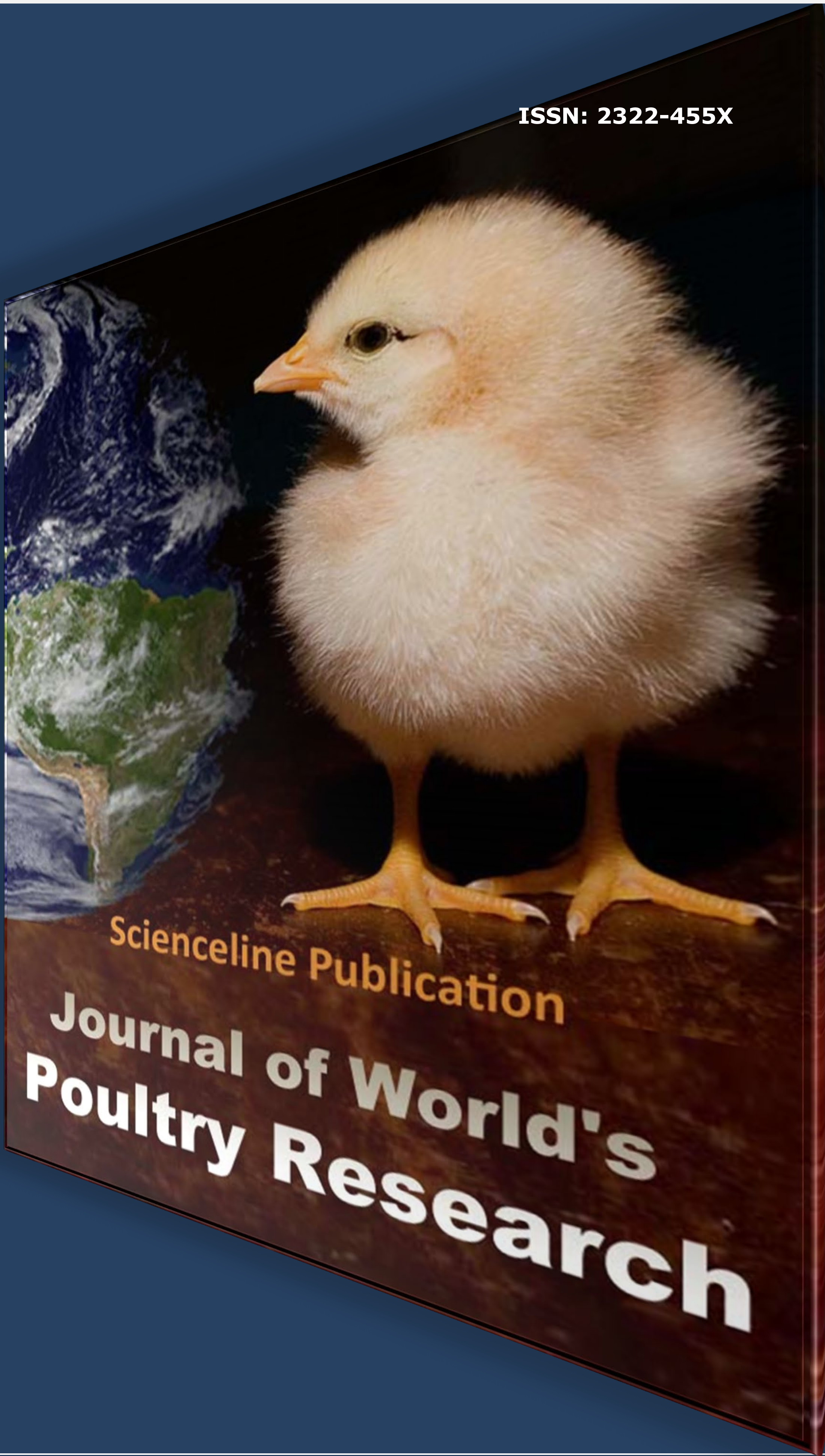
Journal of World's Poultry Research

BOOKLET



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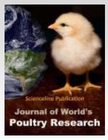
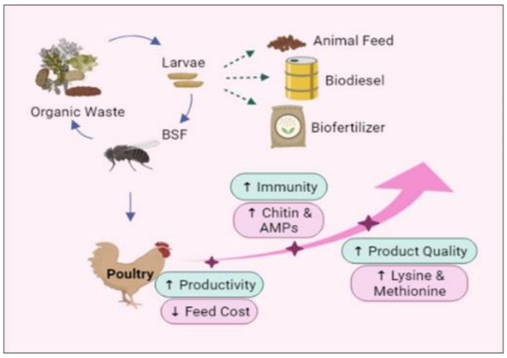


Advisory Board

Volume 12 (1); March 25, 2022

Review

Nutritive Value of Black Soldier Fly (*Hermetia illucens*) as Economical and Alternative Feedstuff for Poultry Diet

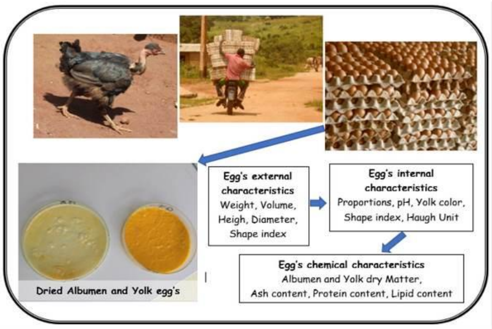


Ali Shah SR and Çetingül IS.

J. World Poult. Res. 12(1): 01-07, 2022; pii: S2322455X2200002-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.1

ABSTRACT: Recently, insects have gained importance as viable protein-rich feedstuff with better productivity and feed efficiency for livestock and pet animal feeds. The most potential species are the black soldier fly (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*), and common house fly (*Musca domestica*). Amongst these insects, the black soldier fly (*Hermetia illucens)* contains high protein and fat with the amino acid profile in *H. illucens* larvae equivalent to that of various protein-rich feedstuffs, such as fish meal and soybean meal. This review aimed to illustrate the reputation of black soldier fly larva meal as a substitute to conservative, expensive, and ecologically threatening crops by guaranteeing a productive, inexpensive, organic, and perpetual source of non-conventional protein feedstuff for poultry production of broilers and layers. It can be concluded that the black soldier fly sometimes has very similar and significant effects on the productivity, health, and product quality of birds, compared to soybean and fishmeal.
Keywords: Black soldier fly, Nutritive potential, Productivity
[Full text-PDF]

Socioeconomical Survey and Physicochemical Parameters of Chicken Eggs concerning the Breeding Systems in Cameroon



Djitie KF, Mohamadou B, Radu-Rusu RM, and Tchiegang C (2022). **Socioeconomical Survey and Physicochemical Parameters of Chicken Eggs concerning the Breeding Systems in Cameroon.** *J. World Poult. Res.*, 12 (1): 8-21. DOI: https://dx.doi.org/10.36380/jwpr.2022.2

Djitie KF, Mohamadou B, Radu-Rusu RM, and Tchiegang C.

J. World Poult. Res. 12(1): 8-21, 2022; pii: S2322455X2200002-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.2

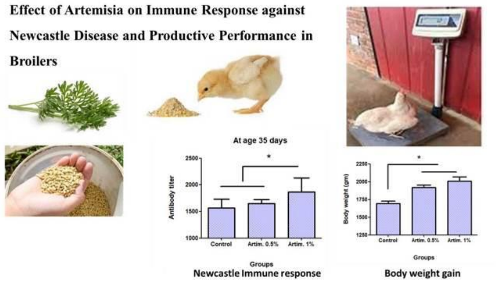
ABSTRACT: Physicochemical characteristics of eggs are still poorly controlled in poultry farming in the city of Ngaoundéré, Cameroon. The present study was thus conducted to characterize the rearing systems in modern poultry farming and to analyze the physicochemical characteristics of eggs from hens reared in deep litter, battery, and backyard systems from August to October 2020 in Ngaoundéré, Cameroon. To this end, 33 farms with approximately 61100 hens (Cobb500 broiler and layer) were surveyed. At the end of this survey, a sample of 180 eggs was collected, with 60 eggs per system for physicochemical analyses. Data included socio-economic and technical characteristics of modern farming systems as well as the physicochemical parameters of the eggs. The obtained results indicated that 90.9% of Cobb500 chicken owners were men. Of the total of the layer's buildings, 73% were equipped with nests. The materials used for feeders were made of wood (54.4%), plastic (21.2%), or cement (6.1%). Moreover, 81.1% of the poultry farmers buy chicken feed on the market while the others prepare their own feed from various ingredients. Preventive and curative prophylactic measures were applied by all livestock farmers, yet 54. 5% were still victims of different diseases. The selling price of a 45-day-old broiler chicken was between 4 and 6 USD, while the price of a 30-egg tray varied between 3 and 4 USD, which contributed to 60-80% of family income for 54.5% of poultry farmers. The high feed cost as well as lack of finance, ingredients, and security were the main issues of poultry farming. Concerning the physicochemical characteristics of eggs, a significant increase in egg weight was noted among backyard (43.50 ± 3.15 g), battery (58.19 ± 4.02 g), and deep litter (63.51 ± 3.91 g) systems. The Haugh's Unit of eggs in the backyard system (72.33 ± 4.42%) was significantly lower than deep litter (82.91 ± 6.76%) and battery systems (86.83 ± 11.42%). The proportions of eggshell and edible contents were similar in all production systems. Yolk lipid (17.63%) and yolk protein (7.11%) in dry matter contents of local breed eggs were higher than those of improved breed from both systems. The findings indicated that modern poultry farming in Ngaoundéré has been poorly developed and backyard eggs were richer in nutrients and consequently highly recommended to use.
Keywords: Breeding Systems, Egg Characteristics, Chicken, Socio-economic survey
[Full text-PDF]

Research Paper

The Effect of Artemisia on Immune Response and Productive Performance Against Newcastle Disease in Broiler Chickens

Kaab HT, Hameed SS, and Sahib AM.

J. World Poult. Res. 12(1): 22-30, 2022; pii: S2322455X2200003-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.3



Kaab HT, Hameed SS, and Sahib AM (2022). **The Effect of Artemisia on Immune Response and Productive Performance Against Newcastle Disease in Broiler Chickens.** *J. World Poult. Res.,* 12 (1): 22-30. DOI: https://dx.doi.org/10.36380/jwpr.2022.3

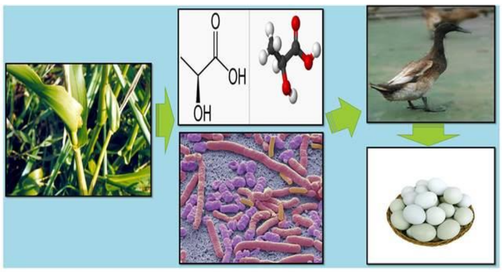
ABSTRACT: Prevention of Newcastle disease has received a lot of interest across the world. The high productivity performance of the commercial chickens' breeds has negative effects on the immune system and animal welfare. As a result, the current study aimed to investigate the benefits of adding Artemisia powder at levels of 0.5% and 1% to broilers' feed as a growth and health promoter. A total of 120 commercial broiler chickens were grown on the floor in a chicken house and separated into three groups, including one control and two treatment groups. Each group contained 40 chickens subdivided into two replicates. The three groups, namely G1 (chickens without Artemisia powder, as a control group), G2, and G3 in which chickens were fed with basal diet plus 0.5% and 1% Artemisia powder, respectively, were differentiated based on their diet throughout 35 days of the experiment. The measured parameters included the immune response to Newcastle disease vaccine, blood biochemical parameters, and growth performance as well as relative weight for the spleen and bursa of Fabricius. A diet containing 1% Artemisia powder significantly improved antibody titer against Newcastle disease, body weight, and weight gain. Thus, the addition of 1% of Artemisia powder to the broiler's diet can improve immune response against Newcastle disease and growth performance.
Keywords: Artemisia, Broiler chicken, Newcastle disease, Productive traits
[Full text-PDF]

Research Paper

The Effect of Probiotic Derived from Kumpai Minyak (Hymenachne Amplexicaulis) Silage on Performance and Egg Quality Characteristcs of Pegagan Ducks

Sandi S, Yosi F, Sahara E, Ali AIM, Gofar N, and Muhamad N.

J. World Poult. Res. 12(1): 31-37, 2022; pii: S2322455X2200004-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.4



Sandi S, Yosi F, Sahara E, Ali AIM, Gofar N, and Muhamad N (2022). **The Effect of Probiotic Derived from Kumpai Minyak (*Hymenachne Amplexicaulis*) Silage on Performance and Egg Quality Characteristcs of Pegagan Ducks**. *J. World Poult. Res.,* 12 (1): 31-37. DOI: https://dx.doi.org/10.36380/jwpr.2022.4

ABSTRACT: The study aimed to determine the effect of probiotics derived from an isolate of silage Kumpai Minyak grass on performance and the physical egg quality of Pegagan ducks. The study was conducted in 16 weeks, from May to September 2020. The sample size was 400 female Pegagan ducks aged five months. The treatments included basal diet (Control) and base diet plus 0.2% (P2), 0.4% (P4), 0.6% (P6), and 0.8% (P8) probiotic silage of Kumpai Minyak grass. The observed variables were performance (egg production, egg weight, feed consumption, and feed conversion ratio) and physical quality (albumen index, albumen weight, yolk weight, and Haugh unit). Observation data on probiotic treatment 0.8% (P8) established a significant effect on egg weight, compared to other treatments. Moreover, P8 probiotic treatment could significantly affect daily egg production and feed conversion ratio, compared to P2 and P4 probiotic treatments. Different results were found in the observations on feed consumption, where the overall treatment diet indicated significant results, compared to the control treatment. Specifically, several variables showed a significant effect, namely albumen index, albumen weight, egg yolk weight, and Haugh unit. Each observed variable value increased along with increasing probiotic treatment levels. However, egg index, egg yolk index, shell weight, and thickness were inversely related to the other variables investigated in this study. The P8 probiotic treatment could increase digestibility and absorption of feed nutrients due to inhibition of pathogenic bacteria and optimization of the digestive tract. The probiotics at the level of 0.8% produced from the Kumpai grass silage process can be used as a growth promoter for laying ducks to replace commercial antibiotic products.
Keywords: Albumen, Antibiotic, Growth promoter, Isolate, Probiotic, Silage
[Full text-PDF]

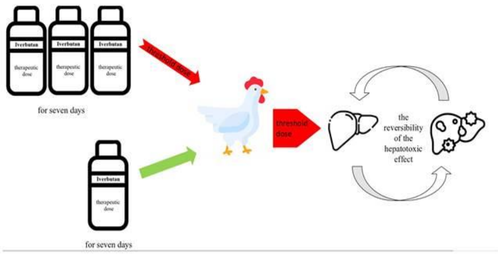
Research Paper

Subchronic Toxicity of Ivermectin and Butaphosphan in Layer Chickens

Indyuhova E, Arisov M, Maximov V, and Azarnova T.

