isolated fowl adenoviruses and evaluate the vaccine used to control fowl adenoviruses in Egypt.

Keywords: Fowl adenoviruses, Hexon, Hydropericardium syndrome, Phylogenetic analysis, Polymerase chain reaction

INTRODUCTION

Fowl adenoviruses (FAdVs) belong to the genus *Aviadenovirus*in the family Adenoviridae. FAdVs are serologically classified into five species (FAdV-A to FAdV-E) according to restriction enzyme digestion pattern (Hess, 2000) and 12 serotypes (FAdV-1 to -8a and -8b to -11) based on serum cross-neutralization tests (Meulemans et al., 2004). The most common diseases caused by FAdVs-infection are inclusion body hepatitis (IBH), hydropericardium syndrome (HPS), and gizzard erosions (Hess, 2000). Hydropericardium syndrome is the most serious form, as it causes a high mortality rate (Kataria et al., 2005).

Hydropericardium syndrome was first observed in Angara Goth, Pakistan, in 1987 and has been named hydropericardium–hepatitis syndrome/Angara disease in Pakistan, Litchi heart disease in India, or inclusion body hepatitis-hydropericardium syndrome (IBH–HPS) in other places (Anjum et al., 1989). This disease affects broiler chickens at 3-6 weeks of age and can be transmitted vertically from infected chicken to young broiler, which becomes more susceptible to infection (Schachner et al.,

2021). The symptoms include sudden onset, anemia, retarded growth, depression, crouching position with ascites, which can lead to an increased mortality rate of up to 60% and immune depression (McFerran and Adair, 2003; Hafez, 2011). Due to their possible immunosuppressive properties, FAdVs can make chickens more susceptible to other microbes by decreasing humoral and cell-mediated immunity (Schonewille et al., 2008). The most prominent lesions are an enlarged, hemorrhagic, friable liver, hydropericardium with a flabby congested heart, swollen and hemorrhagic kidneys, and mottled spleen (Cizmecigil et al., 2020). Pathologically, HPS is characterized by massive degeneration and necrosis of the liver, heart, kidney, and lung with large basophilic intranuclear inclusion bodies in the liver and severe hyperemia and edema in kidney and lung (Maartens et al., 2014; Niu et al., 2018).

FAdVs Species D and E have mainly been associated with outbreaks of IBH (Mohamed et al., 2018; Kiss et al., 2021), while most HPS is caused by (species C) serotype (4, 9 and 10), especially the virulent serotype (FAdV-4; Schachner et al., 2014). However, Chen et al. (2017) reported that any of the twelve FAdV serotypes could cause IBH or HPS in broiler chickens, with a mortality rate ranging from 10% to 60%.

Conventional and molecular methods are employed to diagnose IBH/HPS, followed by sequencing to differentiate FadVsserotypes (Mittal et al., 2014). The serotype-specific gene sequence of the adenovirus is the hexon gene. It is a major adenovirus protein that possesses the neutralizing epitope used for serotyping FAdV (Niczyporuk, 2018). Since the FadVs have not gained much attention in Egypt, no vaccinations are currently available for use in the poultry sector. However, many studies have reported the spread of different serotypes in different Egyptian poultry farms, including FAdVs species D serotype2-11 (El-Tholoth and Abou El-Azm, 2019; Elbestawy et al., 2020), FAdV species E serotype 8a (Radwan et al., 2019), and FAdV serotypes 1, 3 and 8b (Adel et al., 2021).

The present study aimed to identify the current circulating FAdVs in Egypt by focusing on its pathological and molecular characterization using partial hexon gene DNA sequencing and phylogenetic analysis of obtained sequences. The results were then compared with other previously reported sequences in Egypt and other countries, which may be helpful in developing an efficient vaccination program in Egypt.

MATERIALS AND METHODS

Ethical approval

Infected broiler chickens were euthanized humanely then samples were collected following the regulations of the General Organization for Veterinary Services and Animal Health Research Institute, Giza, Egypt (AHRI 191221).

History of investigated broiler chicken flocks

From January 2021 to March 2022, a total of 26 broiler chicken flocks with a history of hydropericardium and variable mortality rates were examined for diagnosis of possible causes. These flocks representing different broiler chicken breeds (5 Cobb, 5 Ross, 4 IR, 7 Modified-Avian, and 5 Arbor Acres) were localized in Beheira (n = 15) and Menofia (n = 11) governorates in Egypt. All of them were unvaccinated against FAdV and negative for chicken anaemia virus, Infectious bursal disease virus, and reovirus using Polymerase chain reaction (PCR). Farms capacity ranged from 5000 to 19000 chicks. The chickens under examination were within the age range of 6-38 days. Infected broiler chickens showed depression, retarded growth, ascites, watery diarrhea, and variable mortality rates (Table 1). The main observed gross lesions in the examined flocks were pale, swollen livers with sub-capsular ecchymotic hemorrhages and hydropericardium.

Samples for histopathological examination

Tissues for histopathological examination from positive broiler flocks were collected from the liver, lung, spleen, kidney, heart, pancreas, proventriculus, bursa of Fabricious, thymus, and intestine of 10 freshly dead or euthanized infected broiler chickens aged 6 to 38 days. The samples were placed immediately in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin for pathological examination (Bancroft and Layton, 2013) using 10-20-40 lenses of the microscope (Micros Austria, Austria).

Samples for molecular detection of fowl adenoviruses

About 270 cloacal swabs from 26 broiler chicken flocks (10 from each flock) were obtained for FAdV molecular detection. Every10 different cloacal swab was pooled and dissolved in 1 mL of 7.4 pH phosphate-buffered saline.

Table 1. Epidemiological data of FAdV positive from broiler chicken flocks

Serial farm Number	NCBI Accession Number	Location in Egypt	Date	Broiler breed	Age (day)	Clinical signs	Mortality percentage
1		Menofia	Jan. 2021	Arbor Acres	12	Ascites	10%
2 *	OP297554	Menofia	Oct. 2021	Ross	36	Ascites	13%
13*	OP297555	Beheira, Shubrakhit	Jan. 2021	Cobb	6	Retarded growth and ascites	28%
16	--	Beheira, Edco	Oct. 2021	Ross	27	Ascites	23%
17*	OP297556	Beheira, Badr	Dec.2021	Indian River (IR)	27	Retarded growth and ascites	27%
21	--	Beheira,Abu El Matamir	Dec.2021	Arbor Acres	22	Retarded growth and ascites	28%
23*	OP297557	Beheira, Mahmoudiyah, Dayrut	Jan. 2022	Modified avian	32	Retarded growth and ascites	25%
24*	OP297558	Beheira, Mahmoudiyah, Fazara	Feb.2022	Modified avian	30	Retarded growth and ascites	20 %
25*	OP297559	Beheira, Mahmoudiyah, Monia Al-Saeed.	Mar. 2022	Modified avian	32	Retarded growth and ascites	12 %
26*	OP297560	Menofia	Feb.2022	Cobb	35	Retarded growth and ascites	22 %

*: Chosen samples for sequence.

Isolation and propagation of fowl adenoviruses in liver chicken embryo

The cloacal swabs supernatant was inoculated in the confluent monolayers of liver chicken embryo cells (CEL) obtained from 14 to 16-day-old specific pathogen-free embryos and incubated for 3-4 days until an intensive cytopathogenic effect was noticed using an inverted microscope (Olympus, Japan) as described by Mohamed Sohaimi et al. (2019). Virus isolation was performed using PCR to detect FAdVs in the collected supernatant of harvested tissue culture fluids.

Fowl adenoviruses molecular detection

Nucleic acid extraction

Total viral nucleic acid was extracted from 200 µL of the cloacal swabs supernatant using QIAampDNAMinikit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol.

PCR detection

The PCR was conducted using the EmeraldAmp Max PCR Master Mix (Takara, Japan) with hexon gene-specific primers (F: ATGGGAGCSACCTAYTTCGACAT, R: AAATTGTCCCKRAANCCGATGTA) according to Raue et al. (2005). As the initial denaturation step, the PCR reaction was heated at 95◦C for 5 minutes. Then, 40 cycles of; denaturation at 94˚C for 30 seconds, primer annealing at 58°C for 30 seconds, and elongation at 72°C for 45 seconds, followed by a final elongation step of 7 minutes at 72°C. The PCR product was analyzed by agarose gel electrophoresis to visualize the specific band at 590 bp by UV Trans illuminator.

Partial hexon gene nucleotide sequencing

Seven FAdVs samples were selected for partial hexon gene sequencing as they gave clear PCR bands indicating a higher viral load. Specific DNA bands with 590 bp size were excised from the gel, and the PCR products were extracted using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The purified PCR products were subjected to sequencing reactions with the forward and reverse primers in two reactions using a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's specifications. The obtained sequences were submitted to NCBI Gene bank (https://www.ncbi.nlm.nih.gov/WebSub/).

Similarity percent between strain sequences in this study and other published sequences in the NCBI database was done using Lasergene software. The nucleotide sequences were analyzed by the BIOEDIT program (Hall, 1999) using the Clustal W alignment algorithm. The obtained sequences in this study were aligned with the adenovirus field and reference strain

sequences from different countries accessible in the NCBI GenBank database. Phylogenetic trees were constructed by the maximum likelihood method using MEGA 6 software (Tamura et al., 2013).

RESULTS

Epidemiological findings

A total of 26 suspected FAdVs-affected broiler chicken flocks were investigated from January 2021 to March 2022 in Egypt provinces (Beheira and Menofia). The obtained data are presented in Table 1. The owner's complaints were generally about ascites, depression, retarded growth, increased susceptibility to various diseases, vaccination failure, morbidity rates ranging 20-38%, and mortality of 10 to 28%

Clinical signs and postmortem lesions

The infected broiler in investigated flocks showed retarded growth, depression, ruffled feathers, and a crouching position with ascites (Figure1 A and B). All infected chickens had hydropericardium, which is an accumulation of extra straw or amber-colored fluid in the pericardial sac (Figure 1 C). The pericardial sac contained a flabby, floating heart having congested blood vessels with hemorrhagic pericardial fat (Figure 1 D). The liver was pale, enlarged, and friable, with variable areas of necrosis and some petechial hemorrhages (Figure 1 C, D, and E). The duodenum was hemorrhagic (Figure1 F). Other lesions included congested and edematous lungs, slightly enlarged spleen, hemorrhagic and swollen kidneys with necrotic foci, and atrophy of the bursa of Fabricius and the thymus.

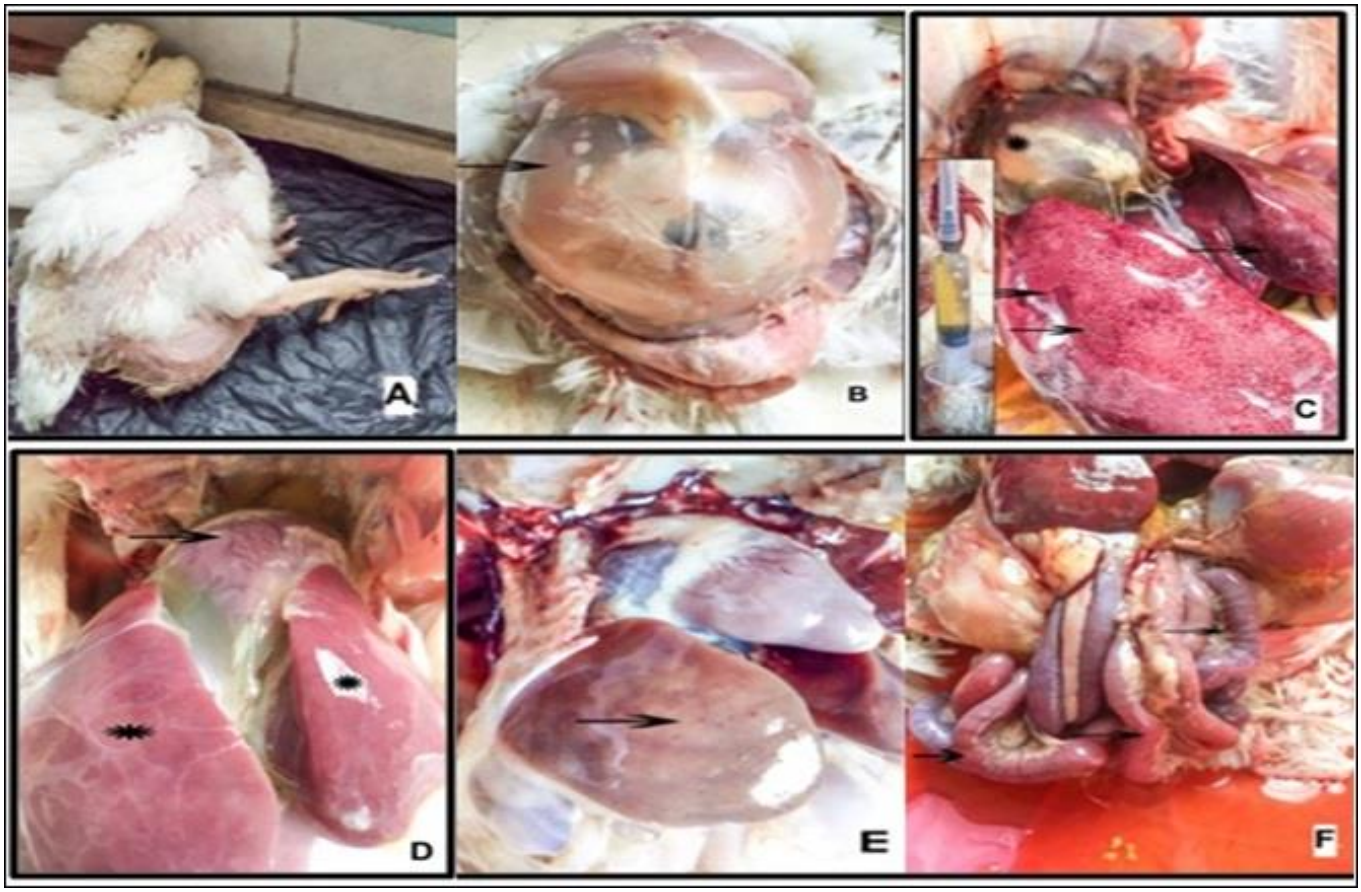


Figure 1. Clinical signs and postmortem lesions of broiler chickens infected by Hydropericardium syndrome. **A:** Broiler chicken looks listless, with ruffled feathers and in a crouching position. **B:** Severe ascites (Black arrow). **C:** The pericardial sac was distended with clear straw-colored fluid (Asterisk) and the liver was enlarged and mottled, with extensive necrosis (Black arrow). **D:** The heart was seen within the dilated pericardial sac engorged with blood (black arrow) and the liver was swollen and friable (Asterisk). **E:** Necrotized liver with some petechial hemorrhages (Black arrow). **F:** Hemorrhagic duodenitis (Black arrow).