J. World Poult. Res. 12(1): 38-45, 2022; pii: S2322455X2200005-12

DOI: https://dx.doi.org/10.36380/jwpr.2022.5



Indyuhova E, Arisov M, Maximov V, and Azarnova T (2022). **Subchronic Toxicity of Ivermectin and Butaphosphan in Layer Chickens.** *J. World Poult. Res.*, 12 (1): 38-45. DOI: https://dx.doi.org/10.36380/jwpr.2022.5

ABSTRACT: The development of new veterinary drugs to treat and prevent poultry parasitic infections, as well as the study of their safety is a hot topic for modern parasitology. The purpose of this research was to study the subchronic toxicity of the ivermectin and butaphosphan-based drugs at a therapeutic and threefold therapeutic dose during a seven-day oral administration to the Hisex White chickens. The provisional name of the drug is Iverbutan. The chickens from the first experimental group were given the drug at a threefold therapeutic dose of 3 mL of the drug per one liter of drinking water. The chickens from the second experimental group were given the drug at a therapeutic dose of 1 mL of Iverbutan per one liter of drinking water. The chickens from the control group received water without the drug. The chickens were weighed, and then the body temperature and blood samples from the axillary vein were measured on days 1, 8, and 17 of the experiment before the morning feeding. On day 8 of the study, the chickens from the first experimental group showed a 7.4% decrease in body weight and increase in body temperature by 0.8%, an increase in alanine aminotransferase activity by 2.1 times, aspartate aminotransferase activity by 1.6 times, and bile acids by 1.4 times. Moreover, there was a 4.6% decrease in glucose concentration, a 3.5% increase in lactate dehydrogenase activity, a 7.3% decrease in triglycerides, as well as a decrease in hemoglobin by 3.2% and erythrocytes by 10.6% in the first experimental group, compared to the control group. On day 17 of the experiment, the above blood parameters in the chickens from the first experimental group did not significantly differ from the control group, indicating the reversibility of the hepatotoxic effect. In this regard, a three-fold therapeutic dose can be considered a threshold. The chickens from the second experimental group showed no changes in their physiological status as compared to the control. Thus, the study results confirm the safety of the drug in the recommended dosage regimen.
Keywords: Blood, Butaphosphan, Chickens, Ivermectin, Metabolism, Subchronic Toxicity
[Full text-PDF]

Research Paper

The Effect of Kepok Banana (*Musa Paradisiaca* L.) on Immunoglobulin, Vitamins, and Cholesterol Content of Eggs in Laying Hens

Leke JR, Wantasen E, Siahaan R, Sompie F, Kaunang Ch, Widjastuti T, and Natsir MH.

J. World Poult. Res. 12(1): 46-51, 2022; pii: S2322455X2200006-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.6

Leke JR, Wantasen E, Siahaan R, Sompie F, Kaunang Ch, Widjastuti T, and Natsir MH (2022). **The Effect of Kepok Banana (*Musa Paradisiaca* L.) on Immunoglobulin, Vitamins, and Cholesterol Content of Eggs in Laying Hens.** *J. World Poult. Res.*, 12 (1): 46-51. DOI: https://dx.doi.org/10.36380/jwpr.2022.6

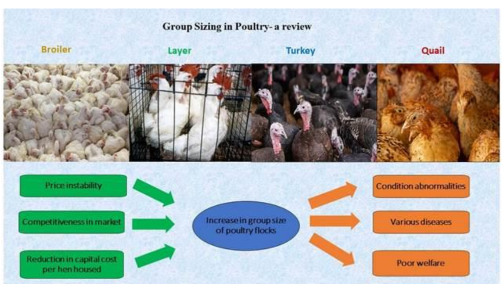
ABSTRACT: Eggs contain all the proteins, lipids, vitamins, minerals, and growth factors necessary for the development of the embryo. Egg white and yolk proteins are considered functional food substances since they have biological activities, such as metal-chelating, antimicrobial, anticancer, antioxidant, and immunomodulatory activities. The current study aimed to determine the effect of banana kepok on the production levels of immunoglobulin Y (IgY), vitamins, and cholesterol of eggs in laying hens. A total of 200 laying hens (medium brown 402) were used at 80 weeks of age with 5 treatments and 5 replications and each entailed 8 chickens. The treatment groups included the use of kepok banana flour (KBF) as R0 (no KBF), R1 (95 Basal feed + 5% KBF), R2 (90 basal feed + 10% KBF), R3 (85 basal feed + 15% KBF), and R4 (80 basal feed + 20% KBF). A total of 50 eggs were used in egg yolk sampling. The investigated variables were egg IgY, vitamins (A, B1, B6, D2, D3), and cholesterol content. The results of the study indicated that the administration of kepok bananas at different levels could provide a significant difference in IgY, vitamins (A, B1, D2, D3), and cholesterol of eggs. However, it did not significantly affect Vitamin B6. The study concluded that KBF can positively affect IgY and vitamins in eggs. Moreover, it could decrease the cholesterol in eggs.
Keywords: Egg cholesterol, Egg IgY, Egg vitamins, Kepok banana flour
[Full text-PDF]

Review

Effects of Group Sizing on Behavior, Welfare, and Productivity of Poultry

Kiani A.

J. World Poult. Res. 12(1): 52-68, 2022; pii: S2322455X2200007-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.7



Kiani A (2022). **Effects of Group Sizing on Behavior, Welfare, and Productivity of Poultry.** *J. World Poult. Res.*, 12 (1): 52-68. DOI: https://dx.doi.org/10.36380/jwpr.2022.7

ABSTRACT: The excessive intensive production of poultry meat and egg caused significant changes in poultry husbandry, behavior, and welfare. Therefore, animal welfare and behavior have become an important issue in poultry production and arises the necessity to reconsider all husbandry practices including group size and density. This review aims to

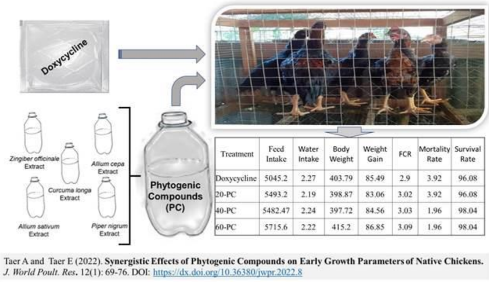
investigate the association of group size with growth performance, detrimental behaviors, and welfare by reviewing current norms and regulations, as well as scientific literature in industrial poultry farms, including chicken, turkey, and quail. It has been found that group size can affect production performance, especially growth rates, feed efficiency, and number of competitors, which can lead to damaging behavior and consequently injuries in poultry. Due to the intensification of the poultry production systems, many natural behaviors of domesticated poultry, including food search strategies, hierarchy formation, and aggressiveness, are changed or modified, compared to their ancestors. Therefore, challenging behaviors in commercialized conditions and large groups of poultry must be investigated. The current recommendations and regulations of the industry for commercial poultry on group size and space requirements differ from scientifically investigated trials. On the other hand, available scientific research about the impact of flock size on poultry welfare, behavior, and production, has been carried out in experimental settings with flock sizes that are varied considerably from those used in the commercial settings. In conclusion, results from studies on optimum group size have indicated some degree of confounding and interactions between enclosure size and density. Furthermore, the social and physical environment can have a significant impact on a variety of welfare-related aspects and behavioral indicators. It is important to note that this evaluation focused on studies conducted in experimental settings, making it difficult to extrapolate the findings to commercial settings where thousands of birds are reared at once.
Keywords: Chicken, Group size, Quail, Turkey, Welfare
[Full text-PDF]

Short Communication

Synergistic Effects of Phytogenic Compounds on Early Growth Parameters of Native Chickens

Taer A and Taer E.

J. World Poult. Res. 12(1): 69-76, 2022; pii: S2322455X2200008-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.8



ABSTRACT: The survival of native chickens during the first 6 weeks of growth is less than 50%. Hence, this study proposes the introduction of prophylactic antibiotics for poultry diseases prevention and treatment. However, the ban on antibiotics has prompted the search for plant-based biomedicines. Therefore, this trial aimed to determine the effect of phytogenic compounds (PC) of five herbs as water additives on the survival and growth responses of native chickens. A total of 204 Bisaya chickens (unsexed) were randomly assigned to 4 treatments replicated three times with 17 chickens in each replicate. Chickens were fed *ad libitum* and received water with 1.5 g antibiotics/1000ml water (control), 20 ml PC/1000ml water (T2), 40 ml PC/1000ml water (T3), and 60 ml PC/1000ml water (T4) for 35 days. No significant differences were observed on feed intake for chickens in antibiotics and PC treatments, however, the 60-PC group consumed slightly higher feed intake, compared to chickens under antibiotics and the other level of PC supplementation. Chickens in 60-PC ate 4-12% more feed than the others at the end of the trial period. Cumulative water used per kg feed did not differ among the experimental groups. The control and the PC supplemented chickens shared homogenous body weight and weight gains patterns, averaging 403.79 to 415.20g and 85.49 to 86.85g, respectively. Supplementation of 40-PC and 60-PC in drinking water for native chickens reduced the mortality rate and comparable feed conversion ratio with antibiotics. The 60-PC as a phytogenic water additive could enhance the growth performance, increase the survival rate, reduce mortality, and improve feed conversion ratio correlative to antibiotics.
Keywords: Antibiotic, Mortality, Native chicken, Phytogenic compounds, Synergistic effect, Survival rate

[Full text-PDF]

ABOUT JOURNAL

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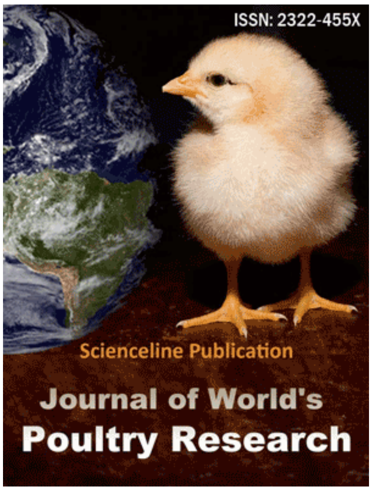

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DOI: https://dx.doi.org/10.36380/jwpr.2022.1

Nutritive Value of Black Soldier Fly *(Hermetia illucens)* as Economical and Alternative Feedstuff for Poultry Diet

Syed Rizwan Ali Shah* and Ibrahim Sadi Çetingül

Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Sciences, Afyon Kocatepe University, PO box 03200, Afyonkarahisar, Turkey

*Corresponding author's Email: rizwansyed604@gmail.com; ORCID: 0000-0002-2752-4854

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ABSTRACT

Recently, insects have gained importance as viable protein-rich feedstuff with better productivity and feed efficiency for livestock and pet animal feeds. The most potential species are the black soldier fly (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*), and common house fly (*Musca domestica*). Amongst these insects, the black soldier fly (*Hermetia illucens)* contains high protein and fat with the amino acid profile in *H. illucens* larvae equivalent to that of various protein-rich feedstuffs, such as fish meal and soybean meal. This review aimed to illustrate the reputation of black soldier fly larva meal as a substitute to conservative, expensive, and ecologically threatening crops by guaranteeing a productive, inexpensive, organic, and perpetual source of non-conventional protein feedstuff for poultry production of broilers and layers. It can be concluded that the black soldier fly sometimes has very similar and significant effects on the productivity, health, and product quality of birds, compared to soybean and fishmeal.

Keywords: Black soldier fly, Nutritive potential, Productivity

INTRODUCTION

The demand for food is probably to upsurge by 70% by the year 2050 because the population of people is being increased to 9.5 billion by that time worldwide (FAO, 2009a). The trend of people has been changed from vegetable-based protein to animal-based protein foodstuff, including milk, fish, meat, and eggs, and this inclination is predictable to heighten over time (Hunter et al., 2017). The inclination towards diets categorized by the increased utilization of animal products tends to increase the demand for animal feed ingredients and this trend probably continues in the near future. Moreover, conventional feedstuffs are being replaced by unconventional feed resources (Belghit et al., 2019). Eating habits are changing extensively due to commercial development and relocation from the countryside to metropolitan regions In developing states, the livestock sector can play a major role in sagging inadequate living standards and enhancing food security (Armanda et al., 2019). Furthermore, corn and soybean meal, the most important requirements for chick feed, may be predisposed by global warming, climate change, and nutritional expenses, thus prompting worldwide food security (Nkukwana, 2018). The high price of soybean has grown into a solemn concern for the economic stability of poultry farming, predominantly in unindustrialized states. Any sort of livestock farming feed budget includes at least 70% of the production expenses (Pica-Ciamarra et al., 2015) which specifies that cost-effective feed and their accessibility could impart an efficacious role in farming (Dumont et al., 2019). Soybean and fish meal increase the expenses of the feeds because human beings are also the consumer of soybean and fish (Kelemu et al., 2015). Fishmeal is being used in nurturing livestock and is also the most important source of protein for fish husbandry (Olsen, 2011). Furthermore, the intensification of soybean cultivation exclusively in the tropic areas could trigger land grabbing and deforestation as well as other adverse public and ecological concerns (Muscat et al., 2020). According to FAO (2017), about 70% of the fish was used for producing fish meal and fish oil in 2012. For the last century, the cost of fish meal globally enhanced up to 200%, approximately 1700 USD per 1000 Kg of the fish meal. Although there are potentials for fish meal replacement in animal nutrition, they are

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generally plant-based and hence of poorer protein digestibility and lower amount of essential amino acid content (FAO, 2009b). Therefore, imperative solutions should be designed to substitute conservative costly feed ingredients with cheaper, eco-friendly, high protein quality ones with ease of digestibility (Goldansaz et al., 2017). Insects have been produced as nutriment for humans since the prehistoric era and also they are presently being used in the human diet in many parts of the world. (Feng et al., 2018). Black soldier fly (BSF) larvae (*Hermetia illucens)*, the common house fly (*Musca domestica),* yellow mealworm (*Tenebrio molitor) a*re the insect species that have been used widely as auspicious unconventional feedstuffs of protein for animal feed (van Huis, 2017). These species can be grown on animal dung, coffee bean pulp, apple pulp, orange pulp, vegetable and fruit wastes, dried distillers grains with soluble, animal corpses, fish offal, rotten eggs, bakery product wastes, and restaurant wastes (Mutungi et al., 2019). However, rearing of the larva on livestock dung, poultry fecal droppings, and dead animal corpse are strictly banned by European Union, and consequently, such larva cannot be used for animal feeding with perspective to food safety. These insect species have the potential to turn the organic wastes into high protein biomass and the remaining substrates act as natural fertilizers for crops (Shelomi, 2020).

This review is intended to explicate the reputation of BSF larva meal as a replacement to conventional feedstuffs, including soybean meal, fish meal, soybean fats, and coconut fats in terms of productivity, gut health, blood chemistry, feasibility, and environmental impact, regarding its utilization in animal feedstuffs.

Scope in Turkey

Turkish scientists believe that black soldier fly larva is an excellent example of sustainable bioconversion because it is not just breaking down the wasted food the larva help with, they also serve as excellent livestock feed. The scientists assume that commercial production of black soldier fly larva will bring down animal feed costs and provide much-needed relief to farmers. Scientists claim that these worms have an exceptionally high nutritional value and are one of the richest sources of protein on earth. One larva can consume 100 mg of feed per larvae per day, which is the best ratio for organic wastes and it produces high-grade organic compost (Diener et al., 2011). For one m^2 of larvarium, BSF larvae need 3-5 kg per day for market wastes and one larva needs 100 mg chicken feeds per day (Diener et al., 2009). Scientists consider it a perfect replacement for chemical fertilizers. Turkey imports 90% of its chemical fertilizers and about 5.5 million tons of fertilizers are consumed each year in Turkey (MFAL, 2015). According to the World Bank, the global livestock feed industry has a worth of 370 billion USD. Livestock feed accounts for 70% of global food production costs and in the last decade, the price of livestock feed has increased by 200% (World Bank, 2013).

Nutritional value of black soldier fly larva

Black soldier larvae meal has an ironic amount of protein and fat, which strengthens the probability of using it in livestock, aquatic, and pet animal feed (Malla and Opeyemi, 2018). The nutritive potential of BSF varies concerning the substrates used for its growth and developmental stage during harvest.

Crude protein and amino acids

Crude protein increases just after hatching, it gradually decreases over time as it would be around 38-39% on day 14 of the larval stage. It then increases and reaches 45-46% and 56-57% at the pre-pupa and pupa harvested stages, respectively (Liu et al., 2017). De-fattening of BSF increases the protein content more than the full-fat BSF larva meal (Veldkamp and Bosch, 2015). The crude protein of fully defatted BSF is reported to be 66% that is higher than incompletely defatted BSF reported as 55% (Schiavone et al., 2017; Crosbie et al., 2020) and these values were nearly parallel to meat and fish meal. The lowermost documented crude protein content of BSF is 35-36% (De Marco et al., 2015) and is similar to and higher than plant-originated protein stuff, including sunflower, cottonseed, and linseed meal, wheat distillers grains, and beans (Sauvant et al., 2002). The limiting amino acids in poultry cereal-originated diets containing soybean and corn are lysine, methionine, and threonine and insects have extraordinary levels of these essential amino acids. As compared to corn gluten meal 60%, BSF larva has a higher content of leucine, lysine, and arginine (Liu et al., 2017). Histidine is reported to be four times greater in BSF than fish meals. As far as non-essential amino acids are concerned, the amounts of proline, alanine, and tyrosine are more in BSF, compared to soybean and fish meal (Taufek et al., 2021).

Crude fat

The fatty acids obtained from insects are more suitable and nutritious, compared to soybean and palm kernel cake without any unfavorable outcomes on productivity, digestibility, and intestinal health (Gasco et al., 2019a). On the first day after hatching, BSF larva has crude fat of about 5% that tends to rise gradually during development and reach 28-30% at the pupa stage (McGuckin et al., 2011). Lauric acid, a saturated fatty acid having antimicrobial action, is 35-50% of total fatty acids in BSF (Oonincx et al., 2015). In a study, BSF fed to freshwater Atlantic salmon reduced deposits of lipids in the liver (Belghit et al., 2019). Myristic acid is also higher in BSF, compared to soybean meal (Leni et al., 2017). According to Hoc et al. (2020), the fatty acid content of BSF is high in Saturated fatty acids (C12:0, C14:0, C16:0), and moderate in MUFA, and about 15% of it is PUFAs, and it was similar to the obtained results reported by Zarantoniello et al. (2020). Higher levels of linoleic acid (31.4%) and α-linolenic acid (1.6%) were found at the end of the first week of development, compared to day 14 of development when it was found to be 7% and 1.5%, respectively (Paul et al., 2017). Rendering to substrate for BSF larva growth, a reasonable content of oleic acid (10-15%) is reported in BSF (Michaelsen et al., 2009).

Vitamins and minerals

BSF contains a significant amount of calcium, iron, zinc, phosphorous, and Vitamin E which has noteworthy significance in animal nutrition (Liland et al., 2017). The content of Vitamin E in the pre-pupa stage (3.2 mg/100g) is reported to increase at day 14 tends to be approximately 6.7 mg/100g. Some of the minerals like calcium and phosphorous were double in the early stage, compared to the final stage while sodium, zinc, and iron content were more at the mature stage (Liu et al., 2017). BSF larva fed with horse fecal droppings showed almost 915 mg/100 g of phosphorous at the mature stage that is normally 320 mg/100 g on the chicken feed but the larva grown on fecal droppings are restricted by European Union and it should not be used in animal feed because of chances of disease transmission (Moula et al., 2018).

Implications in poultry

Affirmative outcomes have been observed about insects in relation to animal well-being and performance, gut health characteristics, and product preeminence (FAO, 2019; FAO, 2021). The use of insects as a substitute for soybean and fish meal has been increased for the last decade because they have immunity-enhancing bioactive constituents, including antimicrobial peptides, lauric acid, and chitin. (Gasco et al., 2018). The commercialization of insect farming has led to the establishment of a number of enterprises in India, Canada, the USA, North and South Korea, China, Japan, Italy, Australia, South Africa, Netherlands, and Europe since 2000 (Hubert, 2019). The development of the insect raising business is predominantly interrelated to the advancement in the *Hermetia illucens* (HI) Larva production (Ipema et al., 2020). According to Salomone et al. (2017), HI larva has progressed very rapidly in its production and processing, for example, the net weight of HI larva shifted from 8 thousand tons in 2015 to 14 thousand tons in 2016. This ensures the decline of the dumping expenses of biological wastes and conversion to worthy alternative protein feedstuffs in animal feed production, and hence, validate the continuous supply of healthy and organic animal-based protein stuffs in terms of meat and eggs for human consumption (Alexander et al., 2017). As insects are eaten unsurprisingly by a lot of animals, including fowls, ducks, quail, chukar partridge, pheasants, turkey, pigeons, doves, parrots, parakeet, cat, dogs, pigs fish, and shrimps, it is assumed that these adapting insects can be considered as a persistent and economical source of protein (De Castro et al., 2018). The BSF larva contains 40-50% crude protein, 35-40% lipids, and amino acid content that is very comparable to soybean meal and fish meal (Nyangena et al., 2020). Black soldier fly larvae serve as natural protein feedstuff for poultry, fish, shrimps, pets, and pigs (Nyakeri et al., 2017). The adult BSF can be alive for two weeks deprived of ingesting anything since fatty reserves developed during larval stages and can even live longer when water is being provided for their mating (Chia et al., 2020). Black soldier fly does not act as a vector for disease transmission and BSF larva has the potential of decreasing *E. coli* and *Salmonella enterica* in cow dung and poultry feces because of the secretion of special sort of chemicals that repel these pathogens (Liu et al., 2017). For the last decade, insects are intensely explored as prospective protein alternatives in broiler feed presenting optimistic effects on the growth and health of the birds (Józefiak et al., 2016). In a feeding trial where the hen was fed with BSF larva meal as a full replacement to soybean meal, the results showed higher content of butyric acid, a volatile fatty acid that prevents intestinal mucosa from wear and tears, due to microbial alteration by chitin in caeca, compared to the control group (Borrelli et al., 2017). Leiber et al. (2018) conducted research on layers and broilers by half replacement of soybean with BSF larva meal and they found no significant variance among all groups of layers on feed efficiency and egg quality and among all groups of broilers there was no substantial difference on weight gain and carcass parameters. According to Secci et al. (2020), hy-line brown layers fed with partially defatted BSF larva meal as a 25%

replacement to soybean depicted better egg quality parameters as compared to control. Similarly, soybean meal was replaced fully with defatted BSF larva meal in shaver white hens with no significant effect on egg production, feed intake, and haugh unit. Similarly, defatted BSF as a replacement to soybean meal increased body weight, eggshell thickness, and yolk color in Shaver white layers compared to the control (Mwaniki et al., 2020).

According to Moniello et al. (2019), more acetate and butyrate production along with better egg quality was reported in BSF-fed layers when compared to the soybean meal fed layers. The meat of rabbits nourished with the diets containing fats of BSF and yellow meal worn (*Tenebrio molitor)* was less vulnerable to oxidative damage and it was reported that the level of MDA (malondialdehyde), a marker of oxidative stress, in meat was 0.23 mg/kg of meat, compared to control as 0.40mg/kg (Gasco et al., 2019b). A study was conducted on broilers where they were fed with BSF larva as a replacement to the fishmeal and meat quality. The results showed a significant increase in meat protein level and muscle yield of the breast, compared to control, and no effect on the thigh and abdominal fat content were observed between all groups (Mlaga et al., 2020). In a study on broilers fed with BSF as compared to soybean meal, the carcass quality characteristics, including pH, color, flavor, juiciness, tenderness, cooking loss, and thawing loss were the same amongst all of the experimental groups (Pieterse et al., 2019). Full fat and extruded BSF larva meal in layers have presented a high percentage of laying eggs with no dissimilarity on egg quality parameters amongst all tentative groups (Jansen, 2018). Insects are not only being utilized in poultry but they are also successfully being used in quail, turkey, and duck farming. In a research trial soybean meal was replaced with BSF meal and its effects were seen on turkey carcass characters, growth, and health parameters. The results showed a better physiological response, immune status, compared to control, with no significant difference among all groups on productive parameters and carcass characteristics except gizzard weight that was more in BSF fed group, compared to control (Lalev et al., 2020). The negative effects of BSF larvae on productivity depend upon the inclusion level of BSF. For example, in a study defatted BSF larvae meal was used as 25% and 50% replacement of soybean meal in Hy-line brown layers. The results showed that the eggshell thickness and albumin foaming capacity were decreased significantly in the 50% replacement group as compared to the control and 25% replacement group (Secci et al., 2020). Similarly in Shaver White hens, 10% and 15% defatted BSF larvae meal as a substitute for soybean meal indicated that BSF larva improved eggshell thickness and yolk color but the FCR and egg mass reduced along with increased body weights (Mwaniki et al., 2020). In research on broiler (Ross 308) chicks at hatching fed with 50% and 100% replacement of soybean oil with BSF fat, it was concluded that the level of saturated and monounsaturated fatty acids in breast meat was highest in the 100% replacement group as compared to control and 50% replacement group. Polyunsaturated fatty acids decreased significantly in 100 replacement (22.74%) which was 27.84% for 50 % replacement to soybean oil and 29.49% for the control group. In short, 50 % replacement of soybean oil was more acceptable, compared to 100% replacement (Kim et al., 2020). Similarly in another study on 21 day-old broilers (Ross 308), 50 % and 100% replacement of soybean oil were done with BSF fat until 48 days of the age. The results showed that there was no significant difference between the performance and hematological parameters. Only little histopathological findings were obtained in the 100% replacement group. Consequently, it was safer to use 100% BSF only during the finisher stage rather than in chicks at hatching (Schiavone et al., 2018).

CONCLUSION

Insects have gained emergent utilization in livestock feed as an alternative protein feedstuff. Insects decrease environmental pollution in terms of little greenhouse gases emission and efficient utilization of bio-wastes. Instantly, the production of the insect as feed and decline of carbon-based wastes, such as cafeterias waste, domestic waste, and food street waste can support the giveaway of the ecological encumbrances and enhance natural standards to these types of wastes. To accomplish this objective, there is a need to choose appropriate insects for extensive production with better amino acid profile, growth performance, feed conversion, reduced mortality, and morbidity. From this standpoint, BSF is an exceptional candidate because it is very cheaper to rear larva on biowastes. Concerning feed safety and security issues, BSF larvae should not be grown on animal dungs and corpses. It has been concluded from the present-day literature that BSF larva is represented as a potential feed ingredient for poultry as a substituent for soybean or fish meals. Moreover, to prevent any negative impact of BSF larvae on animal production and product quality, it should be partially replaced with conventional feedstuffs during the growing stages. However, more exploration must be done to evaluate the highest inclusion levels of BSF larvae meal deprived of undesirable consequences on animal welfare, immunity, gut health, productivity, meat and egg quality, and consumer preferences.

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Socioeconomic Survey and Physicochemical Parameters of Chicken Eggs concerning the Breeding Systems in Cameroon

Djitie Kouatcho François[1], Mohamadou Bachirou[1], Radu-Rusu Razvan Mihail[2*], and Tchiegang Clergé[3]

[1]*Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, Po Box 454 Ngaoundere Cameroon*
[2] *Iași University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490 Iași, Romania*
[3] *University Institute of Technology, University of Ngaoundere, Po Box 455 Ngaoundere Cameroon*

*Corresponding author's Email: radurazvan@uaiasi.ro; ORCID: 0000-0001-7735-9028

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ABSTRACT

Physicochemical characteristics of eggs are still poorly controlled in poultry farming in the city of Ngaoundéré, Cameroon. The present study was thus conducted to characterize the rearing systems in modern poultry farming and to analyze the physicochemical characteristics of eggs from hens reared in deep litter, battery, and backyard systems from August to October 2020 in Ngaoundéré, Cameroon. To this end, 33 farms with approximately 61100 hens (Cobb500 broiler and layer) were surveyed. At the end of this survey, a sample of 180 eggs was collected, with 60 eggs per system for physicochemical analyses. Data included socio-economic and technical characteristics of modern farming systems as well as the physicochemical parameters of the eggs. The obtained results indicated that 90.9% of Cobb500 chicken owners were men. Of the total of the layer's buildings, 73% were equipped with nests. The materials used for feeders were made of wood (54.4%), plastic (21.2%), or cement (6.1%). Moreover, 81.1% of the poultry farmers buy chicken feed on the market while the others prepare their own feed from various ingredients. Preventive and curative prophylactic measures were applied by all livestock farmers, yet 54. 5% were still victims of different diseases. The selling price of a 45-day-old broiler chicken was between 4 and 6 USD, while the price of a 30-egg tray varied between 3 and 4 USD, which contributed to 60-80% of family income for 54.5% of poultry farmers. The high feed cost as well as lack of finance, ingredients, and security were the main issues of poultry farming. Concerning the physicochemical characteristics of eggs, a significant increase in egg weight was noted among backyard (43.50 ± 3.15 g), battery (58.19 ± 4.02 g), and deep litter (63.51 ± 3.91 g) systems. The Haugh's Unit of eggs in the backyard system (72.33 ± 4.42%) was significantly lower than deep litter (82.91 ± 6.76%) and battery systems (86.83 ± 11.42%). The proportions of eggshell and edible contents were similar in all production systems. Yolk lipid (17.63%) and yolk protein (7.11%) in dry matter contents of local breed eggs were higher than those of improved breed from both systems. The findings indicated that modern poultry farming in Ngaoundéré has been poorly developed and backyard eggs were richer in nutrients and consequently highly recommended to use.

Keywords: Breeding Systems, Egg Characteristics, Chicken, Socio-economic survey

INTRODUCTION

Poultry plays an important role in meeting the animal protein needs of people. Poultry meat and eggs are relatively cheap products of good dietary quality, rich in protein and low in fat (Bastianelli and Prin,1999; Sanfo et al., 2012). Because of its many potentialities (a short time in reaching the aim of the production, easier production, low investment requirements, access to all segments of the developing country populations), poultry farming currently possesses a significant position in poverty alleviation strategies of most developing countries (Sonaiya and Swan, 2004). More than 80% of the population, mostly rural in sub-Saharan Africa, is involved in village poultry farming (Fanou, 2006).

The poultry population in Cameroon is about 45 million heads of which 70% are traditional chickens, compared to 24% from improved breeds and 6% from non-conventional species of poultry (Ngandeu and Ngatchou, 2006; Fotsa et al., 2007). Despite this potential, the country is unable to meet the ever-increasing market annual demand for eggs and poultry meat due to population growth.

Indeed, the poultry meat shortage was estimated at 98000 tons in 2015, meaning that an additional 24 million chickens were needed, with an annual increase of 1.2

million heads per year, to avoid imports and encourage local production. Generally, in the northern part of the country, particularly in Ngaoundéré, modern poultry farming contributes significantly to the consumption of poultry meat. However, its production yield remains low due to the specificities and techniques of production that are not well known. The heavy losses that poultry farmers face originate from the lack of knowledge about environmental factors and breeding techniques. Given its importance, better profitability in this sector must be targeted by identifying the weaknesses in the poultry farming system which can provide solutions for the real development of modern poultry farming in the northern part of Cameroon in general.