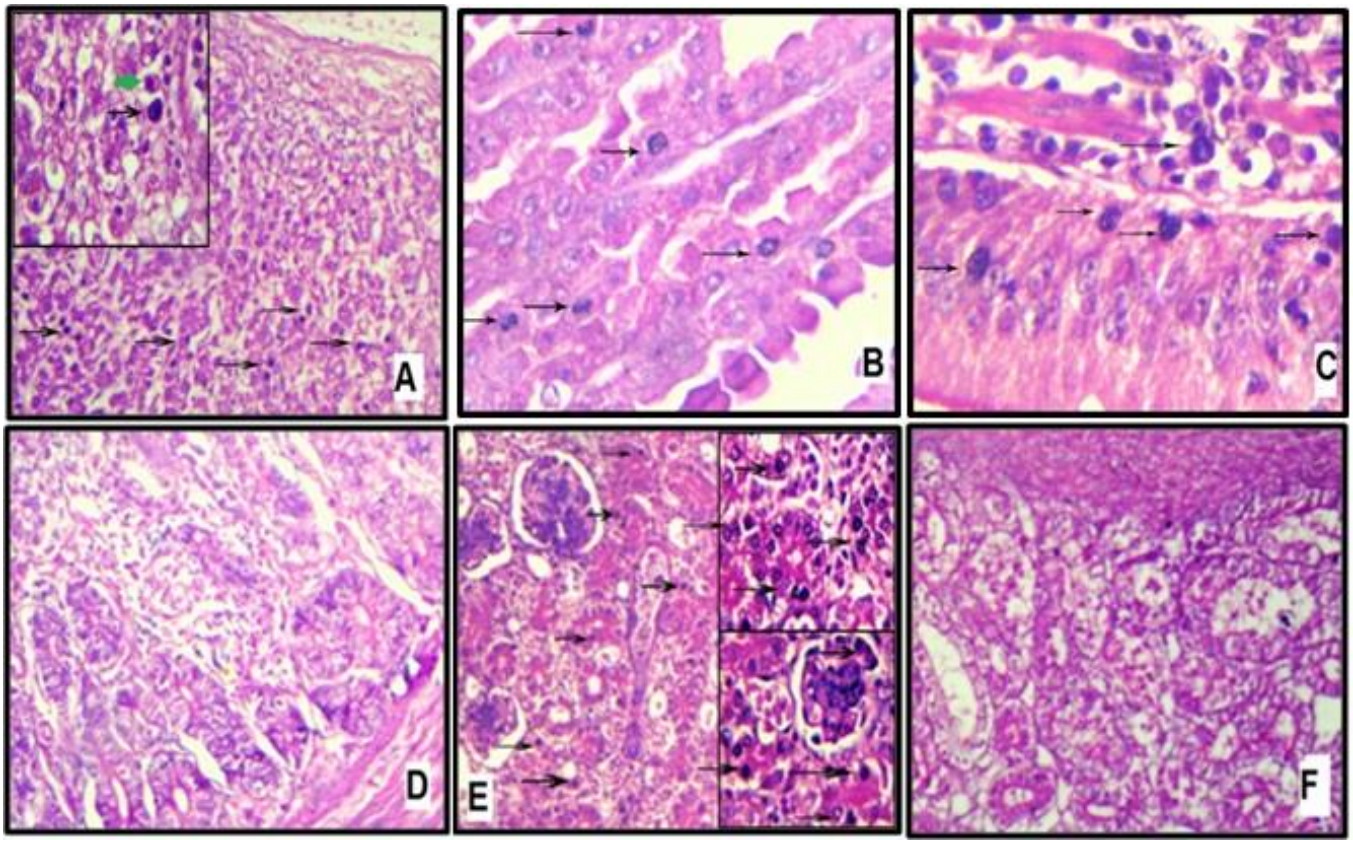


Figure 2. Pathological lesions of liver, proventriculus, duodenum and kidney of infected broiler chickens with FAdVs. **A:** Liver tissue, hepatic vacuolar degeneration, and necrosis with large basophilic (black arrows) and eosinophilic (green arrow) intranuclear inclusion bodies. **B:** Proventriculus tissue, the presence of intranuclear inclusion bodies in the epithelial cells of glandular lobules. **C** and **D:** Duodenumtissue, intranuclear inclusion bodies (Black arrow) with necrosis of intestinal villi with lymphocytic infiltration, edema, and hemorrhage in the submucosa. **E** and **F:** Kidney tissue, interstitial edema, and severe degeneration and necrosis of renal tubules and intranuclear inclusion bodies in the glomerular and tubular epithelium (Black arrow, H&EX200).

Histopathological lesions

Different organs of 10 dead or euthanized chickens from each positive flock were selected for histopathological examination and showed similar histopathological lesions. Massive intranuclear basophilic and eosinophilic inclusion bodies were the obvious feature of the liver which was widely distributed but frequently observed near the areas of necrosis (Figure 2 A). Other than the liver, intranuclear inclusion bodies were detected in a variety of organs, as in the epithelial cells of glandular lobules of the proventriculus (Figure 2 B), duodenum (Figure 2 C), renal glomeruli and tubules (Figure 2E), glandular cells of the pancreas (Figure 3 C) and inside the lymphocytes of the spleen (Figure 3 D). In addition, the intestines of infected chickens showed enteritis and necrosis with sloughing of the intestinal mucosa by lymphocytic infiltration of the submucosa (Figure 3 D). The kidneys displayed vacuolar degeneration and necrosis of renal tubules with interstitial edema and hemorrhage (Figure 3 F). Heart revealed pericardial edema with noticeable infiltration of heterophils and monocytes between myocytes (Figure 3 A). Lung indicated pneumonia with perivascular edema, hemorrhages, and alveolar infiltration of inflammatory cells (Figure 3 B). Diffuse focal necrosis of glandular cells of the pancreas (Figure 3 C), as well as depletion of lymphocytes in the bursa of Fabricius and thymus, were observed (Figures 3 E and F).

Fowl adenoviruses detection in commercial broiler farms

Of 26 tested samples, 10 (38.46%) were positive for FAdV by PCR. Seven gave intense PCR bands, so they were chosen for DNA sequencing, while the other three samples gave faint bands (Figure 4). Data of positive samples are shown in Table 1.

Isolation of fowl adenoviruses in chicken embryo liver cells

The positive samples of FAdVs species D serotype 2/11 were cultured in CEL cells. The cytopathic effect

appeared after 4 days including cell clumping and sloughing (Figure 5). Successful isolation of the virus on CEL cells was confirmed by positive PCR results for the harvested tissue culture fluids.

DNA sequencing of the partial hexon gene

Blast analysis of seven sequenced FAdVs in this study revealed that the nucleotide identity percent of the detected FAdVs sequences with the available Egyptian strains on the NCBI GenBank database ranged from 53.8% to 100% (Figure 6). The highest identity percent was with FAdV species D strains (93.3%-100%) while with FAdV serotype 8a was 70.6% followed byFAdV serotype 8b (67.8%),FAdVspecies B (63.5%,FAdV species A (56.1%), and lowest identity was forFAdV serotype 4 (53.8%; (Figure6). Sequence alignment of the obtained sequences revealed that the 7 FAdVs strains detected in this study were identical. This suggests that FAdVs species D is an important cause of HPS in Egypt between 2021 and 2022.

The partial hexon gene sequences of seven representative FAdV-D strains (Men1, Beh1, Beh2, Beh3, Beh4, Beh5, and Men2) detected in this study were submitted to the NCBI GenBank under accession numbers (OP297554, OP297555, OP297556, OP297557, OP297558, OP297559 and OP297560), respectively.Phylogenetic analysis of FAdVs indicated that these strains clustered with Egyptian FAdVs species D serotype 2/11strains, such as dmn2, kfr4, bst11, sin1, 2, 3, 4, and AD1, 4, 7, 9 (Figure 6). Moreover, FAdVs strains detected in the current study are closely related to those isolated from other countries, such as Germany (isolate 08-8872), Sweden (strain GB 1340-11), Spain(strain 12-2014), Japan (strain Gunma 2009) and Israel (strains 3346, 3114, 1917, Figure 7).

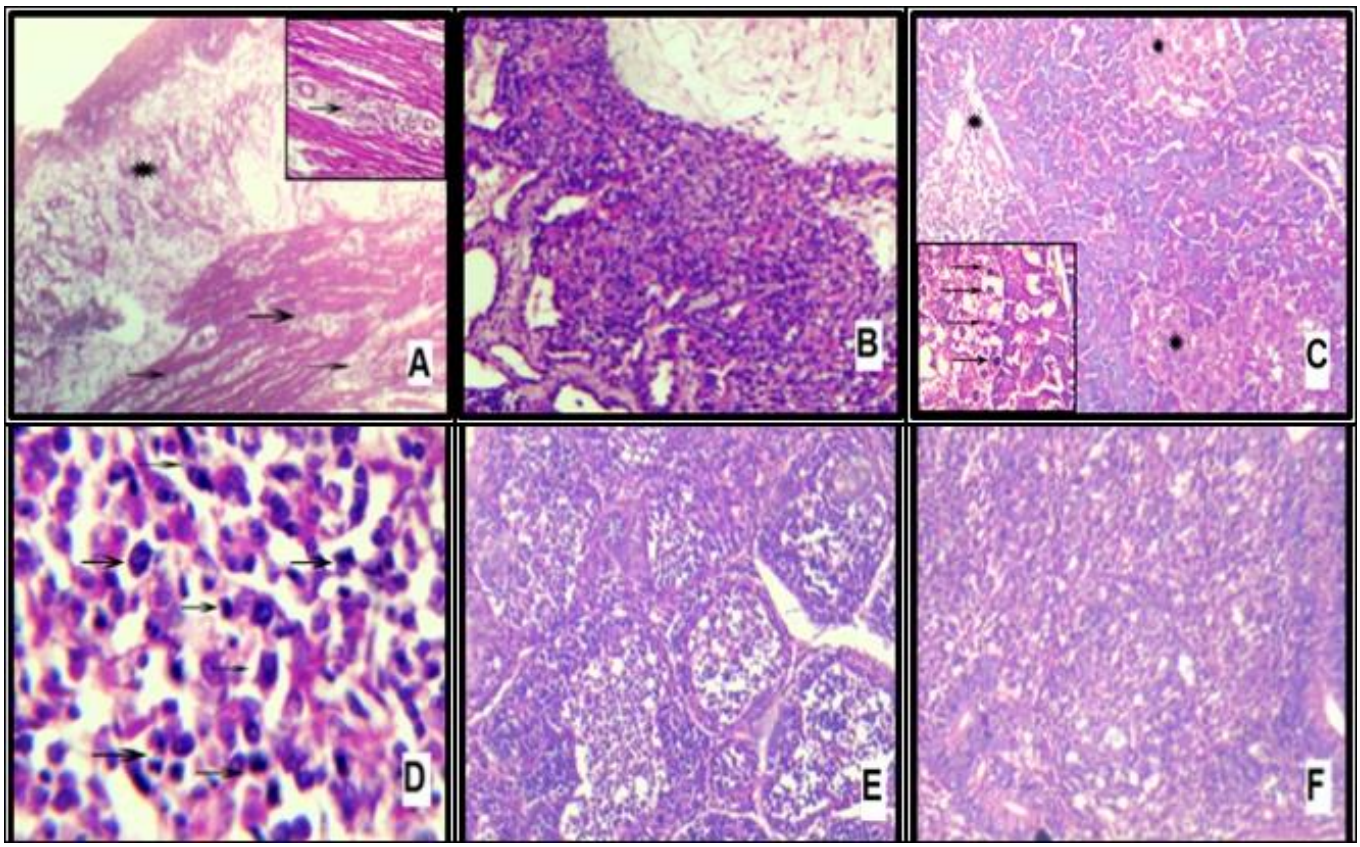


Figure 3. Pathological lesions of heart, lung, pancreas, spleen, bursa of Fabricius, and thymus of positive FAdVs infected broiler chickens**. A:** Heart, severe pericardial edema (asterisk) with myocytic necrosis and heterophilic and monocytic cell infiltration (Black arrow, H&E X 100, 200). **B:** Lung, pneumonia with massive edema and inflammatory cell infiltration (H&E X 200). **C:** Pancreas, diffuse glandular cell necrosis (asterisk) with intranuclear inclusion bodies (Black arrow, H&E X 200- 400). **D:** Spleen, intranuclear inclusion bodies in the splenic lymphocytes (Black arrow, H&E X 400). **E:** Bursa of Fabricius, and **F:** Thymus, showed lymphocytic depletion (H&E X200).

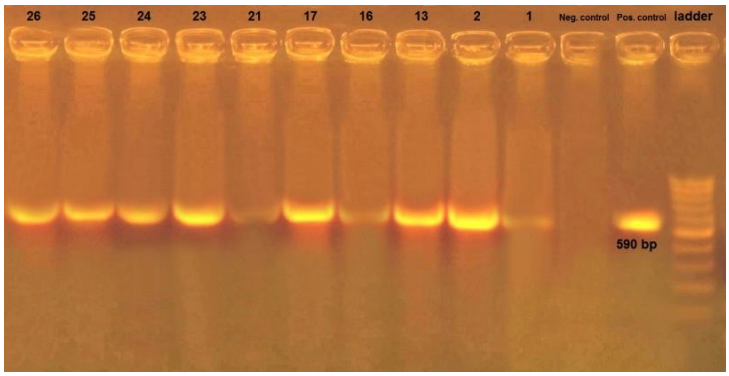


Figure 4. Agar gel electrophoresis of the amplified products of the partial hexon gene. Showing ladder: DNA marker, Pos: positive control with molecular weight 590bp, Neg: Negative control. Samples number 2, 13, 17, 23, 24, 25, and 26 showed clear bands and samples number 1, 16, and 21 showed faint bands.