Few studies on the characterization of poultry farming systems in the city of Ngaoundéré have mainly concerned village poultry farming (Djitie et al., 2015). Chicks (Cobb500 breed) commonly come from regions located over 500 km away leading to a high rate of mortality during transport. Most of the farmers have a very low level of knowledge about poultry farming, which results in a low level of production. In developed countries, such as the EU countries, egg production follows several international standards. However, in third-world countries in general and Cameroon in particular, poultry farming, packing, and transportation logistics are not structured.

Breeding systems are neither standardized nor controlled which affects the quality and quantity of the resulting products (meat and eggs). Most of the eggs consumed in the town of Ngaoundéré come from regions other than Adamaoua, which are more than 500 km away, causing them to deteriorate during transport.

With this in mind, this study aimed to improve the poultry sector in the northern part of Cameroon in terms of farming management and the quality of the products. To achieve this, a survey on the socio-economic and technical characterization of the modern poultry farming systems has been proceeded followed by a study on the physicochemical characteristics of the eggs from various systems found.

MATERIALS AND METHODS

Ethical approval

Experimental protocols used in the current study were approved by the ethic committee of the Faculty of Sciences, University of Ngaoundere, Cameroon, and strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of November 24, 1986.

Presentation of the study area

The study was carried out from August to October 2020 in Ngaoundéré which is the capital of the Adamawa Region in Cameroon. Ngaoundéré is a cosmopolitan town located on the Adamawa plateau (7-8°N and 13-14°E) in the Sudano-Guinean ecological zone of Cameroon.

Sampling and data collection

To understand local practices in modern poultry farming in the city of Ngaoundéré, a socio-economic survey on this sector was conducted between August and September 2020 in the three districts of Ngaoundéré, Cameroon. Using a survey questionnaire prepared for this purpose, information was collected from the farmers on the socio-economic characteristics of the farmers, the characteristics and description of the farm, the feeding method and equipment used, disease cases, product sales, constraints, and prospects. The survey covered a total of 33 farms in the three districts of Ngaoundéré (11 in the Ngaoundéré first zone, 7 in the second zone, and 15 in the third zone). In the next step, to investigate the physicochemical characteristics of the eggs, a total of 180 chicken (*Gallus gallus*) eggs were collected and divided into 3 batches (60 eggs from local hens, 60 eggs from the deep litter system, and 60 eggs from the battery system). Eggs from the backyard system were collected at the Dang village market from rural families, while eggs from the deep litter and battery systems were purchased directly from the farms of each farming system. The collected samples were transported to the laboratory of quality control of feed products of the University Institute of Technology, University of Ngaoundéré, in cardboard trays for physicochemical analysis. Once at the laboratory, the eggs were labeled, and then the physical characteristics of 30 eggs from each system were evaluated, while the remaining 30 eggs per batch were used for the evaluation of some physicochemical parameters.

External characteristics of eggs

Weighing and measuring the egg

Eggs were individually weighed on an electronic scale branded Shimadzu UX4200H, Poland, with a capacity of 320 g and 0.01 g accuracy.

Large diameter and height of the eggs were measured using a digital Vernier caliper with a 150 mm range and 0.01 mm accuracy.

Egg Shape Index was obtained by dividing the large diameter by height and multiplying by 100.

$$\text{Shape index} = \left[\frac{\text{Diameter (mm)}}{\text{Height (mm)}}\right] \times 100$$

The specific gravity assessment of eggs was based on Archimedes' principle. Eggs were weighed normally. Displaced water weight (at 22°C) was determined by immersing the eggs in the water of a beaker on the same scale (Zita et al., 2013). The specific gravity of the eggs was then determined using the equation.

$$\text{Specific gravity} = \left[\frac{\text{Normal weight (g)}}{\text{Weight of water displaced (g)}}\right]$$

Egg volume was obtained by placing the egg in a graduated cylinder containing a known volume of water. Egg volume (Ve) was determined by the difference between the volume after the introduction of the egg (Vf) and the initial volume of water (Vi) (Markos et al., 2017).

Ve = Vf-Vi

Internal characteristics of the egg

After 60 eggs of each poultry farming system were broken, the data related to diameter and height of the dense albumen and yolk, yolk color, weight of the albumen, shell and yolk, as well as shell thickness were recorded.

Measuring and weighing albumen, yolk, and shell

Eggs were broken individually and their contents were carefully placed on a 40 cm × 40 cm glass slab on a flat and stable surface. The diameter and then the height of the dense albumen and yolk were measured using the digital Vernier caliper (My Project, Germany). For height measurement, the caliper was attached to tripods to be perpendicular to the glass plate (Radu-Rusu et al., 2014). Yolk color was assessed according to the method of Vuilleumier (1969) which uses a range of yolk colors (Yolk Color Fan® scale, Roche).

After separating albumen from yolk using a 100 ml syringe, yolk was weighed using an electronic balance Model PI-214 (Denver Instruments 214, USA) with a capacity of 210 g and an accuracy of 0.0001 g.

The shells were washed with water to remove the remaining albumen and then dried before being weighed with the same digital scale as previously used (Radu-Rusu et al., 2014). Albumen weight was obtained by calculating the difference between whole egg weight and the weight of the yolk and shell.

Pb = Po-(Pj+Pc)

Where, Pb is albumen egg weight, Pj refers to yolk weight, Po denotes whole egg weight, and Pc signifies shell weight

Using the previously described digital caliper attached to a tripod, the thickness of the shell was taken from shell fragments of the large side, the large diameter, and the small side of the egg (Radu-Rusu et al., 2014).

The different weights and measurements on the internal and external egg characteristics were used to calculate the following parameters:

- Proportion of egg components (%) = $\left[\frac{\text{Shell weight/yolk/white weight (g)}}{\text{Egg weight (g)}}\right] \times 100$

- Percentage of edible matter (%) = proportion of yolk + proportion of albumen

- Egg constituent index (%) = $\left[\frac{\text{Yolk/white height (mm)}}{\text{Yolk/white diameter (mm)}}\right] \times 100$

- Haugh unit (HU) = 100 log ($H+7.57 - 1.7P^{0.37}$)

Where, H means albumen height (mm), P signifies egg weight (g), 7.57 is albumen height correction factor, and 1.7 represents the egg weight correction factor (Haugh, 1937).

Albumen and yolk were homogenized separately in large glass Petri dishes. The pH values were obtained by inserting the tip of a pre-calibrated electronic pH meter into each of them. The pH values were noted once the number had stabilized on the display of the pH meter (Bluelab, Germany). The procedure was repeated 5 times for each sample and the average was measured (Radu-Rusu et al., 2014).

Analysis of chemical characteristics

Albumen and yolk were homogenized separately in large glass Petri dishes. The pH values were obtained by inserting the end of a previously calibrated electronic pH meter into each of them. The pH values were recorded once the number stabilized on the pH meter screen.

AOAC (1990) Method No. 925.30 was used to evaluate the dry matter in pre-dried eggs obtained by dehydration in a Memmert SEU 700 forced-air oven at 70ºC.

Crude ash content was evaluated by incineration at 550ºC in a Super Therm C311 incinerator after pre-combustion with a Bunsen tray until the cessation of smoking in accordance with AOAC Specification 900.02 (AOAC, 1990).

Crude protein (CP) was obtained from the evaluation of total nitrogen content by the Kjeldahl method, applied on a Velp Scientifica DK 6 digestion system and UDK 7 distillation system (Italy), according to AOAC Method

No. 925.31. Finally, the total nitrogen content was multiplied by 6.25, which generated the crude protein content. Total lipid content in the form of crude fat was determined by AOAC method No. 925.32 (AOAC, 1990) using a Velp Scientifica Soxhlet SER 148 extractor, Italy.

Statistical analysis of the data

The obtained data were expressed as mean ± standard deviation on the mean. One-way analysis of variance (ANOVA) was used following the general linear model to compare the means of the different parameters. When differences between means were significant, they were statistically separated by Duncan's test at the 5% significance level. The IBM Statistic SPSS (version 25) and Excel 2016 were also used for data analysis and illustration.

RESULTS

Socio-economic and technical survey

Socio-economic status of poultry farmers

Most poultry farmers in the city of Ngaoundéré (90.9%) were men. Of the investigated population, 69.7% of the farmers were Christians, followed by Muslims (30.3%). Regarding the age, 78.8% of the farmers were between 25 and 45 years old, 9.1% were over 55 years old, and only 3% were less than 25 years old. In terms of education, 45.5% of the people surveyed had a university degree, 42.4% could complete secondary education, and only 9.1% had basic education. Poultry farming was the main activity in 84.8% of cases, while traders and civil servants constituted only 9.1% and 6.1%, respectively. Agriculture was the secondary activity for 42.4% of all interviewees. In addition, 75.8% were married and 69.7% had between one and five children. This activity had been carried out for more than 5 years in 39.4% of cases. Poultry production was 100% for profit, 90.9% of farmers had their own sources of financing, and only 9.1% were supported by financing.

Breeding management

Housing

The animals were all sheltered and the buildings were made of long-lasting materials. In 97% of cases, they lived in adapted buildings, and all of them lived in buildings made of brick with sheet metal roofs. These buildings, with sheet metal roofs, varied in size depending on the number of animals they contain. Only 73% of the farmers had a nesting box.

Feeding

All farms were in charge of animal feeding of whom 81.1% supply the required feed from the animal feed store and others (18.2%) made the feed on their own. The equipment used varied from one farmer to another, and the majority (90.9%) had plastic troughs while others (9.1%) used only cement or automatic troughs (Figure 1). Drinking water came from wells (69.7%) and taps (30.3%). Water was provided when the water container was empty in 75.8% of cases. Other farmers provided water twice (6.1%) or three times a day (18.2%). Furthermore, 54.5% of respondents implemented a wooden feeder, 21.2% used a plastic feeder, and only 6.1% utilized an aluminum feeder.

Health management

Mortalities occurred in 30.3% of the farmers each year that affected 1-10% of the chickens. Among these losses, 57.6% were attributed to management problems and 30.3% to disease. Moreover, 84.8% had experienced a disaster and 54.5% attributed it to disease. Based on the description of symptoms (cough, sneeze, weight loss, loss of appetite, diarrhea, and runny nose), the suspected diseases for these losses were New Castle disease, infectious bronchitis, and salmonellosis. All farmers were visited by a veterinary officer. The frequency of the visit was three times a month for 54.5% of the producers, others only asked for a visit in case of necessity (24.2%), once a month for some (15.2%), and finally twice a month for 6.1% of other producers.

Product marketing

For 69.7% of farmers, products are exclusively sold on the farm, compared to 21.1% who sold at the market. The cost of bags of droppings was 4 USD/bag for 75.8% of farmers and 5 USD for 21.2%. According to the respondents, the contribution of poultry farming to their household income was respectively 20 to 40% and 40 to 60% for 18.2%, 60 to 80% for 54.5%, and only 9.1% of the respondents reported that this activity contributed between 80 and 100% to their household income. All farmers agreed that there was no market for poultry alone that operated properly, and all would like to see a market established for the sale of poultry exclusively. Depending on the size, weight, and shape of the animals, the price of a chicken was 5 USD for 84.8% of the farmers (broilers and layers), while the price of chicks varied between 1 and 1.8 USD (broilers and layers) for 51.5% and 23.7% of the respondents respectively.

A: Aluminium feeder **B:** Plastic feeder **C:** Automatic drinker

D: Wooden feeder **E:** Plastic drinker

Figure 1. Some materials used in the modern poultry feeding in the city of Ngaoundéré, Cameroon, from August to October 2020

Problems and prospects

Modern poultry farming is an activity that requires a high level of financial and managerial involvement as well as strict monitoring of animals to obtain optimal results. It should be noted that, however, many problems were encountered and contributed to significant losses. The general distribution of the problems encountered is illustrated in Figure 2. In view of this, for 27.3% of the farmers surveyed, the high cost of feed was the main problem encountered, while 18.2% were victims of problems with the supply of feed and theft. Lack of funding and feed ingredients were the real constraints for 12.1% and 9.4% of the participants, respectively. Only 6.1% and 3% of farmers complained about lack of supervision and marketing problems respectively. Faced with these constraints, 66.7% of the farmers were looking forward to improving the conditions and breeding techniques. Despite the difficulties faced by others, 30.3% wished to increase the size of their livestock, and 3% were looking for outlets.

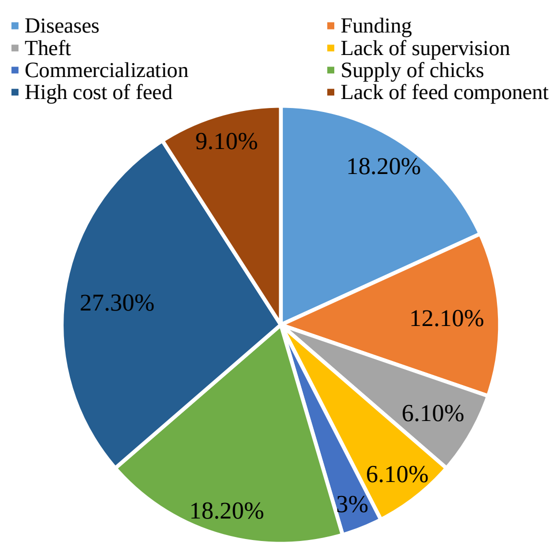

Figure 2. Constraints of modern poultry farming in the city of Ngaoundéré, Cameroon, from August to October 2020

Farmers' demands to the public authorities

The majority of farmers were not a member of a Common Initiative Group or a cooperative (81.8%). In order to improve their activity and obtain satisfactory results, 97% of the breeders requested financial support from the competent authorities and 3% wanted to be supervised.

External characteristics of eggs according to the farming system

Figure 3 illustrates the external characteristics of eggs according to the production system. There was a significant increase ($p < 0.05$) in egg weight among the backyard (43.50 ± 3.15 g), battery (58.19 ± 4.02 g), and deep litter (63.51 ± 3.91 g) systems. The same trend was observed for egg height, with values of 52.51 ± 1.73 mm, 55.35 ± 2.27 mm, and 59.89 ± 2.12 mm for the backyard, battery, and deep litter systems, respectively ($p < 0.05$). Regarding egg diameter, the lowest significant value was recorded for eggs from hens kept in the backyard system (39.59 ± 2.1 mm), compared to eggs from the battery (43.77 ± 0.9 mm) and deep litter (43.86 ± 1.19 mm) systems, which otherwise were not significantly different ($p \geq 0.05$). The same observations were made ($p \geq 0.05$) with density where the values obtained were 1.05 ± 0.06; 1.09 ± 0.06 and 1.10 ± 0.08 respectively for the battery, backyard, and deep litter systems. Both egg volume and weight were significantly affected by the rearing systems. The lowest ($p < 0.05$) value was recorded for eggs from the backyard system (41.75 ± 3.89 ml), followed by battery (53.80 ± 4.01 ml) and finally deep litter (58.05 ± 5.52 ml). The shape index of eggs was significantly ($p < 0.05$) higher with the battery system (79.16 ± 2.46%) compared to the backyard (75.45 ± 4.45%) and deep litter (75.86 ± 3.12%) systems where any significant difference ($p < 0.05$) was noticed.

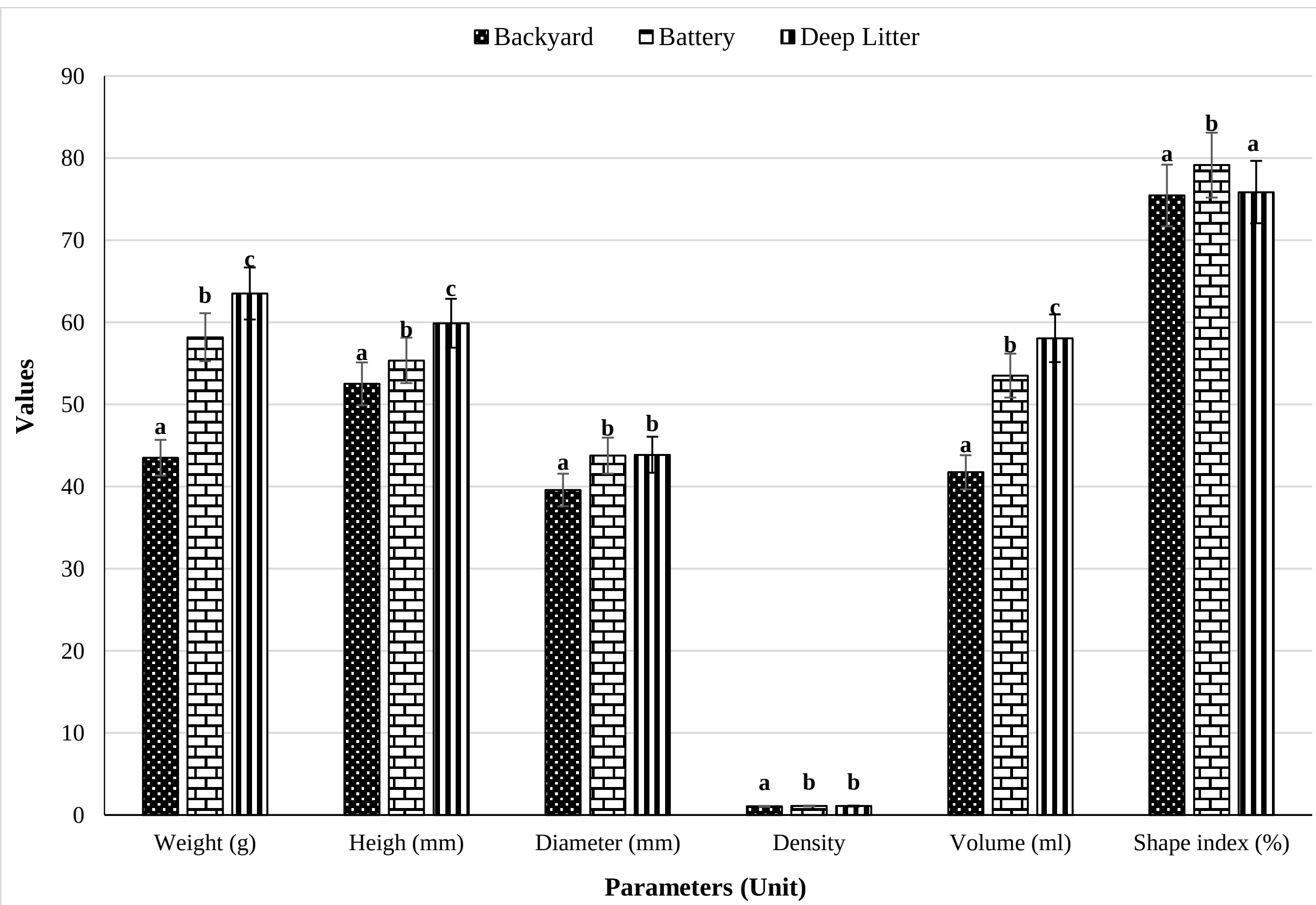

Figure 3. External characteristics of layer hen eggs according to production systems in the city of Ngaoundéré, Cameroon, from August to October 2020. [a,b,c]: Values with the same letter are not significantly different per parameter ($p > 0.05$)

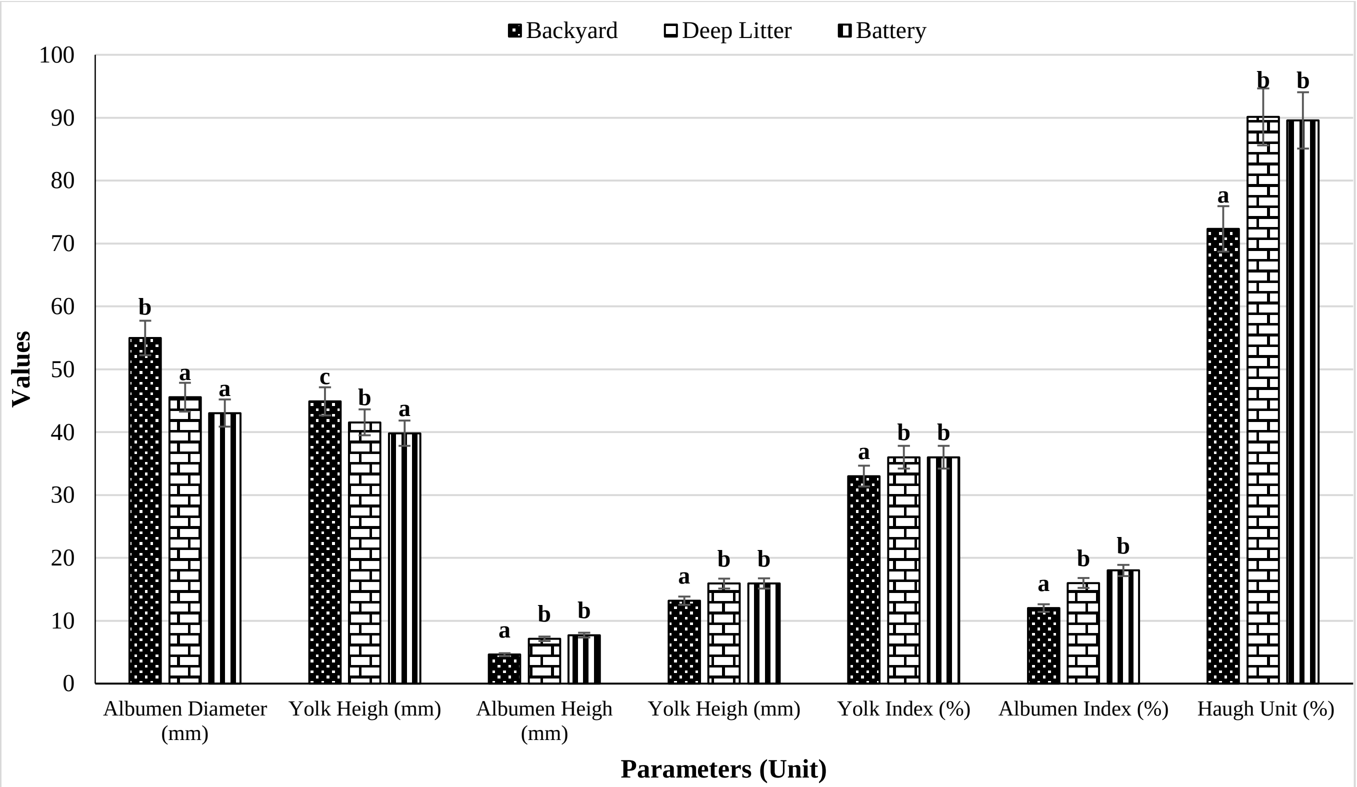

Figure 4. Internal characteristics of layer hen eggs according to production systems in the city of Ngaoundéré, Cameroon from August to October 2020. [a,b,c]: Values with the same letter are not significantly different per parameter ($p > 0.05$)

Egg internal characteristics

Measurements, yolk index, egg albumen index, and Haugh unit according to the production system

The evolution of the internal characteristics of the eggs according to the production system is shown in Figure 4. Albumen and yolk diameters were significantly ($p < 0.05$) higher in eggs from backyard hens (54.99 ± 18.31 mm and 44.91 ± 1.95 mm, respectively), compared to the other systems. The values obtained for albumen diameter were similar for the deep litter and battery systems, while the yolk diameter of eggs from the deep litter system (41.56 ± 1.74 mm) was significantly ($p < 0.05$) higher than that of the battery system (39.83 ± 1.72 mm). Regarding the yolk and albumen height parameters, the significantly lower values were recorded for eggs from hens reared in the backyard system (13.18 ± 1.18 mm and 4.61 ± 0.51 mm), compared to the deep litter and battery systems, which were otherwise comparable ($p < 0.05$). Similar observations were made for yolk and albumen indexes as well as the Haugh Unit. Eggs from the backyard system exhibited values of 12 ± 0.01%, 33 ± 0.03%, and 72.33 ± 4.42%, respectively, for albumen index, yolk index, and Haugh Unit which were significantly lower ($p < 0.05$), compared to the battery and deep litter systems.

Weight and proportions of yolk and albumen according to the production system

Table 1 shows the variation in weight of egg constituents, yolk, and albumen proportions according to the production system. It can be seen that the highest yolk weight (15.43 ± 1.3 g) was recorded in the deep litter system eggs and was similar to that of the backyard system. However, the albumen weight (41.83 ± 3.08 g) of the deep litter system was significantly ($p < 0.05$) higher than the other two systems. Regarding the shell weight, there was no significant difference between the eggs from the deep litter system and the battery system, but it was higher than that from the backyard system. As indicated, the proportions of albumen and yolk of eggs from the deep litter system were similar to those of the backyard system.

Shell characteristics according to production systems

Shell characteristics according to production systems are presented in Table 2. The production system showed no significant effect in shell proportions. The highest value of shell thickness at the large side of eggs was recorded for eggs from the battery system (0.60 ± 0.16 mm), which was significantly higher than those recorded for eggs from the deep litter and backyard systems ($p < 0.05$). Same patterns were found for the shell thickness at the medium and small ends. Moreover, the highest average shell thickness was recorded for eggs from the battery system (0.56 ± 0.08mm), compared to eggs from the deep litter and battery systems.

Egg chemical characteristics according to production systems.

Yolk and albumen pH

Table 3 presents the variations in yolk and egg albumen pH according to production systems. The highest yolk pH value was recorded for eggs from the backyard system (6.96 ± 0.1), which was similar to the battery system (6.81 ± 0.1) pH, but higher than that of the eggs from the deep litter system. With regard to albumen pH, there was no significant difference among the three groups regardless of the production system considered ($p \geq 0.05$). In all investigated systems, the albumen pH was higher than that of the yolk.

Dry matter, protein, and lipid content

In Figure 5, the chemical characteristics of the eggs are presented in relation to the different production systems. It appeared that the dry matter of the albumen was similar ($p \geq 0.05$) regardless of the production system. The highest dry matter content was recorded in battery eggs (14.64 ± 1.01%), followed by eggs from the deep litter (13.92 ± 1.02%), and backyard systems (13.56 ± 0.94%). The yolk dry matter represented almost half of the fresh sample. No significant differences were found in the values recorded in the three systems. However, the highest yolk dry matter content was obtained in eggs from the deep litter system (44.36 ± 3.26%) followed by the battery (42.02 ± 1.33%) and backyard (35.34 ± 12.6%) systems (Figure 5A).

The determination of soluble protein in egg albumen and yolk showed that egg yolk contained less protein than egg albumen. The protein content in egg albumen of the deep litter (5.11 ± 0.45%) differed significantly from that of the other two groups ($p < 0.05$). Battery (7.07 ± 0.48%) and backyard (7.11 ± 0.15) systems had comparable ($p \geq 0.05$) values of protein content. The same trend was observed for the protein content of the yolk in the deep litter, backyard, and battery systems as 8.87 ± 0.48%, 9.34 ± 0.6%, and 9.52 ± 0.29%, respectively (Figure 5B).

The lipid content of the yolk as a percentage of dry matter varied according to the farming systems. The significantly ($p < 0.05$) highest value of yolk lipid content (Figure 5D) were recorded in backyard system eggs (41.97 ± 1.2%), compared to deep litter (34.57 ± 0.6%) and battery (33.38 ± 0.9%) systems, which also presented similar ($p \geq 0.5$) values (Figure 5C). The same observations were made with regard to protein content as a percentage of fresh matter.

Table 1. Weight and proportions of layer hen egg constituents according to farming systems between August and October 2020 in the city of Ngaoundéré, Cameroon

Farming systems \ Parameters	Yolk weight (g)	Albumen weight (g)	Shell weight (%)	Yolk proportion (%)	Albumen proportion (%)
Backyard	15.20 ± 1.07[b]	23.88 ± 2.87[a]	4.43 ± 0.70[a]	35.10 ± 3.3[b]	54.75 ± 3.31[a]
Battery	14.23 ± 0.90[a]	37.92 ± 3.87[b]	6.03 ± 0.4[b]	24.53 ± 1.83[a]	65.06 ± 2.36[b]
Deep Litter	15.43 ± 1.3[b]	41.83 ± 3.08[c]	6.25 ± 0.5[b]	24.31 ± 1.67[a]	65.84 ± 1.7[b]

[a,b,c]: Values with the same letter are not significantly different per parameter ($p > 0.05$)

Table 2. Shell characteristics of layer hen according to farming systems between August and October 2020 in the city of Ngaoundéré, Cameroon

Parameters \ Farming systems	Backyard	Battery	Deep Litter
Proportion (%)	10.15 ± 1.27[a]	10.42 ± 1.05[a]	9.85 ± 0.73[a]
Large side thickness (mm)	0.33 ± 0.03[a]	0.60 ± 0.16[c]	0.43 ± 0.03[b]
Medium side thickness (mm)	0.33 ± 0.04[a]	0.51 ± 0.09 [c]	0.44 ± 0.03 [b]
Small side thickness (mm)	0.37 ± 0.06[a]	0.57 ± 0.17[c]	0.43 ± 0.05[b]
Average thickness (mm)	0.35 ± 0.03[a]	0.56 ± 0.08[c]	0.44 ± 0.03[b]

[a,b,c]: Values with the same letter are not significantly different per parameter ($p > 0.05$)

Table 3. pH variations of yolk and albumen according to farming systems between August and October 2020 in the city of Ngaoundéré, Cameroon

Parameters \ Farming systems	Backyard	Battery	Deep Litter
Yolk pH	6.96 ± 0.10^{b}	6.81 ± 0.10^{ab}	6.63 ± 0.19^{a}
Albumen pH	9.42 ± 0.06^{a}	9.40 ± 0.06^{a}	9.36 ± 0.16^{a}

^{a,b,c}: Values with the same letter are not significantly different per parameter ($p > 0.05$)