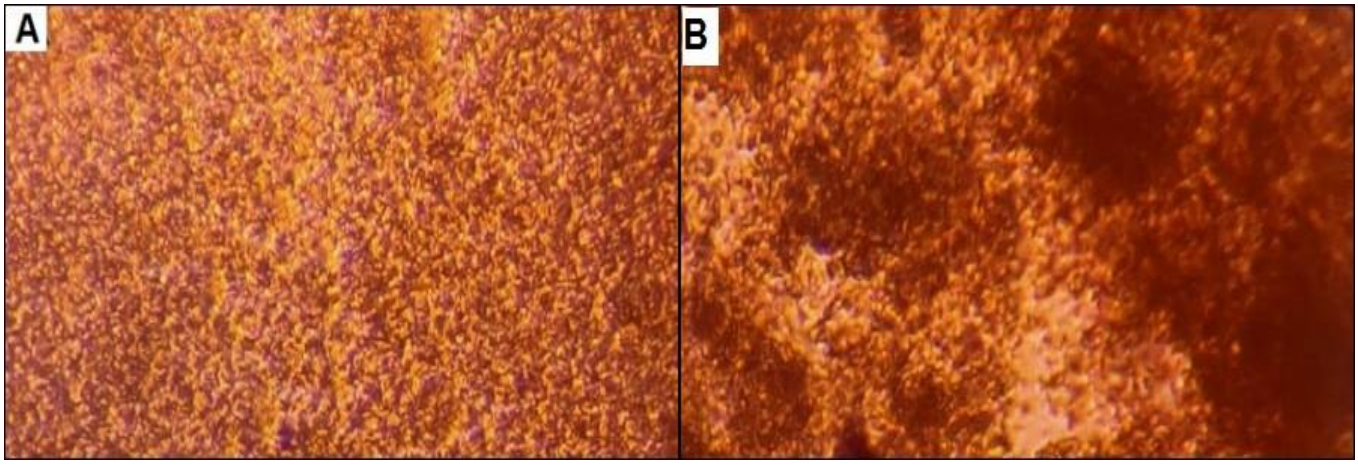


Figure 5. Cytopathic effect of FAdV on CEL cells in comparison with normal CEL cells**. A:** spindle shape of the liver cells (Control). **B:** Growth of FAdV on CEL cell culture in form of small and large areas of focal cell death with the beginning detachment of the cells at 4 days post-infection (X10).

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
1		100.0	100.0	100.0	100.0	100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	1	OP297554-FAdV-D-Men1-Egypt-2021
2	0.0		100.0	100.0	100.0	100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	2	OP297555-FAdV-D-Beh1-Egypt-2021
3	0.0	0.0		100.0	100.0	100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	3	OP297556-FAdV-D-Beh2-Egypt-2021
4	0.0	0.0	0.0		100.0	100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	4	OP297557-FAdV-D-Beh3-Egypt-2022
5	0.0	0.0	0.0	0.0		100.0	100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	5	OP297558-FAdV-D-Beh4-Egypt-2022
6	0.0	0.0	0.0	0.0	0.0		100.0	99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	6	OP297559-FAdV-D-Beh5-Egypt-2022
7	0.0	0.0	0.0	0.0	0.0	0.0		99.8	100.0	99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	7	OP297560-FAdV-D-Men2-Egypt-2022
8	0.2	0.2	0.2	0.2	0.2	0.2	0.2		99.8	100.0	100.0	100.0	99.8	99.4	99.6	99.6	99.8	99.6	99.4	93.5	97.0	55.9	63.7	70.4	67.8	53.8	8	OK634392.1-FAdV-D-Sin-4-Egypt-2017
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2		99.8	99.8	99.8	99.6	99.3	99.4	99.4	99.6	99.4	99.3	93.3	96.8	56.1	63.5	70.6	67.8	53.8	9	MH782425.1-FAdV-D-kom3-Egypt-2018
10	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2		100.0	100.0	99.8	99.4	99.6	99.6	99.8	99.6	99.4	93.5	97.0	55.9	63.7	70.4	67.8	53.8	10	MH782424.1-FAdV-D-dmn2-Egypt-2018
11	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2	0.0		100.0	99.8	99.4	99.6	99.6	99.8	99.6	99.4	93.5	97.0	55.9	63.7	70.4	67.8	53.8	11	MH782423.1-FAdV-D-bst11-Egypt-2018
12	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2	0.0	0.0		99.8	99.4	99.6	99.6	99.8	99.6	99.4	93.5	97.0	55.9	63.7	70.4	67.8	53.8	12	MH782426.1-FAdV-D-kfr4-Egypt-2018
13	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.4	0.2	0.2	0.2		99.6	99.4	99.8	100.0	99.8	99.6	93.3	97.2	56.1	63.9	70.2	67.6	53.8	13	MW699421.1-FAdV-D-AD1-Egypt-2019
14	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.6	0.8	0.6	0.6	0.6	0.4		99.1	99.4	99.6	99.4	99.3	92.9	96.8	56.1	63.5	70.0	67.4	53.8	14	MW699424.1-FAdV-D-AD4-Egypt-2020
15	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.4	0.6	0.4	0.4	0.4	0.6	1.0		99.3	99.4	99.3	99.1	93.5	97.0	55.7	63.7	70.8	68.0	53.6	15	MW699427.1-FAdV-D-AD7-Egypt-2020
16	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.4	0.6	0.4	0.4	0.4	0.2	0.6	0.8		99.8	99.6	99.4	93.1	97.0	55.9	63.9	70.2	67.6	53.6	16	FN869962.1-FAdV-D-08-8872-Germany-2016
17	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.4	0.2	0.2	0.2	0.0	0.4	0.6	0.2		99.8	99.6	93.3	97.2	56.1	63.9	70.2	67.6	53.8	17	JX257176.1-FAdV-D-GB-1340-Sweden-2011
18	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.4	0.6	0.4	0.4	0.4	0.2	0.6	0.8	0.4	0.2		99.4	93.1	97.0	55.9	63.9	70.0	67.8	53.8	18	LN907534.1-FAdV-D-12-2014-Spain-2012
19	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.6	0.8	0.6	0.6	0.6	0.4	0.8	1.0	0.6	0.4	0.6		93.3	97.2	56.2	63.7	69.8	67.4	54.2	19	LC505993.1-FAdV-D-Gunma-Japan-2009
20	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.0	7.2	7.0	7.0	7.0	7.2	7.6	7.0	7.4	7.2	7.4	7.2		92.9	55.9	63.1	69.1	67.0	53.6	20	MG029109.1-FAdV-D-SA4-Saudi-Arabia-2016
21	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.1	3.3	3.1	3.1	3.1	2.9	3.3	3.1	3.1	2.9	3.1	2.9	7.6		56.1	63.7	69.3	67.8	53.8	21	MG832884.1-FAdV-D-685-Canada-2007
22	62.2	62.2	62.2	62.2	62.2	62.2	62.2	62.7	62.2	62.7	62.7	62.7	62.2	62.2	63.1	62.7	62.2	62.7	61.7	62.6	62.2		59.0	57.4	56.2	62.6	22	MW689188.1-FAdV-A-AD17-Egypt-2020
23	48.3	48.3	48.3	48.3	48.3	48.3	48.3	48.0	48.3	48.0	48.0	48.0	47.6	48.3	48.0	47.6	47.6	47.6	48.0	49.1	48.0	55.0		65.4	63.3	56.2	23	MW699419.1-FAdV-B-AD18-Egypt-2020
24	38.7	38.7	38.7	38.7	38.7	38.7	38.7	39.0	38.7	39.0	39.0	39.0	39.4	39.7	38.4	39.4	39.4	39.7	40.1	41.5	41.2	59.5	45.1		79.3	56.4	24	KT781516.1-FAdV-8a-MMR-B15-Egypt-2015
25	42.8	42.8	42.8	42.8	42.8	42.8	42.8	42.9	42.8	42.9	42.9	42.9	43.2	43.5	42.6	43.2	43.2	42.9	43.6	44.2	42.9	63.0	49.6	24.2		54.7	25	MW712888.1-FAdV-8b-AD15-Egypt-2020
26	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	69.1	69.1	68.6	68.6	67.6	69.0	68.6	49.2	60.3	62.2	64.5		26	MW660887.1-FAdV-C-Alex-1-Egypt-2021

Figure 6. Pairwise analysis for nucleotide sequences of seven targets Egyptian FAdVs strains isolated from broiler chickens and other related strains.

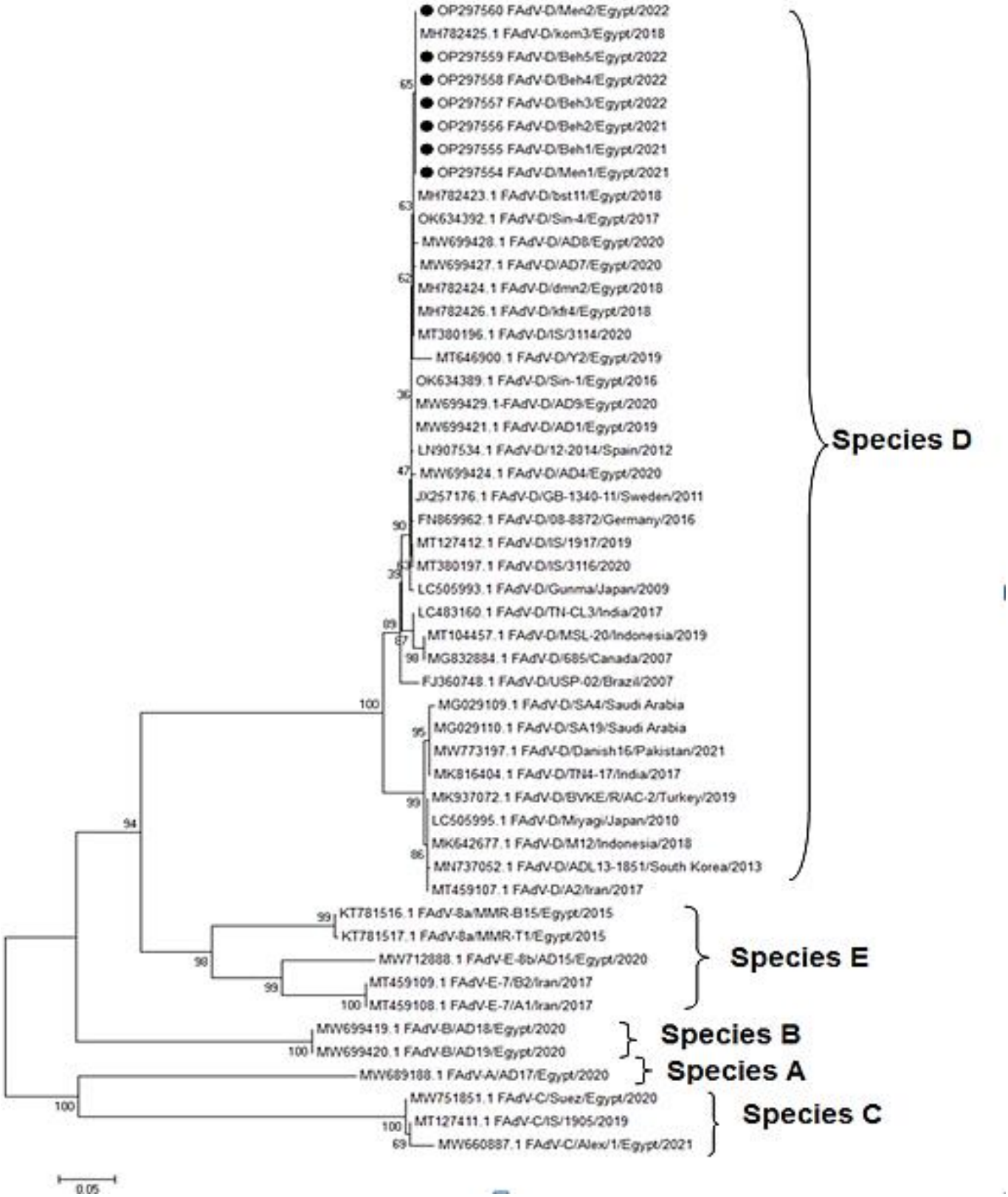


Figure 7. Phylogenetic tree for the nucleotide sequence of Loop1 region on hexon gene of FAdVs shows that the seven Egyptian strains of this study (labeled with black circle) which were isolated from broiler chickens are related genetically to FAdVs species D

DISCUSSION

Several avian species, including wild birds and domestic poultry, have recently been infected by fowl adenoviruses (Mohamed et al., 2018). Due to FAdVs spreading in the last years among chicken farms in Egypt and its effect on the elevation of mortalities and poor performance (El bestway et al., 2020; Adel et al., 2021), the current study was performed on 26 suspected chicken flocks to detect FAdVs incidence and genetic characterization of obtained viruses.

In the present study, clinical signs and postmortem lesions, especially hydropericardium, the most pathognomic postmortem lesions for HPS, were extensively detected, which is similar to previous studies (Chen et al., 2017; Schachner et al., 2018; Sultan et al., 2021). The variation in mortalities (from 10% to 28%) could depend on the age and susceptibility of the chicken's breed, the condition of the chicken's immune system, virus infection load, and/or concurrent infection with other infectious agents (Schachner et al., 2018).

Microscopically, the liver showed vacuolar degeneration and acute hepatic necrosis with intranuclear inclusion bodies in hepatocytes, which are signs of HPS and IBH infection. There were two types of intranuclear inclusions, namely basophilic and eosinophilic inclusions. The dense basophilic inclusions occupied most of the nucleus, which was enlarged with chromatin margination, and the eosinophilic inclusions with a halo around them. Presence of both basophilic and eosinophilic inclusions was similar to previous studies by Elbestway et al. (2020), Abouzied et al. (2021), and Ishag et al. (2022). The basophilic inclusions were found to be virus particle aggregation, whereas the eosinophilic inclusions were fibrillar-granular material and crystal aggregation (Itakura et al., 1977). In most cases, basophilic inclusions were formed first, followed by eosinophilic inclusions. Basophilic inclusions in the liver, kidney, proventriculus, intestine, pancreas, and spleen indicate a severe and rapid infection with FadVs (Nakamura et al., 2011). The cause of hydropericardium and ascites in chickens was acute hepatic necrosis leading to hepatic failure and consequently heart circulatory failure (Nakamura et al., 2002). Heart circulatory failure causes acute hydremia and hypoproteinemia resulting in the large immediate effusion of pericardial fluid through the blood capillaries of the epicardium into the pericardial sac, leading to hydropericardium that might impair heart function by decreasing sound, filling with blood and pulse pressure leading to death (Nakamura et al., 1999). The heart had severe edema and hyperemia in the pericardium, and cardiac myocytes were necrotized with moderate neutrophil and monocyte infiltration. These lesions were according to the findings of previous studies by Niu et al. (2018) and Ishag et al. (2022). There was also severe pneumonia, perivascular edema, and hemorrhage in infected chickens, which was reported in studies by Kataria et al. (2013) and Niu et al. (2018). In the kidneys, there were considerable edema and hemorrhage in the renal interstitium, as well as massive vacuolar degeneration and necrosis of renal tubules similar to that mentioned by Kataria et al. (2013), Zhao et al. (2015) and Niu et al. (2018). Zhao et al. (2015) also reported severe enteritis of the intestinal mucosa, edema, and hemorrhage in the submucosa of the duodenum. The current results agreed with previous studies regarding intranuclear inclusion bodies in epithelial cells of renal tubules (Schachner et al., 2018; Ishag et al., 2022), glandular cells of the pancreas (Nakamura et al., 2011), glandular lobules of proventriculus and duodenum (Nakamura et al., 1999), and inside the lymphocytes of the spleen (Ishag et al., 2022) as well as lymphocytic depletion of the bursa of Fabricius and thymus (Matos et al., 2016).