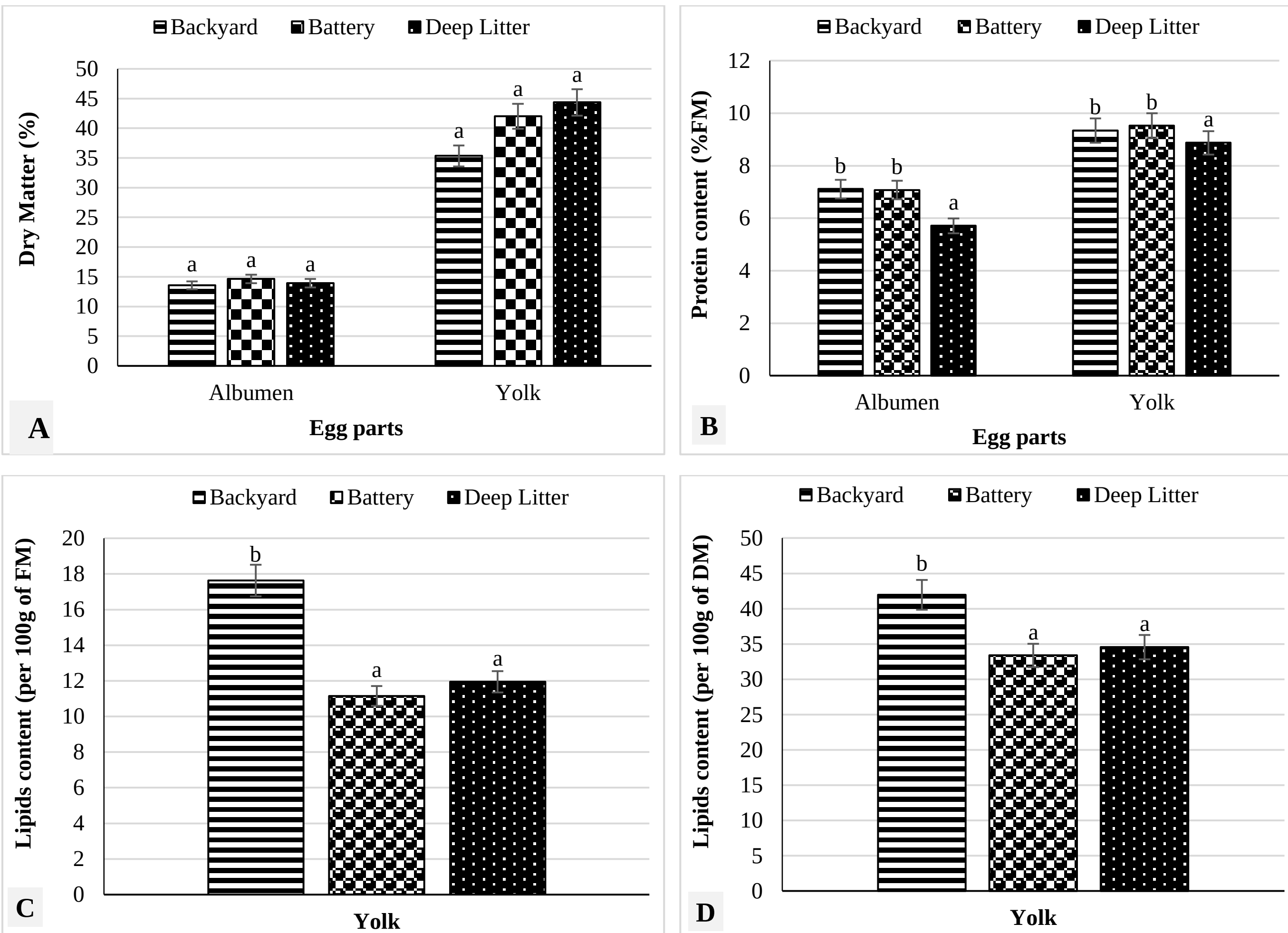

Figure 5. Chemical characteristics of layer hen eggs according to production systems in the city of Ngaoundéré, Cameroon from August to October 2020. A: Dry Matter B: Protein, C: Lipids (per 100g FM: fresh matter), and D: lipids (100g DM: dry matter). ^{a,b,c}: Values with the same letter are not significantly different per parameter ($p > 0.05$).

DISCUSSION

The predominance of men (90.9%) in modern poultry farming recorded in the current study contradicted with the general trends of a study by Fotsa et al. (2007) concluding that family poultry farming was mostly (56.6%) carried out by women. This was also the case for Moula et al. (2012) whose work in village poultry farming in Bas-Congo in the Democratic Republic of Congo revealed that in 42.9% of the families visited, hen rearing was exclusively carried out by women, compared to only 15.6% of men. Similar results were reported by Pousga (2009), and Djitie et al. (2015), who indicated that village or family poultry farming is mainly considered as an activity for women and children. Such discrepancy can be due to traditions in the Ngaoundéré region that all family burdens should be supported by men and women are not required to be economically active. Modern poultry practice also requires a strong financial and managerial involvement which cannot easily be supported by women.

Poultry farming and trade have been the main activities of the respondents. Agriculture as the second dominant activity is an integral part of the lives of urban populations. These observations contrast with previous studies (Ekue et al., 2002; Sonaiya and Swan, 2004; Djitie et al., 2015) who argued that in African family poultry farming is rarely the household livelihood, but represents one of the many integrated and complementary activities that contribute to its overall well-being. These observations in the case of the present study would be justified by the fact that modern poultry farming is an income-generating activity, which helps to support the farmers. All animals were housed and buildings were made of long-lasting materials that provide good protection for the animals against bad weather, theft, and predators. These observations contrasted to those reported by Pousga (2009) and Moula et al. (2012) who indicated that in rural areas, 80% of poultry houses were built in the traditional style with old sacks (73%) or straw (7%). They also reported that in some cases, chickens have no shelter and spend the night perched on trees. The same observations were made by Fotsa et al. (2007); Moula et al. (2012) and Djitie et al. (2015) whose husbandry practices seemed to be a little more serious. In their studies, the farmers said that they locked up chickens in the evening and only opened them in the morning. Badudi and Ravindra (2002) and Hassen et al. (2007) in Botswana and Ethiopia respectively, reported that a few farmers could provide habitat for their animals. However, the results of the present study were in line with a study by Ayssiwede et al. (2011) who noted that in the Gambia, only 10% of livestock keepers had acceptable shelters. Kondombo (2007) and Ouedraogo et al. (2015) in Burkina Faso indicated an improvement in the use of improved housing as the sub-sector has been supported by several programs and projects.

Feeding was entirely the responsibility of the farmers. In this regard, most farmers bought the feed directly from companies while others made their own feed formulation. The drinkers, feeders, and nesting boxes were adapted to modern poultry farming. Results of the present study were in contrast to studies by Sonaiya and Swan, (2004) and Ayssiwede et al. (2011) in poultry farming of a village in Zimbabwe, indicating that in 39.58% of poultry farms, a mixture consisting mainly of maize waste and barley grains was fed to the animals. This observation was also made in family poultry farming by Fotsa et al. (2007) in the Cameroonian plateau, Djitie et al. (2015) in Vina Division, Adamaoua region, Cameroon, and Ouedraogo et al. (2015), in Ouagadougou, Burkina Faso. According to them, the current chicken feeding system was based on rambling consisting of pigeon food, insects, and other items.

The mortality rate was 1-10% per year for 30.3% of the farmers. The low mortality rate can be explained by the fact that battery and deep litter poultry farming systems were more closely monitored than in the traditional system. These observations corroborated those reported for chickens raised in stations (9.8%, Diagne, 2012). The selling price of broilers at 45 days of age and of re-laying hens at the end of the laying period varies from 5 to 6 USD as a function of chicken weight and size, as well as market demand. This was in line with the observation made by Djitie et al. (2015). The most frequently cited constraints were the high cost of feed as well as lack of financial support, feed supply, and security. These constraints were in contradiction with those reported in village poultry farming in numerous studies generally in developing countries, such as a study by Kugonza et al. (2008) in Uganda, Raach-Moujahed et al. (2011) in Tunisia, Bett et al. (2012) in Kenya, Moula et al. (2012) in the Democratic Republic of Congo and Djitie et al. (2015) in Cameroon, who observed that the most frequently encountered constraints were diseases, predators, lack of monitoring and theft. In the case of the present study, it could be explained by the fact that the animals were confined in large numbers, which increases their feed consumption. Another important factor, stealing, occurred since most poultry farms were housed in a peri-urban area with poor security.

Egg weights from the backyard system (43.50 g) were lower than those from the battery (58.19 g) and deep litter (63.51g) systems. These differences in weight can be explained by genetic diversity, rearing systems, feed, age of the hen, climactic changes, and vegetation (Egahi et al., 2013). It could also be due to the fact that backyard hens are mainly characterized by a small size, which produces smaller eggs in contrast to layers, which are hens selected only for laying. Results of the present study were similar to those obtained by Keambou et al. (2009) in Cameroon, who reported an average egg weight of 44.49 g for local hens, compared to the commercial breed. Several authors (Dafaalla et al., 2005; Fotsa et al., 2007), reported similar weights to those obtained in the present study ranging from 37.95 to 44.9 g on local eggs in the West and Central Africa. Dahloum et al. (2015) in Algeria working on commercial hens, recorded results close to observation (61.54 g) on egg's weight from the battery and deep litter systems in the present study. On the other hand, Moula et al. (2012) reported average weights between 50.23 and 54.32g on local hen eggs in lower Kabylia. Depending on the genetic variability, the weight of local hen eggs is in the range of 27-54.7g (Alkan et al., 2013). There was also a significant difference in density, with the lowest value observed in backyard eggs (1.05 ± 0.06), compared to the battery (1.09 ± 0.06) and deep litter (1.10 ± 0.08) systems.

The diameter and height average of eggs from backyard systems were lower than those of eggs from the battery and deep litter systems. These observations were higher than those recorded by Fayeye et al. (2005) in Morocco, Keambou et al. (2009) in West Cameroon, but lower than those reported by Samandoulougou et al. (2016) in Burkina Faso, Djitie et al. (2020), in the city of Iasi in Romania. The values recorded in the present study were higher than those reported by Salifou (2007) in Dakar on commercial eggs. All these variabilities in egg size could be related to breeds, the laying period of the hen, and the protein content of the feed. They could also be due to genetic type (Küçükyılmaz et al., 2012), and geographical areas (Athias, 2003) that affect egg weight, size, and even color (Sauveur, 1988). Shape indexes recorded in the present study were higher than those of other studies. Markos et al. (2017) reported the shape indexes of 66.5 to 71.3% for local hen eggs, Keambou et al. (2009) found values close to 73% for eggs from rural families, and Samandoulougou et al. (2016) estimated the range of 72-75% for commercial breed eggs. According to King'ori (2012), size, age, health status of the hen is among the factors that can strongly influence the egg shape index. Backyard eggs showed a significantly lower egg volume of 41.75, compared to those from deep litter and battery systems. This lower egg volume observed in backyard eggs could be attributed to the lower egg weight, diameter, and height (Jessy et al., 2016). These results corroborate those reported by Kanagaraju et al. (2013) on quail, who indicated that egg volumes vary between phenotypes. Observations on yolk quality highlighted that eggs from backyard systems contained more yolk correlated with the higher diameter and yolk proportion. The same trends were observed by several authors on the assessment of eggs from local and commercial breeds (Alewi et al., 2012; Zaaboube and Benrahou, 2014). However, higher egg volumes than in the present study were observed in different agro-ecological zones in Ethiopia (Melesse et al., 2010; Melesse et al., 2013) on eggs from rural hens. Although eggs from deep litter and battery systems showed no significant difference in yolk height and index, they were higher, compared to those from backyard eggs. The average albumen weight of eggs from deep litter and battery systems was higher than that from the backyard. Similar values to those of eggs from deep litter and battery systems were observed by Moula et al. (2012) with eggs from Isa Brown and CoqArd breeds. Lower weights within the range of 30.92 and 33.18 g were found in eggs from the White Leghorn strain (Sreenivas et al., 2013). They reported significant relationships between albumen and whole egg weights. Albumen percentage and height were higher in backyard eggs, compared to eggs from the battery and deep litter systems, the opposite was recorded for diameter.

Haugh unit did not differ significantly for deep litter and battery eggs, which were also higher than the value obtained with backyard eggs. This would be justified by the fact that backyard eggs were collected from the market, and therefore the age could be different, yet eggs from deep litter and battery systems were of the same age. Haugh units from the present study were higher than that reported by Melesse et al. (2013) in Ethiopia, Egahi et al. (2013) in Nigeria in local hens. The highest shell weight value was observed in eggs from deep litter and battery systems. Similar observations were made by Moula et al. (2012) who reported that the shell weight of eggs from Isa Brown hen was higher than that of the traditional breed. Several authors reported that this trait is strongly related to the weight of the whole egg (Alipanah et al., 2013; Sreenivas et al., 2013). The same remark was made by Dahloum et al. (2015) in Algeria, according to whom the commercial layer hen strains had a higher shell weight compared to the local breed with normal feathers. The values recorded in the present study could be explained by the fact that the feeding of the commercial breed of hens was optimal and generally met the required standards, whereas, backyard hens were free-range. Observations made for shell proportions revealed that they were around 9.85%, 10.42%, and 10.45% for battery, deep litter, and backyard systems eggs, respectively. Previous works by El-Safty et al. (2006) on bare-necked heterozygous hens and local hens in Egypt and Djitie et al. (2020) on backyard eggs in Romania indicated lower values than values obtained in the present study. However, Samandoulougou et al. (2016) observed higher values of shell proportions in Burkina Faso. Egg shell thickness obtained in the current study was close to that recorded by Djitie et al. (2020) revealing similar values to those of eggs from deep litter and battery poultry farming systems, but lower than those of backyard eggs.

Egg yolk pH was acidic while egg albumen pH was basic regardless of the farming systems considered. A significant increase in pH was observed between deep litter, battery, and backyard systems. These results were in agreement with those mentioned by Dahloum et al. (2015) in Algeria and Samandoulougou et al. (2016) in Burkina Faso who mentioned values of 5-6 and 8-9, respectively, for yolk and albumen in local and commercial hens. This increase could be justified by the fact that backyard eggs were collected from rural families and their production date associated with the feed ingredients and feeding program while eggs from the battery and deep litter poultry farming systems were of the same age and standardized feeding program. This variation of pH could also be due to the genetic type and rearing system. Yolk contained more dry matter and lipids than the albumen in all three systems, except for the protein content where the reverse case was recorded. Results obtained for eggs from deep litter and battery systems were in line with those found by Nys and Sauveur (2004) in France on layer eggs, Samandoulougou et al. (2016) in Ouagadougou on eggs from improved breeds, Djitie et al. (2020) in Romania on from local hens eggs. The lipid content of backyard eggs was higher than that of deep litter and battery systems eggs. These observations were contrary to those of AEB (2006) on eggs from rural families in the United States of America, which reported a value of (52.9%) and Samandoulougou et al. (2016), who recorded 32.76% for eggs from local hens in Burkina Faso. This difference in results could be explained by genetic type, rearing systems, environment, and animal feeding. Given the very low lipid content (0.2-0.4%) in egg albumen (Sauveur, 1988; AEB, 2006), the effect of production systems on lipid content has not been studied.

The yolk dry matter content of battery and deep litter system eggs was higher than that obtained in backyard eggs. The yolk dry matter recorded was higher than that reported by Arzour (2006) in Algeria (26%) and Samandoulougou et al. (2016) in Burkina Faso (28.68%) on improved breed eggs. Results obtained were also in contrast to the work of Djitie et al. (2020) which showed that local breed eggs contained more dry matter than commercial breed eggs. The protein content of yolk and albumen was similar between the backyard and battery eggs but higher than values recorded for the deep litter system. This observation contrasted with those of Lahouari et al. (2015) who recorded the values of 13.07% and 12.61% for eggs from commercial and local breeds, respectively. This difference in results could be explained by the influence of breed, age, and diet on the egg protein content (Sauveur, 1988; Nys and Sauveur, 2004).

CONCLUSION

In conclusion, modern poultry farming was generally carried out in poorly developed deep litter systems. Poultry farmers were not trained for this activity despite the need for real intensive training and improvement of the modern poultry farming sector in the area. Generally, eggs from deep litter systems exhibited better characteristics in terms of weight and freshness, eggs from local hens showed better characteristics in terms of chemical or nutritive elements and were, therefore, more recommended.