Of 26 samples, 10 (38.46%) from Beheira and Menofia were positive for FAdV using primers that target the L1 region of the hexon gene. Similarly, previous studies in Egypt indicated FAdV in 45% of flocks in Alexandria, Beheira, and Kafr El Sheikh governorates (Elbestawy et al., 2020), and 22% in Behira governorate (Radwan et al., 2019). However, Abouzied et al. (2021) reported 11 positive samples (out of 14) from the North Sinai governorate, Egypt. The difference in virus detection from suspected flocks may be due to the course of the virus, virus infection load, age of the chicken, and its immune status.

In the present study, genetic sequencing for the 590bp DNA fragment of loop 1 of the hexon gene of seven FAdVs was done using the same PCR primers since this gene is used for FAdVs serotyping and produces the main protein against which the neutralizing epitope was directed (Niczyporuk, 2018).

Neighbor-joining based phylogenetic tree was constructed using sequences of this study and obtained sequences from Egypt and other countries representing different FAdVs species and serotypes. The analysis showed that present sequences were clustered into FAdV species serotype 2/11. This serotype was previously recorded in Egypt in different governorates, such as

Alexandria, Beheira, Kafr El Sheikh, Sharkia, North Sinai, Assiut, Daqahlia, Sohag, and El Wadi El gedid (Elbestawy et al., 2020; Abouzied et al., 2021; Adel et al., 2021; Safwat et al., 2022) indicating the dominance and wide spread of this serotype in Egypt. Globally, 34% of FAdV isolates from 38 countries were classified as FAdV Species D (Kiss et al., 2021). Moreover, the sequences of the current study were close to other published sequences from countries, such as Germany, Spain, and Japan (Marek et al., 2010; Mase et al., 2012; Schachner et al., 2016, Figure 7).

Nucleotide identity between sequences in this study and other selected sequences from Egypt and other countries was performed using Lasergene software. The tested strains and the reference strain had a high nucleotide similarity of 99.3% to 100% with other FAdV-D strains previously detected in Egypt during the last four years (Elbestawy et al., 2020), which previously detected the same serotype from 17 different poultry farms. This study revealed that the virus is still present, creating significant losses in Egyptian poultry flocks.

The Beh1, 2, 3, 4, 5 and Men1, 2 showed high divergence from the FAdVs strains previously detected in Egypt that belong to FAdVs species A, B, C, and E (Figure 6) as the identity percent with them ranging 53.8-70.6%.

The studied strains showed high similarity with FAdVs species D detected in Germany, Spain, Sweden, and Japan (more than 99%), while the similarity percent was lower with FAdVs-D detected in Canada (96.8%) and Saudi Arabia (93.3%, Figure6) indicating its continuous circulation in different geographic areas of the world causing significant losses.

CONCLUSION

This study identified FAdV in 10 broiler flocks suffering from HPS and located in Beheira and Menofia provinces of Egypt using molecular and histopathological techniques during 2021-2022. Partial hexon gene DNA sequencing of detected FAdVs revealed that all of them are identical and classified as FAdV species D suggesting that this FAdV species is the most important cause of HPS in Egypt. Ascitis and hydropericardium are the main postmortem lesions, while intra-nuclear inclusion bodies in the liver are the main histopathological finding observed in infected flocks. Further investigations are essential to determine the epidemiology of the FAdVs subtypes in all geographic areas of Egypt and investigate its pathogenicity and full genome characterization to implement protective control measures, including the application of homogenous or multivalent vaccines to prevent further losses in poultry flocks.

DECLARATIONS

Acknowledgments

The authors would like to thank the farm owners and veterinarians who assisted with this study.

Funding

There was no funding support for this study.

Authors' contributions

Eid Hussein put the research protocol, collected the samples from chicken farms, made postmortem examinations, virus isolation, and participated in manuscript writing. Nessreen Fouad Anwar and Marwa Ali Abdelmagid made pathological and histopathological analyses and contributed to the manuscript writing. Osama Mahana and Hossam Shabaan Elsebaey made molecular tests, DNA sequence analyses and participated in manuscript writing. Mohammed Abo Elkhair revised the final draft of the manuscript. All authors have checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors claim that they have no conflicts of interest.

Ethical consideration

Authors considered ethical concerns, such as (plagiarism, misconduct, permission to publish, double publishing, data fabrication and/or falsification, and/or submission, and redundancy).

Availability of data and materials

The data described in this study are accessible from the relevant authors upon request.

REFERENCES

Abouzied H, Soliman M, El-Sayed E, Ali N, and El-Tarabilli M (2021). Molecular investigation of fowl aviadenovirus in broiler flocks in North Sinai Province. Suez Canal Veterinary Medical Journal, 26(2): 449-467. DOI: https://www.doi.org/10.21608/scvmj.2021.217942

Adel A, Mohamed AA, Samir M, Hagag NM, Erfan A, Said M, Arafa A, Hassan WM, El Zowalaty ME, and Shahien MA (2021). Epidemiological and molecular analysis of

circulating fowl adenoviruses and emerging of serotypes 1, 3, and 8b in Egypt. Heliyon, 7(12): e08366. DOI: https://www.doi.org/10.1016/j.heliyon.2021.e08366

Anjum AD, Sabri MA, and Iqbal Z (1989). Hydropericarditis syndrome in broiler chickens in Pakistan. The Veterinary Record, 124(10): 247-248. DOI: https://www.doi.org/10.1136/vr.124.10.247

Bancroft JD and Layton C (2013). The Hematoxylin and eosin. In: S. K. Suvarna, C. Layton, and J. D. Bancroft (Editors), Theory practice of histological techniques, 7th Edition. Churchill Livingstone of El Sevier., Philadelphia. Chapter 10, pp. 173-186. DOI: https://www.doi.org/10.1016/B978-0-7020-4226-3.00010-X

Chen H, Dou Y, Zheng X, Tang Y, Zhang M, Zhang Y, Wang Z, and Diao Y (2017). Hydropericardium hepatitis syndrome emerged in Cherry Valley Ducks in China. Transboundary and Emerging Diseases, 64(4): 1262-1267. DOI: https://www.doi.org/10.1111/tbed.12500

Cizmecigil UY, Umar S, Yilmaz A, Bayraktar E, Turan N, Tali B, Aydin O, Tali HE, Yaramanoglu M, Yilmaz SG et al. (2020). Characterisation of fowl adenovirus (FAdV-8b) strain concerning the geographic analysis and pathological lesions associated with inclusion body hepatitis in broiler flocks in turkey. Journal of Veterinary Research, 64(2): 231-237. DOI: https://www.doi.org/10.2478%2Fjvetres-2020-0026

Elbestawy AR, Ibrahim MH, Ammam H, Noreldin AE, El Bahrawy A, and Ellakany HF (2020). Molecular characterization of fowl adenovirus D species in broiler chickens with inclusion body hepatitis in Egypt. Alexandria Journal of Veterinary Sciences, 64(1): 110-117. DOI: https://www.doi.org/10.5455/ajvs.74411

El-Tholoth M and Abou El-Azm KI (2019). Molecular detection and characterization of fowl adenovirus associated with inclusion body hepatitis from broiler chickens in Egypt. Tropical Animal Health and Production, 51: 1065-1071. DOI: https://www.doi.org/10.1007/s11250-018-01783-0

Hafez MH (2011). Avian adenoviruses infections with special attention to inclusion body hepatitis/ hydropericardium syndrome and egg drop syndrome. Pakistan Veterinary Journal, 31(2): 85-92. DOI: https://www.doi.org/10.17169/REFUBIUM-18486

Hall TA (1999). BIOEDIT: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series, 41: 9598. DOI: https://www.doi.org/10.14601/Phytopathol_Mediterr-14998u1.29

Hess M (2000). Detection and differentiation of avian adenoviruses. Avian Pathology, 29(3): 195-206. DOI: https://www.doi.org/10.1080/03079450050045440

Ishag HZA, Terab AMA, El Tigani-Asil ETA, Bensalah OK, Khalil NAH, Khalafalla AI, Al Hammadi ZMAH, Shah AAM, and Al Muhairi SSM (2022). Pathology and molecular epidemiology of fowl adenovirus serotype 4 outbreaks in broiler chicken in Abu Dhabi Emirate, UAE. Veterinary Sciences, 9(4): 154. DOI: https://www.doi.org/10.3390/vetsci9040154

Itakura C, Matsushita S, and Goto M (1977). Fine structure of inclusion bodies in hepatic cells of chickens naturally affected with inclusion body hepatitis. Avian Pathology, 6(1): 19-32. DOI: https://www.doi.org/10.1080/03079457708418209

Kataria JM, Mohan C, Dey S, Dash BB, and Dhama K (2005). Diagnosis and immunoprophylaxis of economically important poultry diseases: A review. Indian Journal of Animal Sciences, 75(5): 555-567. Available at: https://epubs.icar.org.in/index.php/IJAnS/article/view/9536/4259

Kataria JM, Dhama K, Nagarajan S, Chakraborty S, Kaushal A, and Deb R (2013). Fowl adenoviruses causing hydropericardium syndrome in poultry. Advances in Animal and Veterinary Sciences, 1(4S): 5-13. Available at: http://nexusacademicpublishers.com/table_contents_detail/4/133/html

Kiss I, Homonnay ZG, Mato T, Banyai K, and Palya V (2021). An overview on distribution of fowl adenoviruses. Poultry Science, 100(5): 101052. DOI: https://www.doi.org/10.1016/j.psj.2021.101052

Maartens LH, Joubert HW, Aitchison H, and Venter EH (2014). Inclusion body hepatitis associated with an outbreak of fowl adenovirus type 2 and type 8b in broiler flocks in South Africa. Journal of the South African Veterinary Association, 85(1): 1-5. DOI: https://www.doi.org/10.4102/jsava.v85i1.1146

Marek A, Günes A, Schulz E, and Hess M (2010). Classification of fowl adenoviruses by use of phylogenetic analysis and high-resolution melting-curve analysis of the hexon L1 gene region. Journal of Virological Methods, 170(1-2): 147-154. DOI: https://www.doi.org/10.1016/j.jviromet.2010.09.019

Mase M, Nakamura K, and Minami F (2012). Fowl adenoviruses isolated from chickens with inclusion body hepatitis in Japan, 2009-2010. The Journal of Veterinary Medical Science, 74(8): 1087-1089. DOI: https://www.doi.org/10.1292/jvms.11-0443

Matos M, Grafl B, Liebhart D, Schwendenwein I, and Hess M (2016). Selected clinical chemistry analytes correlate with the pathogenesis of inclusion body hepatitis experimentally induced by fowl aviadenoviruses. Avian Pathology, 45(5): 520-529. DOI: https://www.doi.org/10.1080/03079457.2016.1168513

McFerran JB and Adair BM (2003). Hydropericardium syndrome. Diseases of poultry, 11th Edition, Iowa State University Press., Ames, USA. pp. 220-221.

Meulemans G, Couvreur B, DecaessteckerM, Boschmans M, and van den Berg TP (2004). Phylogenetic analysis of fowl adenoviruses. Avian Pathology, 33(2): 164-170. DOI: https://www.doi.org/10.1080/0307945031000165208 6

Mittal D, Jindal N, Tiwari AK, and Khokhar RS (2014). Characterization of fowl adenoviruses associated with hydropericardium syndrome and inclusion body hepatitis in broiler chickens. Virusdisease, 25(1): 114-119. DOI: https://www.doi.org/10.1007/s13337-013-0183-7

Mohamed MHA, El-Sabagh IM, Abdelaziz AM, Al Ali AM, Alramadan M, Lebdah MA, Ibrahim AM, and Al-Ankari AS (2018). Molecular characterization of fowl aviadenovirusesspecies D and E associated with inclusion body hepatitis in chickens and falcons indicates possible cross-species transmission. Avian Pathology, 47(4): 384-390. DOI: https://www.doi.org/10.1080/03079457.2018.1457769

Mohamed-Sohaimi N, Bejo MH, Omar AR, Ideris A, and Mat-Isa N (2019). Molecular characterization of fowl adenovirus

isolate of Malaysia attenuated in chicken embryo liver cells and its pathogenicity and immunogenicity in chickens. PLoS One, 14(12): e0225863. DOI: https://www.doi.org/10.1371/journal.pone.0225863

Nakamura K, Mase M, Yamaguchi S, Shibahara T, and Yuasa N (1999). Pathologic study of specific-pathogen-free chicks and hens inoculated with adenovirus isolated from hydropericardium syndrome. Avian Disease, 43(3): 414-423. Available at: https://pubmed.ncbi.nlm.nih.gov/10494409

Nakamura K, Tanaka H, Mase M, Imada T, and Yamada M (2002). Pancreatic necrosis and ventricular erosion in adenovirus associated hydropericardium syndrome of broilers. Veterinary Pathology, 39(3): 403-406. Veterinary Pathology, 39(3): 164-170. DOI: https://www.doi.org/10.1354/vp.39-3-403

Nakamura K, Mase M, Yamamoto Y, Takizawa K, Kabeya M, Wakuda T, Matsuda M, Chikuba T, Yamamoto Y, Ohyama T et al. (2011). Inclusion body hepatitis caused by fowl adenovirus in broiler chickens in Japan, 2009-2010. Avian Disease, 55(4): 719-723. DOI: https://www.doi.org/10.1637/9813-052511-Case.1