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Authors’ contribution

FDK and CT designed the study, interpreted the data, and drafted the manuscript. MB was involved in data collection and manuscript preparation. RMRR took part in the study design, preparation, and critical checking of the manuscript.

Competing interests

The authors declare that there is no conflict of interest in the outcome of this research.

Ethical consideration

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

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The Effect of Artemisia on Immune Response and Productive Performance Against Newcastle Disease in Broiler Chickens

Haider Tuma Kaab[1*], Samer Sadeq Hameed[2], and Ali Mahdi Sahib[3]

[1]*Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine, University of Kufa, Iraq*
[2]*Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Baghdad, Iraq*
[3]*Department of Public Health, College of Veterinary Medicine, University of Kufa, Iraq*

*Corresponding author's email: haidert.mehde@uokufa.edu.iq; ORCD: 0000-0002-8016-4595

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ABSTRACT

Prevention of Newcastle disease has received a lot of interest across the world. The high productivity performance of the commercial chickens' breeds has negative effects on the immune system and animal welfare. As a result, the current study aimed to investigate the benefits of adding Artemisia powder at levels of 0.5% and 1% to broilers' feed as a growth and health promoter. A total of 120 commercial broiler chickens were grown on the floor in a chicken house and separated into three groups, including one control and two treatment groups. Each group contained 40 chickens subdivided into two replicates. The three groups, namely G1 (chickens without Artemisia powder, as a control group), G2, and G3 in which chickens were fed with basal diet plus 0.5% and 1% Artemisia powder, respectively, were differentiated based on their diet throughout 35 days of the experiment. The measured parameters included the immune response to Newcastle disease vaccine, blood biochemical parameters, and growth performance as well as relative weight for the spleen and bursa of Fabricius. A diet containing 1% Artemisia powder significantly improved antibody titer against Newcastle disease, body weight, and weight gain. Thus, the addition of 1% of Artemisia powder to the broiler's diet can improve immune response against Newcastle disease and growth performance.

Keywords: Artemisia, Broiler chicken, Newcastle disease, Productive traits

INTRODUCTION

Considering the poultry diet, feed additives, including antibiotics, enzymes, diet dyes, flavors additives, probiotics, (Vahdatpour and Babazadeh, 2016), phytobiotics (Saeed et at., 2018), and antioxidants (Pirgozliev et al., 2019) are added to enhance poultry production performance although they are not nutritious. These substances are added to a diet mainly for the improvement of feed intake and growth rate in chickens (Bali et al., 2011; Abouelfetouh and Moussa, 2012; Ahsan et al., 2018). Most growth promoter antibiotics were banned by the European Union countries in 2006 due to their remains in body tissues and the development of pathogen resistance (Millet and Maertens, 2011). Researchers have investigated the use of aromatic plants as an alternative to antibiotics. Aromatic plants or herbs as feed additives are used widely in the Middle East for a long time ago helping to enhance food taste (Saeed et al., 2017; Daramola, 2019; Christaki et al., 2020). Medical plants supplementation in the broiler chicken diet could affect performance characteristics, serum metabolites, and antioxidant status (Kostadinović et al., 2015; Jin et al., 2020). According to the World Health Organization, about 80% of medical plants in humans are used for disease prevention and treatment particularly in developing countries (Deniz et al., 2021).

Chicken production is mainly correlated to gastrointestinal tract (GIT) efficiency, where its main role is feed digestion and metabolism (Ravindran and Abdollahi, 2021). Thus, GIT health is playing a substantial role in productivity as it affects the utilization of energy, proteins, and consequently disease resistance. Phytogenic feed additives (PFAs) are substances of plant origin used as diet additives in the poultry industry for the improvement of performance characteristics (Noonan, 2018). In modern animal production, PFAs are used for their antimicrobial effect, digestibility enhancement, and performance improvement (Hafeez et al., 2016; Ahsan et al., 2018). Artemisia is a class of plant that belongs to the Asteraceae family and is used as human and animal medicine. The nutrition contents of Artemisia are presented by Iqbal et al. (2012) which includes carbohydrates, fibers, fat, protein, phytate, tannin, and tocopherol. Artemisia has been utilized in poultry production as an enhancer of weight gain, feed conversion ratio (Gholamrezaie et al., 2013), and gut microbiota (Saracila et al., 2018; Song et al., 2018; Panaite et al., 2019). Abdullah and Al-Barwary (2020) indicated that the addition of Artemisia to the broilers' diet can lead to improved performance, gut morphology, and serum biochemical parameters during a coccidiosis challenge. Saracila et al. (2018) found that intestinal microflora improved in broilers reared under heat stress after the administration of Artemisia in the diet. Guo et al. (2020) found that Artemisia could improve growth performance and antioxidant function in broilers. Liu et al. (2009) demonstrated that Artemisia inhibited the proliferation of Newcastle disease virus in chicken embryos in an *in vivo* study. In Iraq, the commercial poultry industry aims to reach the highest body weight gain from feed intake. However, productivity and immune response for diseases are adversely correlated (Lwelamira et al., 2009; Sahib, 2021).

Many countries have tended to minimize or prohibit the chemical components because of their deleterious side effects on both animals and humans (Hao et al., 2014; Osman et al., 2018). Therefore, the aim of the current research was to investigate the effects of adding Artemisia powder at levels of 0.5% and 1% to the basal diet on immune response against Newcastle disease and the productive performance of broiler chickens.

MATERIALS AND METHODS

Ethical approval

All the procedures involved in the current study were confirmed by the scientific and animal care committee at the University of Kufa, Iraq (Certificate number: 100821.139).

Chickens

This experiment was conducted at the poultry farm of the University of Kufa, Iraq. A total of 120 Cobb 500 broiler chickens aged one day old were purchased from a local hatchery in Najaf, Iraq. One-day-old chickens with an average weight of 39.8 g were randomly split into three nutritional groups with two replicates (20 birds per replicate). All the management requirements (temperature, light, and ventilation) were met based on the Cobb 500 production guide management (2021). Chicks were raised on wood shaving floor pens. The first group (G1) was provided with a basal diet as a control group. While the second group (G2) and the third group (G3) were daily fed on a basal diet plus 0.5% and 1% Artemisia powder respectively. Feed and water were available *ad libitum*. Two formulas of diets (starter and finisher) were utilized during 35 days of the experiment (Table 1). All birds received a companied Newcastle disease (ND) (B1 strain, (Poulvac®, USA)) and infectious bronchitis vaccine (Poulvac®, USA) by spraying each of them on the first day at the hatchery.

On the other hand, booster dose was administered orally in water, these vaccines included ND (Lasota strain Poulvac®, USA) on day 10 as well as Gumboro (IBD2, Bursine®, USA) on days 14 and 23 (Lasota strain of ND, Poulvac®, USA). Moreover, Vitamin C was administered (1 gm/liter) orally for 3 days after each vaccination. The chickens cared in accordance with animal welfare principles and with attention to ethics committee roles.

Table 1. Composition of diet in broiler chickens during 5 weeks of rearing

Ingredient (%)	Initial diet (1- 20 day)	Closer diet (21-35 day)
Corn (yellow)	53	44
Soybean meal (48% protein)	30	21
Wheat	10	26
Protein concentrate	5	5
Oil	1	3
Premix (VAPCO®, Jordan)	0.2	0.2
Limestone	0.5	0.5
Salt	0.2	0.2
Dicalcium phosphate	0.1	0.1
Overall	100	100
Chemical analysis		
Energy (kcal/kg)	2951.5	3128.5
Crude protein (%)	22	19

The balanced diet based on the NRC (1994). Premix is a mixture of vitamins and minerals.

Production traits

The electronic balance was used to measure live body weight and feed consumption to calculate body weight gain and feed conversion ratio at weekly intervals (Aguilar et al., 2013). However, the mortality rate was recorded daily as it occurred.

Blood collection and analysis of cells count and enzymes

About 1 ml blood sample from brachial vine was collected from all chickens at age of 20 and 35 days by one-use syringe then transferred to test blank tubes to separate sera after clotting blood by centrifuge (10 minutes at 3000 rpm). In the next step, serum was frozen at -20°C according to Mitchell and Johns (2008). Antibodies of Newcastle disease were measured in serum by utilizing a specific ELISA Kit (Proflok®, USA) run by following the instructions of the manufacturer. Blood components (WBCs and RBCs) measures were conducted using counter medionic cells. Blood biochemistry analyses of total protein, albumin, Hb, and AST were achieved by Micro Lab 300 analyzer (Merck®, France) and using kits from Merck (France). By following the instructions of the manufacturer after mixing the sample with provided reagents with the kits, and then the mixture was brought up to 37°C by incubation for 5 minutes. Measurement was achieved with equivalent wavelength to the designated parameters.

Measurement of lymphatic organs

On day 35 of age, three chicks were randomly taken from each replicate and slaughtered after a cervical dislocation to avoid pain. The weight of each chick was measured using a digital balance. The internal organs, such as the spleen, bursa of Fabricious were weighted after collection by digital balance to calculate the ratio between them. Remove feather, head, legs, and viscera and kept only the carcass.

Statistical analysis

The obtained data were analyzed in a complete randomized design (Steel and Torrie, 1980). These data were analyzed by one way ANOVA test following the procedure of the general linear model (SAS, system-version 9.1). Duncan's multiple range tests were employed to determine the differences among group means at a significance level of $p < 0.05$. The following is the model: $Y_{ij} = \mu + T_i + e_{ijk}$, where, Y_{ij} represents individual observation, μ is the overall mean, T_i indicates the effect of treatment (i = 1, 2, 3), and e_{ijk} signifies a random error.

RESULTS

The effects of Artemisia powder additive during the experimental period on immune response (antibodies titer)against Newcastle disease are shown in Figure 1. The antibody titers increased significantly in the chickens fed with Artemisia 1% at 35 days of age, compared to other groups ($p < 0.05$). On the other hand, 0.5% of Artemisia did not lead to a significant increase regarding antibody titers ($p < 0.05$).

The results of Artemisia's effects on the blood cells count and some biochemical tests are presented in Figure 2. There was a significant difference among treatment groups in terms of red blood cells (RBC) counts with the peak (3.2 ± 0.04) in the group fed 1% Artemisia ($p < 0.05$). The Hb significantly increased in diet plus Artemisia with 0.5% and 1%, compared to the diet without Artemisia additive ($p < 0.05$). White blood cells (WBC) count was significantly higher (32 ± 0.7) in the control group, compared to other groups (Artemisia 0.5% and 1%). Aspartate aminotransferase (AST) measurements revealed significant differences ($p < 0.05$) and recorded a peak in the basal diet without Artemisia (242.5 ± 2.02), compared to chickens fed 0.5% Artemisia (218.5 ± 4.47). There were no significant differences among groups regarding both albumin and total blood protein.

The final body weight at 35 days of age enhanced significantly in G3 and G2 respectively, compared with G1 ($p < 0.05$, Figure 3). Weight gain of broiler chickens for all-treatments groups are presented in Table 2 and the total gain at the end of the experiment (day 35) is presented in Figure 4. The body weight gain was significantly different at week 5 when the highest was in G3 (764.9 ± 32.9 gm). The second highest record for body weight gain was for G2 (679.40 ± 33.09) and then the least was for G1 (556.20 ± 12.85). There were no significant differences among the three groups in terms of feed consumption and feed conversion (Tables 3 and 4). The same trend was true for the relative weight of lymphatic organs (bursa of Fabricious and spleen) among the three groups ($p > 0.05$, Table 5).

Table 2. Weight gain (g) of broiler chickens after dietary supplementation with different levels of Artimasia during 5 weeks of growing

Treatment / Age	First week	Second week	Third week	Fourth week	Fifth week	Total
G1 (control)	130.50±2.12[a]	218.50±5.70[a]	417.10±6.54[b]	469.60±7.73[a]	556.20±12.85[c]	1791.90±8.30[b]
G2 (Artemisia 0.5%)	127.70±1.54[a]	217.30±3.30[a]	424.50±4.06[ab]	469.40±6.51[a]	679.40±33.09[b]	1918.30±40.90[ab]
G3 (Artemisia 1%)	127.70±1.15[a]	214.20±1.91[a]	436.60±3.85[a]	466.40±9.78[a]	764.90±32.95[a]	2009.80±40.60[a]

Mean (gm) ± standard errors, Alteration in superscript letters in each column refers to a significant difference between treatments at $p < 0.05$.

Table 3. Feed intake (g) of broiler chickens after dietary supplementation with different levels of Artimasia during 5 weeks of growing

Treatment / Age	First week	Second week	Third week	Fourth week	Fifth week	Total
G1 (control)	157.50± 4.50[a]	352± 9[a]	666.90± 15[a]	821± 20[a]	1081± 25[a]	3078.40± 73.50[a]
G2 (Artemisia 0.5%)	156.40± 2.50[a]	371± 8[a]	657.10± 13[a]	813.50± 19.50[a]	1082.50± 24.50[a]	3080.50± 67.50[a]
G3 (Artemisia 1%)	157.40± 4.50[a]	362± 9[a]	673.70± 13[a]	814± 18[a]	1052.50± 24.50[a]	3059.60± 69[a]

Mean ± standard errors, Alteration in superscript letters in each column refers to a significant difference between treatments at $p < 0.05$.

Table 4. Feed conversion ratio of broiler chickens after dietary supplementation with different levels of Artimasia for 5 weeks

Treatment / Age	First week	Second week	Third week	Fourth week	Fifth week	Total
G1 (control)	1.206± 0.022[a]	1.610± 0.021[a]	1.598± 0.001[a]	1.751± 0.100[a]	1.944± 0.067[b]	1.622± 0.042[a]
G2 (Artemisia 0.5%)	1.224± 0.016[a]	1.707± 0.034[a]	1.548± 0.057[a]	1.733± 0.005[a]	1.595± 0.045[a]	1.561± 0.011[a]
G3 (Artemisia 1%)	1.233± 0.049[a]	1.689± 0.024[a]	1.543± 0.026[a]	1.745± 0.048[a]	1.378± 0.043[a]	1.517± 0.021[a]

Mean ± standard errors, Alteration in superscript letters in each column refers to a significant difference between treatments at $p < 0.05$.

Table 5. The relative weight of lymphatic organs of broiler chickens at age 35 days after dietary supplementation with different levels of Artemisia

Treatment / Lymphatic organ (%)	Spleen	Bursa of Fabricius
G1 (control)	0.06 ± 0.006[a]	0.08 ± 0.014[a]
G2 (Artemisia 0.5%)	0.07 ± 0.006[a]	0.09 ± 0.012[a]
G3 (Artemisia 1%)	0.07 ± 0.007[a]	0.10 ± 0.012[a]

Mean ± standard errors, Alteration in superscript letters in each column refers to a significant difference between treatments at $p < 0.05$.