Niczyporuk JS (2018). Deep analysis of loop L1 HVRs1-4 region of the hexon gene of adenovirus field strains isolated in Poland. PLoS One, 13(11): e0207668. DOI: https://www.doi.org/10.1371/journal.pone.0207668

Niu Y, Sun Q, Zhu M, Zhao J, Zhang G, Liu X, Xiao Y, and Liu S (2018). Molecular epidemiology and phylogenetic analysis of fowl adenoviruses caused hydropericardium outbreak in China during 2015. Poultry Science, 97(3): 803-811. DOI: https://www.doi.org/10.3382/ps/pex338

Radwan MM, El-Deeb AH, Mousa MR, El-Sanousi AA, and Shalaby MA (2019). First report of fowl adenovirus 8a from commercial broiler chickens in Egypt: Molecular characterization and pathogenicity. Poultry Science, 98(1): 97-104. DOI: https://www.doi.org/10.3382/ps/pey314

Raue R, Gerlach H, and Müller H (2005). Phylogenetic analysis of the hexon loop 1 region of an adenovirus from psittacine birds supports the existence of a new psittacine adenovirus (PsAdV). Archives of Virology, 150(10): 1933-1943. DOI: https://www.doi.org/10.1007/s00705-005-0578-x

Safwat MM, Sayed AR, Elsayed MFA, and Ibrahim AA (2022). Genotyping and pathogenicity of fowl adenovirus isolated from broiler chickens in Egypt. BMC Veterinary Research, 18: 325. DOI: https://www.doi.org/10.1186/s12917-022-03422-1

Schachner A, Marek A, Jaskulska B, Bilic I, and Hess M (2014). Recombinant FAdV-4 fiber-2 protein protects chickens against hepatitis-hydropericardium syndrome (HHS). Vaccine, 32(9): 1086-1092. DOI: https://www.doi.org/10.1016/j.vaccine.2013.12.056

Schachner A, Marek A, Grafl B, and Hess M (2016). Detailed molecular analyses of the hexon loop-1 and fibers of fowl aviadenoviruses reveal new insights into the antigenic relationship and confirm that specific genotypes are involved in field outbreaks of inclusion body hepatitis. Veterinary Microbiology, 186: 13-20. DOI: https://www.doi.org/10.1016/j.vetmic.2016.02.008

Schachner A, Matos M, Grafl B, and Hess M (2018). Fowl adenovirus-induced diseases and strategies for their control-a review on the current global situation. Avian Pathology, 47(2): 111-126. DOI: https://www.doi.org/10.1080/03079457.2017.1385724

Schachner A, Grafl B, and Hess M (2021). Spotlight on avian pathology: Fowl adenovirus (FAdV) in chickens and beyond–an unresolved host-pathogen interplay. Avian Pathology, 50(1): 2-5. DOI: https://www.doi.org/10.1080/03079457.2020.1810629

Schonewille E, Singh A, Gobel TW, Gerner W, Saalmüller A, and Hess M (2008). Fowl adenovirus (FAdV) serotype 4 causes depletion of B and T cells in lymphoid organs in specific pathogen-free chickens following experimental infection. Veterinary Immunology and Immunopathology, 121(1-2): 130-139. DOI: https://www.doi.org/10.1016/j.vetimm.2007.09.017

Sultan H, Arafa AES, Adel A, Selim K, El-Hoseni M, and Talaat S (2021). Genetic characterization of novel fowl aviadenovirus-4 (FADV-4) from the outbreak of hepatitis-hydropericardium syndrome in commercial broiler chickens in Egypt. Avian Diseases, 65(3): 385-390. DOI: https://www.doi.org/10.1637/aviandiseases-D-21-00023

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30(12): 2725-2729. DOI: https://www.doi.org/10.1093/molbev/mst197

Zhao J, Zhong Q, Zhao Y, Hu YX, and Zhang GZ (2015). Pathogenicity and complete genome characterization of fowl adenoviruses isolated from chickens associated with inclusion body hepatitis and hydropericardium syndrome in China. PLoS One, 10(7): e0133073. DOI: https://www.doi.org/10.1371/journal.pone.0133073

JWPR
Journal of World's Poultry Research

2023, Scienceline Publication

J. World Poult. Res. 13(1): 161-167, March 25, 2023

Research Paper, PII: S2322455X2300018-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.18

The Efficiency of *Urtica dioica* Extract in Feeding of Laying Hens

Olga Bagno[1], Sergey Shevchenko[2], Antonina Shevchenko[2], Ekaterina Izhmulkina[3], Oleg Prokhorov[1], Elena Ulrikh[4]*, and Anna Berezina[5]

[1]*Department of Animal Science, Kuzbass State Agricultural Academy, Russia*
[2]*Department of Agricultural Technologies and Veterinary Medicine, Gorno-Altai State University, Russia*
[3]*Rector, Kuzbass State Agricultural Academy, Russia*
[4]*Department of Production and Quality Expertise of Agricultural Products, Kaliningrad State Technical University, Russia*
[5]*Center for Digital Education and Scientific Educational Resources, Kuzbass State Agricultural Academy, Russia*

*Corresponding author's E-mail: elen.ulrich@mail.ru

Received: December 30, 2022
Accepted: February 11, 2023

ABSTRACT

Currently, poultry specialists are working hard to find feed additives of natural origin. Medicinal plants are a source of a wide range of biologically active compounds with multifunctional effects, including antimicrobial ones. To understand the potential use of various medicinal plants and their extracts in poultry farming, the current study aimed to investigate the effect of feeding different doses of water-ethanol extract of *Urtica dioica* (*Urtica dioica* L.) on the egg productivity of laying hens. A total of 300 laying hens were divided to control and five experimental groups of chickens, each with 5 replicates. During the entire experiment, the laying hens of the control group were fed complete compound feeds according to the egg-laying phase, and the chickens of the experimental groups were additionally fed *Urtica dioica* extract in different doses. The results indicated that feeding laying hens with *Urtica dioica* extract in doses of 5, 10, 15, 20, and 25 mg/kg of body weight had a positive effect on their egg productivity. An increase in egg production per average laying hen in the experimental groups was 2.6-6.1%, and the intensity of egg production was 2.1-5.4%, compared to the control. However, the feed consumption in all experimental groups decreased. When introducing Urtica dioica extract into full-fledged compound feeds for laying hens, there was an increase in the relative egg white content, egg white/egg yolk ratio, and a decrease in relative yolk and shell content. Accordingly, it is suggested to include Urtica dioica extract at a dose of 15 mg/kg in the diet of laying hens which can improve economic efficiency and egg parameters.

Keywords: Chemical composition, Egg morphology, Egg production, Feed conversion, Medicinal plant

INTRODUCTION

In recent decades, poultry specialists have been actively interested in the use of plant-based additives in poultry feeding, which can provide high zootechnical indicators, improve the safety of poultry, and contribute to the normalization of metabolism (Abilov et al., 2021; Butt et al., 2022). On the other hand, the modern conditions of the industry necessitate the use of drugs that do not cause drug resistance and have a multifunctional effect. Phyto-biotics, including feed additives derived from *Urtica dioica* medicinal plant (*Urtica dioica* L.), can be effective in this regard. The interest in *Urtica dioica* for its use in feeding poultry is associated with the content of a significant amount of biologically active compounds in its leaves, which can cause different positive effects. Thus, *Urtica dioica* leaves contain terpenoids (Gül et al., 2012) and carotenoids (Kukric et al., 2012), including β-carotene, neoxanthin, violaxanthin, lutein, and lycopene (Guil-Guerrero et al., 2003), fatty acids, especially palmitic, cis-9,12-linoleic and α-linolenic acids (Bağci, 2002; Guil-Guerrero et al., 2003), various polyphenolic compounds (Rutto et al., 2013; Orcic et al., 2014), essential amino acids, chlorophyll, vitamins, tannins, carbohydrates, sterols, polysaccharides, isolectins (Guil-Guerrero et al., 2003; Sajfrtová et al., 2005; Kukric et al., 2012), and minerals (Kara, 2009), the most important of which is iron. A study conducted by Augspole et al. (2017) showed that *Urtica dioica* was richer in individual polyphenols, compared to other wild plants. The advantage of *Urtica dioica* leaves in the content of phenolic compounds,

To cite this paper: Bagno O, Shevchenko S, Shevchenko A, Izhmulkina E, Prokhorov O, Ulrich E, and Berezina A (2023). The Efficiency of *Urtica dioica* Extract in Feeding of Laying Hens. *J. World Poult. Res.*, 13(1): 161-167. DOI: https://dx.doi.org/10.36380/jwpr.2023.18

compared to dandelion leaves, was found by Ghaima et al. (2013). Rutin is noted by Vajic et al. (2015) as the predominant phenolic compound in nettle leaves. According to Nasiri et al. (2011), *Urtica dioica* is the only plant containing choline acetyltransferase synthesizing acetylcholine.

Urtica dioica contains various compounds with antioxidant action, including terpenoid phenol, flavonoids, alpha-tocopherol, and ascorbic acid (Surai et al., 2019). Carvacrol and carvone are the main terpenoids of nettle with antioxidant, growth-stimulating, antibacterial, and antiviral effects (Upton, 2013). Terpenoids and phenolic compounds in *Urtica dioica* suppress oxidative stress through different mechanisms, such as inhibition of lipid peroxidation, activation of antioxidant enzymes, chelation of metals, and an increase in uric acid levels (Behrooj et al., 2012).

Several studies have found that biologically active compounds of *Urtica dioica* can exhibit stronger antibacterial activity than synthetic antimicrobial drugs. Modarresi-Chahardehi et al. (2012) considered the antimicrobial activity of nine extracts of *Urtica dioica* obtained by various methods using organic solvents. Of nine extracts, four types suppressed the growth of Gram-negative and five types could inhibit Gram-positive bacteria. Ethyl acetate extracts of *Urtica dioica* showed the highest antimicrobial activity. Some studies have indicated that the use of *Urtica dioica* in feeding farm animals and poultry has a positive effect on their productivity, resistance, and the state of the microbiota of the gastrointestinal tract (Wenk, 2000).

The use of extracts obtained from *Urtica dioica* in poultry diet is of particular interest. In this regard, the current study aimed to determine the optimal doses of *Urtica dioica* extract in mixed feed for the egg productivity of chickens.

MATERIALS AND METHODS

Ethical approval

The study was conducted and certified based on the ethical rules of Kuzbass State Agricultural Academy, Russia.

Study design

The research was carried out on laying hens (Hisex White cross breed) of a poultry farm located in Kuzbass, Russia, aged 49 weeks. A total of 300 laying hens were randomly selected from the study area and were divided into five experimental groups and one control group. Each group had 5 replicates, including 10 laying hens. The hens were managed based on the last recommendations of the management guidelines of the breed during the study.

When dividing laying hens into groups, the age and body weight of laying hens were taken according to the requirements of the methodology developed in VNITIP (Rinttilä and Apajalahti, 2013). The chickens were fed with a full-fledged compound feed according to the egg-laying phase. The chickens of the experimental groups were additionally fed *Urtica dioica* extract in different doses based on the main biologically active compounds of the plant (Devkota et al., 2022). Laying hens of the first, second, third, and fourth experimental groups received *Urtica dioica* at 5, 10, 15, 20, and 25 mg/kg of body weight, respectively.

Urtica dioica extract was obtained through water-ethanol extraction. It contains flavonoids (in terms of quercetin, 4.26%), 2.53% ascorbic acid, 1.17% caffeic acid, 0.25% ferulic acid, 0.12% carotenoids, and 0.005% coumarins (in terms of scopoletin). The number of biologically active compounds met the requirements of regulatory agents (Bagno et al., 2019). The experiment lasted 6 months. The average egg-laying and the average egg weight were calculated according to the previous study by Rinttilä and Apajalahti (2013). The safety of the chickens was evaluated as a percentage of the initial livestock for the entire period of the research.

The morphological and chemical compositions of eggs were determined according to previously accepted methods (Rinttilä and Apajalahti, 2013; Tikhomirov and Fomin, 2018). The contents of moisture, protein, fat, and ash were determined in the collected egg sample.

The European Efficiency Coefficient (EEC) was used to assess the efficiency of egg production:

$$\text{EEC} = (1{,}4 \times egg\ mass\ per\ head, kg) - (0{,}35 \times feed\ conversion, kg)$$

Economic efficiency was determined by the Poultry Egg Production Efficiency Index (PEPEI; Kavtarashvili, 2015):

$$\text{PEPEI} = \frac{(E \text{ x } ASe) + (M \times Pm)}{(Cf \text{ x } 100 : Sf) + Cgr} \text{ x } 100$$

An increase in the above indices, expressed in units, indicates an increase in the efficiency of egg production.

Where, E denotes the gross yield of eggs (pcs), ASe describes the average selling price of 1 egg (rub), M signifies the gross yield of meat in slaughter weight (kg), Pm is the average selling price of 1 kg of meat (rub), Cf refers to the total cost of feed for the productive period (rub), Sf represents the share of feed in the cost of eggs in percentage, and Cgr is the cost of growing replacements (rub).

Statistical analysis

The obtained data were analyzed by standard statistical methods using SPSS Software Version 22. The data in the tables below are presented in the form of Mean (M) ± Standard Error of the Mean (SEM). The reliability of the differences between the control group and each of the experimental groups was assessed by the Student's t-test. The results at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The results of this study on the effect of feeding different doses of *Urtica dioica* extract on the egg productivity of laying hens are presented in Table 1 and Figure 1. The chickens fed a diet containing *Urtica dioica* extract had higher egg production for the initial and average laying in the experimental groups than chickens in the control group ($p > 0.05$). This production rate increased by 2.7% in the first group, 5.3% and 6.6% in the second group, 8.2% and 6.1% in the third group, 3.6% and 2.6% in the fourth group, and 7.8% and 6.1% in the fifth group. A similar finding was reported by Bruneel et al. (2013), indicating the higher intensity of egg production in the five experimental groups, compared to the control by 2.2, 5.4, 5.0, 2.1, and 5.0%, respectively. The average egg weight rates of laying hens of the first to the third experimental groups were 0.8%, 2.3%, and 1.4%, respectively, and the fourth and fifth experimental groups were 2.6% and 1.0%, which were less than the control group ($p > 0.05$).

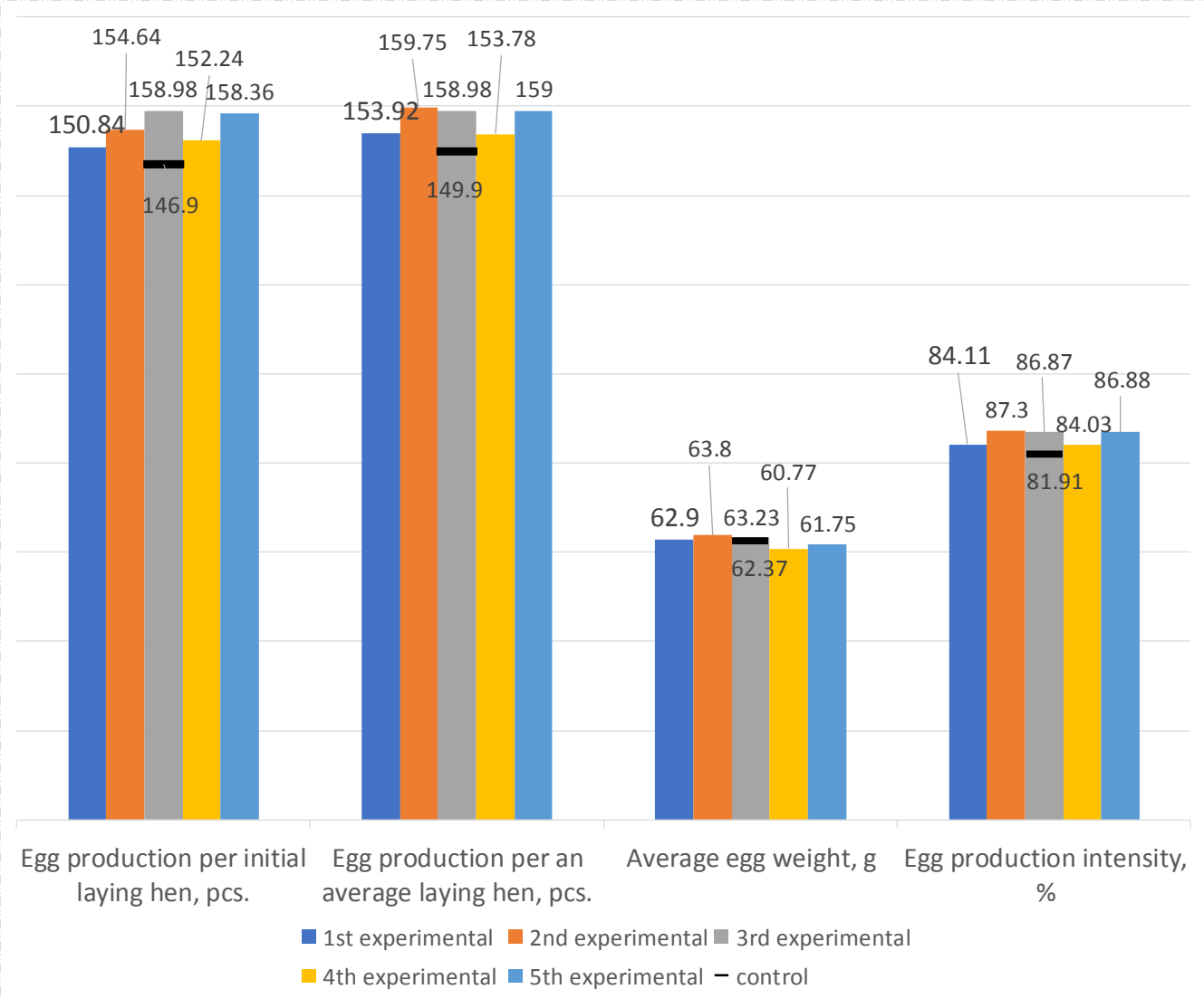


Figure 1. Indicators of egg productivity of laying hens aged 49 weeks consumed different levels of *Urtica dioica* extract

Table 1. The effects of different levels of *Urtica dioica* extract on the performance of laying hens aged 49 weeks

Indicator \ Group	Control	1	2	3	4	5
Safety (%)	94	94	92	100	96	98
Feed consumption per group (kg)	1125	1130.37	1150.50	1111.60	1073.70	1081.82
Feed consumption per 1 head (kg)	22.96	23.07	23.77	22.23	21.69	21.72
Feed conversion per 10 eggs	1.53	1.50 (-2%)*	1.49 (-2.6%)*	1.40 (-8.5%)*	1.41 (-7.8%)*	1.37 (-10.5%)*
Feed conversion per 1 kg of egg mass	2.46	2.38 (-3.2%)*	2.33 (-5.3%)*	2.21 (-10.2%)*	2.32 (-5.7%)*	2.21 (-10.2%)*

*compared to the control group results, %

Table 2. The eggs contents of lying hens aged 49-week-old fed different levels of *Urtica dioica* extract

Parameters \ Group	Control	1	2	3	4	5
Shell thickness (mm)	0.403 ± 0.009	0.401± 0.008	0.407 ± 0.020	0.393 ± 0.022	0.373 ± 0.004*	0.377 ± 0.014
Egg White (%)	57.11 ± 0.99	57.72 ± 0.86	58.40 ± 0.73	59.04 ± 0.64	59.67 ± 0.58*	60.36 ± 0.59*
Egg Yolk (%)	30.19 ± 0.92	29.59 ± 0.77	28.99 ± 0.63	28.38 ± 0.51	27.76 ± 0.44*	27.13 ± 0.48**
Egg Shell (%)	11.09 ± 0.46	11.08 ± 0.42	11.00 ± 0.38	10.98 ± 0.39	10.96 ± 0.40	10.89 ± 0.45
Egg white/egg yolk ratio	1.91 ± 0.09	1.96 ± 0.08	2.02 ± 0.06	2.09 ± 0.06	2.16 ± 0.05*	2.23 ± 0.05**

The safety of laying hens in the third, fourth, and fifth experimental groups was higher than 100, 96, and 98%, respectively, and it was 92% in the second experimental group, which was less than the control hens (94%).

The feed consumption per group in the first and second experimental groups was more than the control, third, fourth, and fifth groups, respectively, compared to the control. Therewith, the feed conversion per 10 eggs in all experimental groups decreased by 2%, 2.6%, 8.5%, 7.8%, and 10.5%, respectively, compared to the control ($p > 0.05$). In all experimental groups, the conversion of feed per 1 kg of egg mass decreased compared to the control group ($p > 0.05$). According to Langhout (2000), Madrid et al. (2003), Alçiçek et al. (2004), and Zhang et al. (2005), *Urtica dioica* extracts may have properties that stimulate appetite and digestion.

The morphological composition of eggs is presented in Table 2. There was a significant decrease in eggshell thickness in the fourth experimental group compared to the control group ($p < 0.05$).

When feeding hens with *Urtica dioica* extract, there was a significant increase in the relative egg white content of the eggs in the fourth and fifth experimental groups compared to the control group ($p < 0.05$), and a decrease in the relative yolk content in the fourth and fifth experimental groups, compared to the control group ($p < 0.05$). The relative content of the shell decreased insignificantly compared to the control group, and the egg white/yolk ratio was elevated significantly in the fourth and fifth experimental groups compared to the control group ($p < 0.05$).

When feeding *Urtica dioica* extract to laying hens, the ash content in the eggs of chickens in the experimental groups did not significantly differ from that of the control group ($p > 0.05$; Figure 2). There was a decrease in protein content in the samples of the first, second, third, and fifth experimental groups by 0.02%, 0.04%, 0.06%, 0.10%, and an increase in the fourth experimental group by 0.69%. The water and fat content in the eggs of chickens of the experimental groups was lower than the control group ($p > 0.05$).

The efficiency indices of chicken egg production are presented in Table 3.The EEC of egg production, which characterizes a set of indicators, such as egg mass per 1 laying hen and feed conversion per 1 kg of egg mass, was higher when feeding the *Urtica dioica* extract to the experimental groups (the first to fifth) by 0.49, 1.22, 1.07,

0.03, 0.74 units, respectively. In PEPEI of laying hens, the parameters of the full technological cycle of poultry production, were higher in the experimental chickens, compared to the control when using *Urtica dioica* extract in the first to fifth experimental groups by 1.18%, 0.93%, 6.76%, 4.17%, 6.71%, respectively.

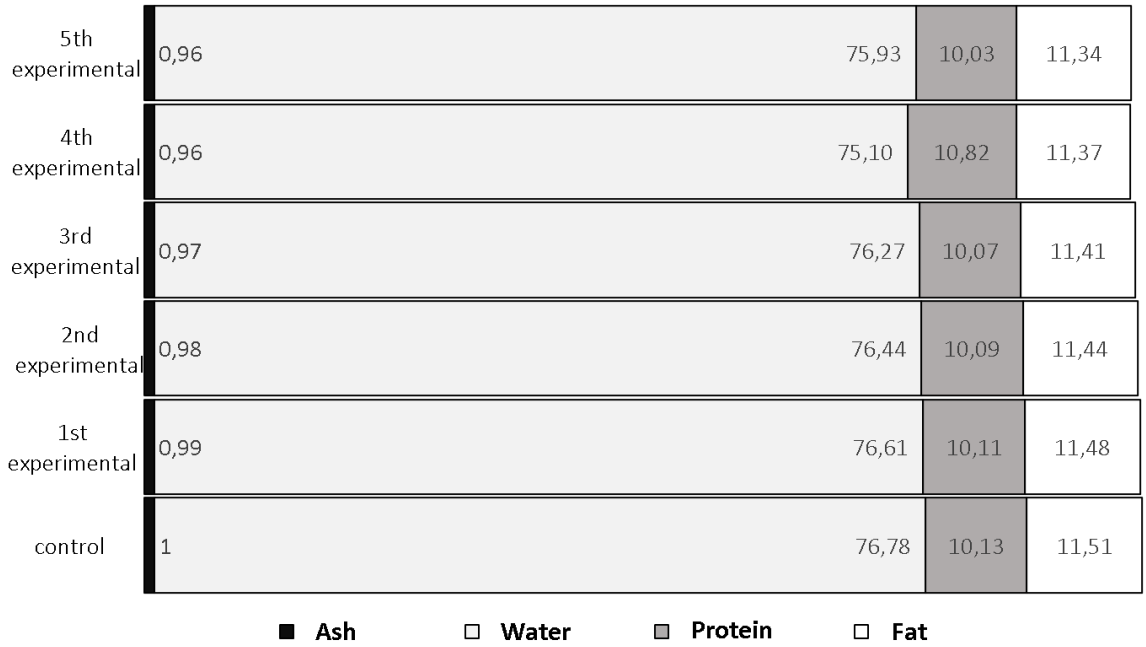


Figure 2. Chemical composition of chicken eggs that used different levels of *Urtica dioica* extracts.

Table 3. Indicators of the efficiency of the production of chicken eggs when feeding *Urtica dioica* extracts

Factor / Group	Control	1	2	3	4	5
EEC (unit)	12.23	12.72	13.45	13.30	12.26	12.97
PEPEI (unit)	100.82	102.0	101.75	107.58	104.99	107.53

EEC: European Efficiency Coefficient, PEPEI: Poultry Egg Production Efficiency Index

The results of the current research in the main provisions are consistent with the results obtained by researchers on the use of *Urtica dioica* in feeding laying hens. Thus, it was found in another study by Hosseini Mansoub (2011) that the use of *Urtica dioica* in the diets of laying hens in the amount of 0.75-2.0% of the feed weight had a significant effect on the productivity and quality of eggs.

Grela et al. (2013) indicated that *Urtica dioica* positively affected the growth and egg-laying of laying hens, and improved the color and taste of egg yolk.

The addition of *Urtica dioica* to the diet of laying hens in an amount of 1 g/kg of live weight in combination with a feed additive from Mukhor-Talin zeolite in an amount of 5% of the dry feed weight increased the safety by 4%, and the intensity of egg production by 1.3% (Luzbaev, 2010).

CONCLUSION

The findings of the present study indicated the use of *Urtica dioica* extracts at a dose of 15 mg/kg of body weight in feeding laying hens contributed to a significant increase in the production and improved the egg parameters.

DECLARATIONS

Authors' contributions

All the authors contributed to conducting the study, collecting data, statistical analysis, and writing the article.

Acknowledgments

The article was prepared as part of a comprehensive project named Development and implementation of a new

series of highly effective phytobiotic feed additives based on extracts of medicinal plants for the transition to a highly productive and environmentally friendly agricultural economy, subsidy agreement dated October 03, 2017 No. 4.610.21.0016, unique project identifier RFMEF161017X0016. This study was supported by the department of animal science, Kuzbass state agricultural academy, Russia.

Ethical considerations

The authors confirmed that attended to all ethical issues, including misconduct, plagiarism, consent to publish, falsification, double publication or submission have been checked by the authors very carefully.

Availability of data and materials

The related data of this study will be available upon request to the authors of this article.

Competing interests

The authors had no competing interests.

REFERENCES

Abilov BT, Marynich AP, Boldareva AV, and Nechaev SA (2021). Efficiency of protein-rich plant and animal additives in feeding broiler chickens. IOP Conference Series: Earth and Environmental Science, 624: 012052. Available at: https://iopscience.iop.org/article/10.1088/1755-1315/624/1/012052/pdf

Bruneel C, Lemahieu C, Fraeye I, Ryckebosch E, Muylaert K, Buyse J, and Foubert I (2013). Impact of microalgal feed supplementation on omega-3 fatty acid enrichment of hen eggs. Journal of Functional Foods, 5(2): 897-904. DOI: https://www.doi.org/10.1016/j.jff.2013.01.039

Butt KY, Nadeem N, Ghori MI, Zain H, and Kanwal N (2022). Analysis of Trigonella foenum-graecum seeds, Musa paradisiaca, and Citrus sinensis peels as a poultry feed supplement. Pakistan Journal of Life and Social Sciences, 20(1): 45-51. Available at: http://www.pjlss.edu.pk/pdf_files/2022_1/45-51.pdf

Alçiçek A, Bozkurt M, and Çabuk M (2004). The effects of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. South African Journal of Animal Science, 33(2): 89-94. DOI: https://www.doi.org/10.4314/sajas.v33i2.3761

Augspole I, Duma M, Ozola B, and Cinkmanis I (2017). Phenolic profile of fresh and frozen nettle, goutweed, dandelion, and chickweed leaves. Proceedings of the 11th Baltic Conference on Food Science and Technology, Food Science and Technology in a Changing World, Jelgava, Latvia, pp. 27-28. Available at: https://www.llu.lv/sites/default/files/files/lapas/FoodBalt_2017_Abstract_book_0.pdf

Bağci E (2002). Fatty acid composition of the aerial parts of *Urtica dioica* (*Stinging nettle*) L. (Urticaceae). In: B. Şener (Editor), Biodiversity. Springer., Boston, MA. pp. 323-327. Available at: https://link.springer.com/chapter/10.1007/978-1-4419-9242-0_40#citeas

Bagno O, Shevchenko S, Shevchenko A, Izhmulkina E, Prokhorov O, and Petruchenko A (2019). Egg productivity in the quails fed on the extract of calendula officinalis. International Journal of Innovative Technology and Exploring Engineering, 8(10): 4108-4112. Available at: https://www.ijitee.org/wp-content/uploads/papers/v8i10/J98560881019.pdf

Behrooj N, Khajali F, and Hassanpour H (2012). Feeding reduced-protein diets to broilers subjected to hypobaric hypoxia is associated with the development of pulmonary hypertension syndrome. British Poultry Science, 53(5): 658-664. DOI: https://www.doi.org/10.1080/00071668.2012.727082

Devkota HP, Paudel KR, Khanal S, Baral A, Panth N, Adhikari-Devkota A, Jha NK, Das N, Singh SK, Chellappan DK et al. (2022). Stinging nettle (*Urtica dioica* L.): Nutritional composition, bioactive compounds, and food functional properties. Molecules, 27(16): 5219. DOI: https://www.doi.org/10.3390/molecules27165219

Ghaima KK, Hashim NM, and Ali SA (2013). Antibacterial and antioxidant activities of ethyl acetate extract of nettle (*Urtica dioica*) and dandelion (Taraxacumofficinale). Journal of Applied Pharmaceutical Sciences, 3(5): 96-99. Available at: https://japsonline.com/admin/php/uploads/904_pdf.pdf

Grela E, Klebaniuk R, Kwiecie´n M, and Pietrzak K (2013). Fitobiotyki w produkcji zwierzecej [Phytobiotics in animal production]. Przegląd Hodowlany, 3: 21-24. Available at: http://ph.ptz.icm.edu.pl/wp-content/uploads/2016/12/10-Grela.pdf

Guil-Guerrero JL, Rebolloso-Fuentes MM, and Isasa MET (2003). Fatty acids and carotenoids from stinging nettle (*Urtica dioica* L.). Journal of Food Composition and Analysis, 16(2): 111-119. DOI: https://www.doi.org/10.1016/S0889-1575(02)00172-2

Gül S, Demirci B, Baser KHC, Akpulat HA, and Aksu P (2012). Chemical composition and *in vitro* cytotoxic, genotoxic effects of essential oil from *Urtica dioica* L. Bulletin of Environmental Contamination and Toxicology, 88: 666-671. DOI: https://www.doi.org/10.1007/s00128-012-0535-9

Hosseini Mansoub N (2011). Effect of nettle (*Urtica dioica*) on performance, quality of eggs, and blood parameters of laying hens. Advances in Environmental Biology, 5(9): 2718-2721. Available at: http://www.aensiweb.com/old/aeb/2011/2718-2721.pdf

Kara D (2009). Evaluation of trace metal concentrations in some herbs and herbal teas by principal component analysis. Food Chemistry, 114(1): 347-354. DOI: https://www.doi.org/10.1016/j.foodchem.2008.09.054

Kukric ZZ, Topalic-Trivunovic LN, Kukavica BM, Matoš SB, Pavicic SS, Boroja MM, and Savic AV (2012). Characterization of antioxidant and antimicrobial activities of nettle leaves (*Urtica dioica* L.). Acta Periodica Technologica, 43: 257-272. Available at: http://www.doiserbia.nb.rs/Article.aspx?ID=1450-71881243257K

Langhout P (2000). New additives for broiler chickens. World Poultry, 16(3): 22-27. Available at: https://www.cabdirect.org/cabdirect/abstract/20001416551

Luzbaev KV (2010). The use of feed additives from natural zeolite and *Urtica dioica* for laying hens. Vestnik Buryatskoi GSKhA, 3: 33-35.

Madrid J, Hernández F, García V, Orengo J, Megías MD, and Sevilla V (2003). Effect of plant extracts on ileal apparent digestibility and carcass yield in broilers at level of farm. 14th European Symposium on Poultry Nutrition, Lillehammer, Norway. p. 187.

Modarresi-Chahardehi A, Ibrahim D, Fariza-Sulaiman S, and Mousavi L (2012). Screening antimicrobial activity of various extracts of *Urtica dioica*. Revista de Biología Tropical, 60(4): 1567-1576. DOI: https://www.doi.org/10.15517/rbt.v60i4.2074

Nasiri S, Nobakht A, and Safamehr A (2011). The effect of different levels of nettle *Urtica dioica* L. (Urticaceae) medical plant in starter and grower feeds on performance, carcass traits, blood biochemical and immunity parameters of broilers. Iranian Journal of Applied Animal Sciences, 1(3): 177-181. Available at: https://www.sid.ir/paper/576168/en

Orcic D, Franciškovic M, Bekvalac K, Svircev E, Beara I, Lesjak M, and Mimica-Dukic N (2014). Quantitative determination of plant phenolics in *Urtica dioica* extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. Food Chemistry, 143: 48-53. DOI: https://www.doi.org/10.1016/j.foodchem.2013.07.097

Rinttilä T and Apajalahti J (2013). Intestinal microbiota and metabolites—Implications for broiler chicken health and performance. Journal of Applied Poultry Research, 22(3): 647-658. DOI: https://www.doi.org/10.3382/japr.2013-00742

Rutto LK, Xu Y, Ramirez E, and Brandt M (2013). Mineral properties and dietary value of raw and processed stinging nettle (*Urtica dioica* L.). International Journal of Food Science, 2013: 857120. DOI: https://www.doi.org/10.1155/2013/857120

Sajfrtová M, Sovová H, Opletal L, and Bártlová M (2005). Near-critical extraction of β-sitosterol and scopoletin from stinging nettle roots. The Journal of Supercritical Fluids, 35(2): 111-118. https://www.doi.org/10.1016/j.supflu.2004.12.008

Surai PF, Kochish II, Fisinin VI, and Kidd MT (2019). Antioxidant defense systems and oxidative stress in poultry biology: An update. Antioxidants, 8(7): 235. DOI: https://www.doi.org/10.3390/antiox8070235

Tikhomirov AI and Fomin AA (2018). Macroeconomic factors in realizing export potential for animal production. International Agricultural Journal, 3: 27-39. Available at: https://cyberleninka.ru/article/n/macroeconomic-factors-in-realizing-export-potential-for-animal-production

Upton R (2013). Stinging nettles leaf (*Urtica dioica* L.): Extraordinary vegetable medicine. Journal of Herbal Medicine, 3(1): 9-38. DOI: https://www.doi.org/10.1016/j.hermed.2012.11.001

Vajic UJ, Grujic-Milanovic J, Živkovic J, Šavikin K, Gođevac D, Miloradovic Z, Bugarski B, and Mihailovic-Stanojevic N (2015). Optimization of extraction of stinging nettle leaf phenolic compounds using response surface methodology. Industrial Crops and Products, 74: 912-917. Available at: https://www.cabdirect.org/cabdirect/abstract/20153316829

Wenk C (2000). Why all the discussion about herbs?. Proceedings of Alltech's 16th Anniversary Symposium Biotechnology in the Feed of Industry. Nottingham University Press. UK. pp. 79-96. Available at: https://cir.nii.ac.jp/crid/1572261550698764928

Zhang KY, Yan F, Keen CA, and Waldroup PW (2005). Evaluation of microencapsulated essential oils and organic acids in diets for broiler chickens. International Journal of Poultry Science, 4(9): 612-619. Available at: https://scialert.net/abstract/?doi=ijps.2005.612.619

Instructions for Authors

SUBMIT AN ARTICLE

Manuscript as Original Research Paper, Short Communication, Case Reports and Review or Mini-Review are invited for rapid peer-review publishing in *the Journal of World's Poultry Research*. Considered subject areas include: Husbandry and management; construction, environment and welfare; exotic and wild birds; Biochemistry and cellular biology; immunology, avian disease control; layer and quail management; nutrition and feeding; physiology, genetics, reproduction and hatching; technology, processing and food safety... view full aims and scope

- JWPR EndNote Style
- Manuscript Template (MS Word)
- Sample Articles
- Declaration form
- Publication Ethics

Submission

The manuscript and other correspondence should preferentially be submit online. Please embed all figures and tables in the manuscript to become one single file for submission. Once submission is complete, the system will generate a manuscript ID and will send an email regarding your submission. Meanwhile, the authors can submit or track articles via editor@jwpr.science-line.com or editorjwpr@gmail.com. All manuscripts must be checked (by English native speaker) and submitted in English for evaluation (in totally confidential and impartial way).

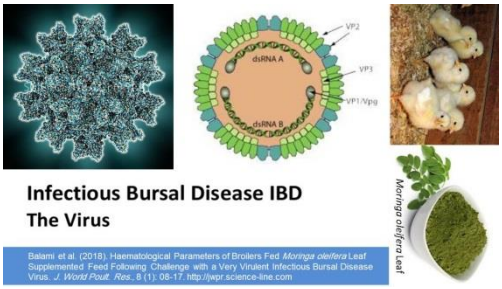
Supplementary information:
The online submission form allows supplementary information to be submitted together with the main manuscript file and covering letter. If you have more than one supplementary files, you can submit the extra ones by email after the initial submission. Author guidelines are specific for each journal. Our Word template can assist you by modifying your page layout, text formatting, headings, title page, image placement, and citations/references such that they agree with the guidelines of journal. If you believe your article is fully edited per journal style, please use our MS Word template before submission.

Supplementary materials may include figures, tables, methods, videos, and other materials. They are available online linked to the original published article. Supplementary tables and figures should be labeled with a "S", e.g. "Table S1" and "Figure S1". The maximum file size for supplementary materials is 10MB each. Please keep the files as small possible to avoid the frustrations experienced by readers with downloading large files.

Submission to the Journal is on the understanding that:
1.The article has not been previously published in any other form and is not under consideration for publication elsewhere;
2.All authors have approved the submission and have obtained permission for publish work.
3.Researchers have proper regard for conservation and animal welfare considerations. Attention is drawn to the *'Guidelines for the Treatment of Animals in Research and Teaching*'. Any possible adverse consequences of the work for populations or individual organisms must be weighed against the possible gains in knowledge and its practical applications. If the approval of an ethics committee is required, please provide the name of the committee and the approval number obtained.

Ethics Committee Approval
Experimental research involving animals should have been approved by author's institutional review board or ethics committee. This information can be mentioned in the manuscript including the name of the board/committee that gave the approval. The use of animals in experiments will have observed the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee.

Graphical Abstract
Authors should provide a graphical abstract (a beautifully designed feature figure) to represent the paper aiming to catch the attention and interest of readers. Graphical abstract will be published online in the table of content. The graphical abstract should be colored, and kept within an area of 12 cm (width) x 6 cm (height) or with similar format. Image should have a minimum resolution of 300 dpi and line art 1200dpi.
Note: Height of the image should be no more than the width. Please avoid putting too much information into the graphical abstract as it occupies only a small space. Authors can provide the graphical abstract in the format of PDF, Word, PowerPoint, jpg, or png, after a manuscript is accepted for publication. For preparing a Professional Graphical Abstract, please click **here**.



Presentation of the article

Main Format

First page of the manuscripts must be properly identified by the title and the name(s) of the author(s). It should be typed in Times New Roman (font sizes: 17pt in capitalization for the title, 10pt for the section headings in the body of the text and the main text, double spaced, in A4 format with 2cm margins. All pages and lines of the main text should be numbered consecutively throughout the manuscript. Abbreviations in the article title are not allowed.
Manuscripts should be arranged in the following order:
1. TITLE (brief, attractive and targeted);

2. Name(s) and Affiliation(s) of author(s) (including post code) and corresponding E-mail; ORCID: 0000-0000-0000-0000
3. ABSTRACT
4. Key words (separate by semicolons; or comma,)
5. Abbreviations (used in the manuscript)
6. INTRODUCTION
7. MATERIALS AND METHODS
8. RESULTS
9. DISCUSSION
10. CONCLUSION
11. DECLARATIONS
12. REFERENCES
13. Tables
14. Figure captions
15. Figures

Results and Discussion can be presented jointly.
Discussion and Conclusion can be presented jointly.

Article Sections Format

Title should be a brief phrase describing the contents of the paper. The first letter of each word in title should use upper case. The Title Page should include the author(s)'s full names and affiliations, the name of the corresponding author along with phone and e-mail information. Present address(es) of author(s) should appear as a footnote.

Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The abstract should be 150 to 350 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 8 key words that will provide indexing references should be listed.

Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and Methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail. The ethical approval for using animals in the researches should be indicated in this section with a separated title.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the author(s)'s experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the results but should be put into the discussion section.

Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Conclusion should be brief and tight about the importance of the work or suggest the potential applications and extensions. This section should not be similar to the Abstract content.

Declarations including Ethics, Consent to publish, Competing interests, Authors' contributions, and Availability of data and materials are necessary.

Acknowledgments of persons, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph forms or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or PowerPoint before pasting in the Microsoft Word manuscript file. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

Declarations section - Please include declarations heading

Please ensure that the sections:
-Ethics (and consent to participate)
-Consent to publish
-Competing interests
-Authors' contributions
-Availability of data and materials
are included at the end of your manuscript in a Declarations section.

Consent to Publish
Please include a 'Consent for publication' section in your manuscript. If your manuscript contains any individual person's data in any form (including individual details, images or videos), consent to publish must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent to publish. You can use your institutional consent form or our consent form if you prefer. You should not send the form to us on submission, but we may request to see a copy at any stage (including after publication). If your manuscript does not contain any individual persons data, please state "Not applicable" in this section.

Authors' Contributions
For manuscripts with more than one author, JWPR require an Authors' Contributions section to be placed after the Competing Interests section.
An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
We suggest the following format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.
For authors that equally participated in a study please write 'All/Both authors contributed equally to this work.' Contributors who do not meet the criteria for authorship should be listed in an acknowledgements section.

Competing Interests
Competing interests that might interfere with the objective presentation of the research findings contained in the manuscript should be declared in a paragraph heading "Competing interests" (after Acknowledgment section and before References). Examples of competing interests are ownership of stock in a company, commercial grants, board membership, etc. If there is no competing interest, please use the statement "The authors declare that they have no competing interests.".
Journal World's Poultry Research adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3. It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Change in authorship
We do not allow any change in authorship after provisional acceptance. We cannot allow any addition, deletion or change in sequence of author name. We have this policy to prevent the fraud.

Acknowledgements
We strongly encourage you to include an Acknowledgements section between the Authors' contributions section and Reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include their source(s) of funding. Please also acknowledge anyone who contributed materials essential for the study.
Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements. Please list the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section. Authors must describe the role of the funding body, if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Data Deposition
Nucleic acid sequences, protein sequences, and atomic coordinates should be deposited in an appropriate database in time for the accession number to be included in the published article. In computational studies where the sequence information is unacceptable for inclusion in databases because of lack of experimental validation, the sequences must be published as an additional file with the article.

References:

A JWPR reference style for **EndNote** may be found **here**.

1. All references to publications made in the text should be presented in a list with their full bibliographical description. DOI number or the link of article should be added to the end of the each reference.
2. In the text, a reference identified by means of an author`s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author`s surname should be mentioned, followed by 'et al`. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a` and 'b` after the date to distinguish the works.
3. References in the text should be arranged chronologically (e.g. Kelebeni, 1983; Usman and Smith, 1992 and Agindotan et al., 2003). The list of references should be arranged alphabetically on author's surnames, and chronologically per author. If an author's name in the list is also mentioned with co-authors, the following order should be used: Publications of the single author, arranged according to publication dates - publications of the same author with one co-author - publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1992a, l992b, etc.
4. Names of authors and title of journals, published in non-latin alphabets should be transliterated in English.
5. A sample of standard reference is "1th Author surname A, 2th Author surname B and 3th Author surname C (2013). Article title should be regular and 9 pt. Journal of World`s Poultry Research, Volume No. (Issue No.): 00-00." DOI:XXX."
6. Journal titles should be full in references. The titles should not be italic.
7. References with more than 10 authors should list the first 10 authors followed by 'et al.'
8. The color of references in the text of article is blue. Example: (Preziosi et al., 2002; Mills et al., 2015).

9. At least 35% of the references of any submitted manuscript (for all types of article) should include scientific results published in the last five years.

-Examples (at the text- blue highlighted)

Abayomi (2000), Agindotan et al. (2003), Vahdatpour and Babazadeh (2016), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993, 1995), (Kumasi et al., 2001).

--Examples (at References section)

a) For journal:

Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. Journal of Dairy Science, 83: 1635-1647.

Kareem SK (2001). Response of albino rats to dietary level of mango cake. Journal of Agricultural Research and Development. pp 31-38. DOI:XXX.

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. African Journal of Biotechnology, 7: 3535-3539. DOI:XXX.

Tahir Khan M, Bhutto ZA, Abbas Raza SH, Saeed M, Arain MA, Arif M, Fazlani SA, Ishfaq M, Siyal FA, Jalili M et al. (2016). Supplementation of different level of deep stacked broiler litter as a source of total mixed ration on digestibility in sheep and their effects on growth performance. Journal of World`ˢ Poultry Research, 6(2): 73-83. DOI: XXX

b) For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (Pampus argentens euphrasen) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. Asian Fisheries Society Manila, Philippine 13: 191-199.

c) For edited symposia, special issues, etc., published in a journal:

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science, 31: 17-27.

d) For books:

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88. Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

e) Books, containing sections written by different authors:

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), Farm Animal Feeding. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg).

In referring to a personal communication the two words are followed by the year, e.g. (Brown, J. M., personal communication, 1982). In this case initials are given in the text.

Nomenclature and Abbreviations:

Nomenclature should follow that given in NCBI web page and Chemical Abstracts. Standard abbreviations are preferable. If a new abbreviation is used, it should be defined at its first usage. Abbreviations should be presented in one paragraph, in the format: "term: definition". Please separate the items by ";".
E.g. ANN: artificial neural network; CFS: closed form solution; ...

Abbreviations of units should conform with those shown below:

Decilitre	dl	**Kilogram**	kg
Milligram	mg	**hours**	h
Micrometer	mm	**Minutes**	min
Molar	mol/L	**Mililitre**	ml
Percent	%		

Other abbreviations and symbols should follow the recommendations on units, symbols and abbreviations: in "A guide for Biological and Medical Editors and Authors (the Royal Society of Medicine London 1977).
Papers that have not been published should be cited as "unpublished". Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published". Papers that have been submitted for publication should be cited as "submitted for publication".

Formulae, numbers and symbols:

1. Typewritten formulae are preferred. Subscripts and superscripts are important. Check disparities between zero (0) and the letter 0, and between one (1) and the letter I.
2. Describe all symbols immediately after the equation in which they are first used.
3. For simple fractions, use the solidus (/), e.g. 10 /38.
4. Equations should be presented into parentheses on the right-hand side, in tandem.
5. Levels of statistical significance which can be used without further explanations are *P < 0.05, **P < 0.01, and ***P < 0.001
6. In the English articles, a decimal point should be used instead of a decimal comma.
7. In chemical formulae, valence of ions should be given, e.g. Ca2+ and CO32-, not as Ca++ or CO3.
8. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
9. Greek letters should be explained in the margins with their names as follows: Aα - alpha, Bβ - beta, Γγ - gamma, Δδ - delta, Eε - epsilon, Zζ - zeta, Hη - eta, Θθ - theta, Iι - iota, Kκ - kappa, Λλ - lambda, Mμ - mu, Nν - nu, Ξξ - xi, Oo - omicron, Ππ - pi, Pρ - rho, Σσ - sigma, Tτ - tau, Yυ - ipsilon, Φφ - phi, Xχ - chi, Ψψ - psi, Ωω - omega.

Review/Decisions/Processing

Firstly, all manuscripts will be checked by one of the plagiarism finding tools (iThenticate, PlagScan and or Docol©c). A double-blind reviewing model is used by JWPR for non-plagiarized papers. The manuscript is edited and reviewed by the English language editor and three reviewers selected by section editor of JWPR respectively. Also, a reviewer result form is filled by reviewer to guide authors. Possible decisions are: accept as is, minor revision, major revision, or reject. See sample of evaluation form. The estimated time from submission to first decision is 5.2 weeks and the estimated time from submission to final decision is 6.6 weeks. The estimated time for final publication of accepted manuscript is 6 weeks

To submit a revision please click here, fill out the form, and mark "Revised" attach the revision (MSword) and submit when completed.
After review and editing the article, a final formatted proof is sent to the corresponding author once again to apply all suggested corrections during the article process. The editor who received the final revisions from the corresponding authors shall not be hold responsible for any mistakes shown in the final publication. Manuscripts with significant results are typically reviewed and published at the highest priority.

Plagiarism
There is a zero-tolerance policy towards plagiarism (including self-plagiarism) in our journals. Manuscripts are screened for plagiarism by one of the plagiarism finding tools (iThenticate, PlagScan and or Docol©c), before or during publication, and if found they will be rejected at any stage of processing. See sample of Docol©c-Report.

Declaration
After manuscript accepted for publication, a declaration form will be sent to the corresponding author who that is responsible to coauthors' agreements to publication of submitted work in JWPR after any amendments arising from the peer review.

Date of issue
The journal will be issued on 25th of March, June, September and December, each year.

Publication charges
No peer-reviewing charges are required. However, the publication costs are covered through article processing charges (APCs). There is a modest APC of 150 Euro(€) editor fee for the processing of each primary accepted paper (1000-4000 words) to encourage high-quality submissions. APCs are only charged for articles that pass the pre-publication checks and are published. A surcharge will be placed on any article that is over 4000 words in length to cover the considerable additional processing costs. Payment can be made by credit card, bank transfer, money order or check. Instruction for payment is sent during publication process as soon as manuscript is accepted. Meanwhile, this journal encourages the academic institutions in low-income countries to publish high quality scientific results, free of charges.

WORD COUNT	PRICE*
1000-4000 words	€150
over 4000 words	€230

* The prices are valid until 30th December 2023.

The Waiver policy
The publication fee will be waived for invited authors, authors of hot papers, and corresponding authors who are editorial board members of the *Journal of World's Poultry Research*. The Journal will consider requests to waive the fee for cases of financial hardship (for high quality manuscripts and upon acceptance for publication). Requests for waiver of the submission fee must be submitted via individual cover letter by the corresponding author and cosigned by an appropriate institutional official to verify that no institutional or grant funds are available for the payment of the fee. Letters including the manuscript title and manuscript ID number should be sent to: editor [at] jwpr.science-line.com. It is expected that waiver requests will be processed and authors will be notified within two business day.

The OA policy
Journal of World's Poultry Research is an open access journal which means that all content is freely available without charge to the user or his/her institution. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the *BOAI definition of Open Access*.

Scienceline Language Editing Services
We suggest that authors whose first language is not English have their manuscripts checked by a native English speaker before submission. This is optional, but will help to ensure that any submissions that reach peer review can be judged exclusively on academic merit. We offer a Scienceline service, and suggest that authors contact as appropriate. Please note that use of language editing services is voluntary, and at the author's own expense. Use of these services does not guarantee that the manuscript will be accepted for publication, nor does it restrict the author to submitting to Scienceline journals. You can send the article/s to the following Emails: daryoushbabazadeh@gmail.com ; info@science-line.com
For more information about editing services please visit here.

Paper Submission Flow

Submission Preparation Checklist

Authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to the following guidelines.
The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
The submission file is in Microsoft Word, RTF, or PDF document file format.
Where available, URLs for the references have been provided.
The text is single-spaced; uses a 12-point font; and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

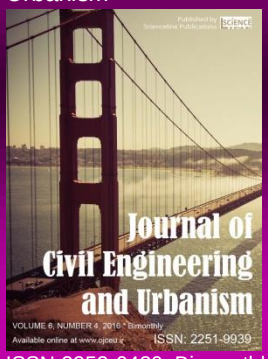
SCIENCELINE PUBLISHING CORPORATION

Scienceline Publication Ltd is a limited liability non-profit non-stock corporation incorporated in Turkey (Company No. 0757086921600001). Scienceline journals that concurrently belong to many societies, universities and research institutes, publishes internationally peer-reviewed open access articles and believe in sharing of new scientific knowledge and vital research in the fields of life and natural sciences, animal sciences, engineering, art, linguistic, management, social and economic sciences all over the world. Scienceline journals include:

Online Journal of Animal and Feed Research

ISSN 2228-7701; Bi-monthly
View Journal | Editorial Board
Email: editors@ojafr.ir
Submit Online >>

Journal of Civil Engineering and Urbanism



ISSN 2252-0430; Bi-monthly
View Journal | Editorial Board
Email: ojceu@ojceu.ir
Submit Online >>

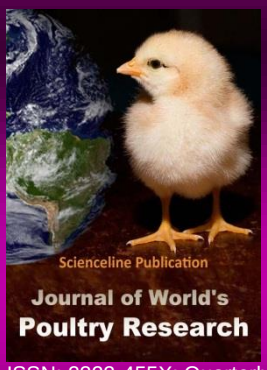
Journal of Life Sciences and Biomedicine

ISSN: 2251-9939; Bi-monthly
View Journal | Editorial Board
Email: editors@jlsb.science-line.com
Submit Online >>

Asian Journal of Medical and Pharmaceutical Researches

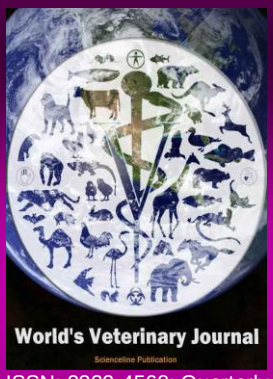
ISSN: 2322-4789; Quarterly
View Journal | Editorial Board
Email: editor@ajmpr.science-line.com
Submit Online >>

Journal of World's Poultry Research



ISSN: 2322-455X; Quarterly
View Journal | Editorial Board
Email: editor@jwpr.science-line.com
Submit Online >>

World's Veterinary Journal

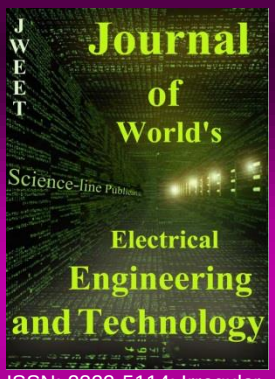


ISSN: 2322-4568; Quarterly
View Journal | Editorial Board
Email: editor@wvj.science-line.com
Submit Online >>

Journal of Educational and Management Studies

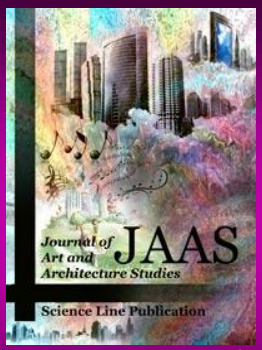
ISSN: 2322-4770; Quarterly
View Journal | Editorial Board
Email: info@jems.science-line.com
Submit Online >>

Journal of World's Electrical Engineering and Technology



ISSN: 2322-5114; Irregular
View Journal | Editorial Board
Email: editor@jweet.science-line.com
Submit Online >>

Journal of Art and Architecture Studies



ISSN: 2383-1553; Irregular
View Journal | Editorial Board
Email: jaas@science-line.com
Submit Online >>

Asian Journal of Social and Economic Sciences

ISSN: 2383-0948; Quarterly
View Journal | Editorial Board
Email: ajses@science-line.com
Submit Online >>

Journal of Applied Business and Finance Researches

ISSN: 2382-9907; Quarterly
View Journal | Editorial Board
Email: jabfr@science-line.com
Submit Online >>

Scientific Journal of Mechanical and Industrial Engineering

ISSN: 2383-0980; Quarterly
View Journal | Editorial Board
Email: sjmie@science-line.com
Submit Online >>

Scienceline is a non-profit organisation inspired by research funders and led by scholars. Our mission is to help researchers accelerate discovery and innovation by operating a platform for research communication that encourages and recognises the most responsible behaviours in science.

Scienceline Publications, Ltd is a limited liability non-profit non-stock corporation registered in the State of Erzurum, Turkey, with company number 0757086921600001, and branch number 18677/25379 at the address: Scienceline Publications, Ltd., Ömer Nasuhi Bilmen Road, Dönmez Apart., G1/6, Yakutiye, Erzurum 25100, Turkey