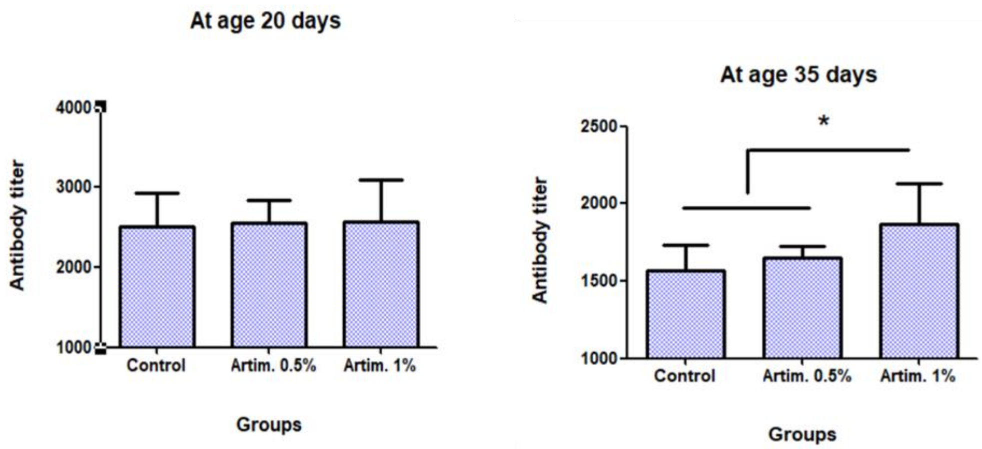

Figure 1. The level of antibody titer against Newcastle disease vaccine in broiler chickens aged 35 days after dietary supplementation of different levels of Artemisia

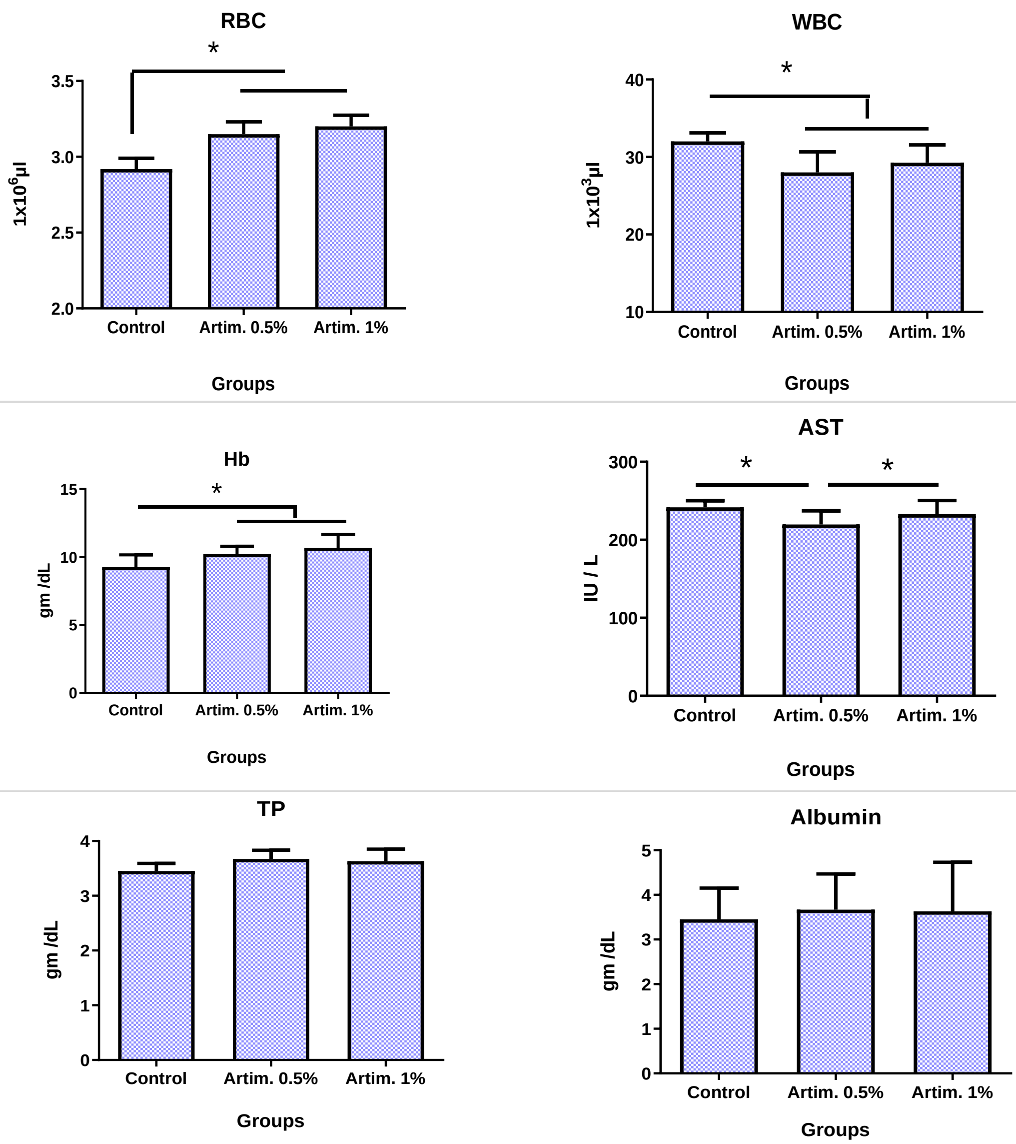

Figure 2. The responses of physiological parameters to dietary supplementation of Artimasia in broiler chickens aged 35 days. These are RBCs, WBCS, Hb and AST, TP, and Albumin

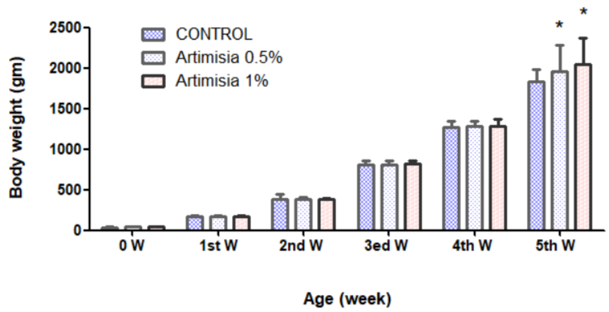

Figure 3. Body weight of broiler chickens from first to fifth week age after dietary supplementation of different levels of Artemisia.

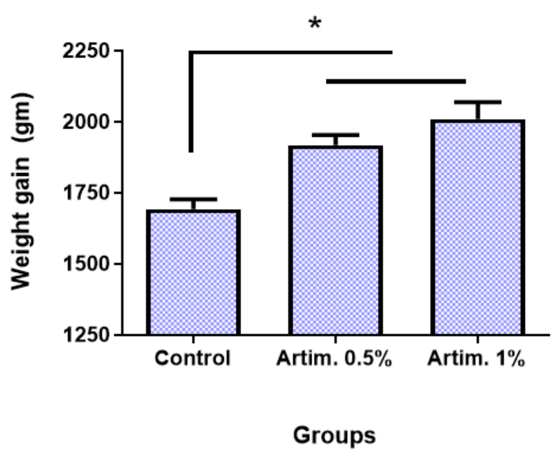

Figure 4. The body weight gain of broiler chickens at 35 days old after dietary supplementation with different levels of Artemisia

DISCUSSION

The immune response was estimated by measuring antibody titer which was significantly higher in G3, compared to other groups (Figure 1). This significant improvement of antibody titer could be due to the promotion of the dendritic cells and their maturation as a result of increased expression of CD86 and CD40. Consequently, antigen phagocytosis decreases, and the function of T-cells is improved (Wang et al., 2019). Other researchers have reported the enhancement of antibody levels after influenza vaccination, in addition to the improvement in lymphocyte proliferation and cytokine secretion (Zhang et al., 2017). This finding was in line with the one reported by AL-Saeedi et al. (2018) indicating an increased level of antibody titer in vaccinated laying hens with Newcastle and Infectious bronchitis diseases vaccine when fed with Artemisia additive.

The mean of the body weight gains of chickens fed diets supplemented with Artemisia 0.5% and 1% in the current study differed significantly from the control group. This finding indicated that the chickens of the current study could positively benefit from the diet containing Artemisia. Ration containing Artemisia has enhanced the broiler productivity performance throughout the experiment period. The increment of body weight and immunity as a result of a diet containing Artemisia additive could lead to promoting the digestion and absorption of nutrients in the intestine. According to previous studies, Artemisia has abundant bioactive composites, such as coumarin, flavonoids, purines, and phenols (Carrà et al., 2014). The polyphenol substances act as numerous biological activities, among which are anti-inflammatory, antipyretic, anti-cancer, anti-fungal, antiparasitic, and cytotoxic activities (Ćavar et al., 2012). Moreover, it improves gut integrity by increasing villous height and villous to crypt depth (Abdullah and Al-Barwary, 2020). Therefore, dietary supplementation of Artemisia leads to an increased concentration of serum globulin. Liu et al. (2019) have reported that Artemisia enhances IgA levels, and decreases IL-2 and IL-6 in the intestine). Furthermore, it reduces the colonization of pathogenic microbes, such as coccidia, Enterobacteriaceae, *Escherichia coli*, and *Staphylococcus aureus* and improves the viable counts of bacteria-produced lactic acid (Lactobacilli, Saracila et al., 2018; Panaite et al., 2019; Abdullah and Al-Barwary, 2020). All these positive properties of Artemisia have consequently enhanced the immune status and performance.

Moreover, Artemisia enhances the activity of catalase, superoxide dismutase, glutathione peroxidase, and total antioxidant capacity, while reducing the concentration of malondialdehyde in serum, liver, and spleen (Guo et al., 2020). Therefore, improved liver enzymes activities lead to better health conditions.

The results of blood biochemical parameters and cells count of the experiment groups at age of 35 days are in agreement with other studies, such as those conducted by El-Latif et al. (2013) and Oleforuh-Okoleh et al. (2015). As the studies suggested the increment of both RBCs and Hb in broiler chickens as a result of different phytogenic feed additives. The measurement of blood components could be employed for interpreting the health status (Maxwell et al., 1990). In the current study, it has been noticed that the levels of blood parameters were in normal ranges which could be interpreted as better health and immune status of the experimental chickens. Nevertheless, the clinical investigations indicated no abnormalities in broiler chickens. The results showed an increase in WBCs count in the control group, compared to the other two experimental groups. On the other hand, the results indicated that the feed additive of 0.5% reduced the level of aspartate aminotransferase, compared to the control group indicating the viability role of Artemisia in enhancing liver activities. Reduced activities of the liver enzyme in the current study confirm the appropriate doses of the investigated herbal additive (Sohail et al., 2012; Ali et al., 2014). No significant differences were recorded in both total serum protein and albumin. These findings were similar to those reported in previous studies (Bhaisare and Thyagarajan, 2014). However, enhancements have been noticed in total protein, which could refer to a better immune state of the chickens fed with Artemisia additives (Kapelański et al., 2004).

CONCLUSION

In conclusion, broiler chickens fed a diet containing Artemisia powder at a level of 1% for 35 days indicated an improved growth rate and immune response. It is suggested to investigate the effect of adding Artemisia to broiler chickens' diet on the immune responses against Gumboro and infectious bronchitis vaccines in extensive research.

DECLARATION

Authors' contribution

Haider Kaab wrote the paper and corresponded to complete further requirements of writing. Samer and Ali conducted the experiment. All authors confirmed the statistical results and the final version of the article.

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Competing interest

No conflict of interest for this manuscript has been stated by the authors.

Ethical considerations

The results (or any part of them) used in the manuscript have not been sent for publication to any other journal nor have they already been published. The authors declare that they have checked the manuscript for plagiarism and there is no data fabrication or redundancy. The performed experiment complied with current laws and written consent of the Scientific Ethnic Committee in the department of the pathology and poultry diseases in the College of Veterinary Medicine University of Kufa and Baghdad.

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The Effect of Probiotic Derived from Kumpai Minyak (*Hymenachne Amplexicaulis*) Silage on Performance and Egg Quality Characteristics of Pegagan Ducks

Sofia Sandi[1]*, Fitra Yosi[1], Eli Sahara[1], Asep Indra Munawar Ali[1], Nuni Gofar[2], and Nur Muhamad[3]

[1]*Department of Animal Science, Faculty of Agriculture, University of Sriwijaya, Palembang, South Sumatera, Indonesia*

[2]*Department of Soil Science, Faculty of Agriculture, University of Sriwijaya, Palembang, South Sumatera, Indonesia*

[3]*Department of Animal Science, Jember polytechnic, Jember, East Java, Indonesia*

*Corresponding author's E-mail: sofiasandi_nasir@yahoo.com; ORCID: 0000-0002-1920-4906

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ABSTRACT

The study aimed to determine the effect of probiotics derived from an isolate of silage Kumpai Minyak grass on performance and the physical egg quality of Pegagan ducks. The study was conducted in 16 weeks, from May to September 2020. The sample size was 400 female Pegagan ducks aged five months. The treatments included basal diet (Control) and base diet plus 0.2% (P2), 0.4% (P4), 0.6% (P6), and 0.8% (P8) probiotic silage of Kumpai Minyak grass. The observed variables were performance (egg production, egg weight, feed consumption, and feed conversion ratio) and physical quality (albumen index, albumen weight, yolk weight, and Haugh unit). Observation data on probiotic treatment 0.8% (P8) established a significant effect on egg weight, compared to other treatments. Moreover, P8 probiotic treatment could significantly affect daily egg production and feed conversion ratio, compared to P2 and P4 probiotic treatments. Different results were found in the observations on feed consumption, where the overall treatment diet indicated significant results, compared to the control treatment. Specifically, several variables showed a significant effect, namely albumen index, albumen weight, egg yolk weight, and Haugh unit. Each observed variable value increased along with increasing probiotic treatment levels. However, egg index, egg yolk index, shell weight, and thickness were inversely related to the other variables investigated in this study. The P8 probiotic treatment could increase digestibility and absorption of feed nutrients due to inhibition of pathogenic bacteria and optimization of the digestive tract. The probiotics at the level of 0.8% produced from the Kumpai grass silage process can be used as a growth promoter for laying ducks to replace commercial antibiotic products.

Keywords: Albumen, Antibiotic, Growth promoter, Isolate, Probiotic, Silage

INTRODUCTION

Pegagan ducks are local ducks originating from Indonesia and are widely available in the southern part of Sumatra. Pegagan ducks are dual-purpose avians meaning that they can produce meat and eggs. Sari et al. (2011) reported that the average weight of Pegagan duck eggs was over 70 g, and this value was relatively high, compared to other local duck eggs. However, the poor maintenance system left the large potential of Pegagan duck unfulfilled leading to a high risk of disease, insufficient nutrient needs, and consequently, low productivity (Sari et al., 2014). One of the efforts to answer this problem is to provide feed additives, such as antibiotics, in animal feed to improve performance and protect poultry production to be more resilient in the face of various invading diseases (Raphael et al., 2017; Amine et al., 2020). However, the use of antibiotics as feed additives has been banned because of the residues they produce (Costa et al., 2018; Sweeney et al., 2018). Antibiotics are generally used to maintain the digestive tract condition by controlling the balance of microflora in ducks' digestive tract. Several experiments have been carried out to overcome or find alternative solutions to replace these antibiotics, including probiotics and organic acid compounds (Sandi et al., 2019; El-Kholy et al., 2020).

Probiotics are live microorganisms that are added to animal feed to increase the balance of the intestinal microflora in order to increase nutrient absorption and increase livestock performance (Chen and Yu, 2020). Until now, many studies have been carried out to find effective and efficient probiotics against poultry in general, such as the use of isolated microorganisms to produce the expected probiotics (Al-Khalaifah, 2018). Furthermore, the use of probiotics from silage isolates has become a new trend among researchers to find probiotics or derivative compounds produced to benefit the world of animal husbandry (Sari et al., 2019; Sandi et al., 2021).

Indonesia is a tropical country whose territory consists of various islands. It has multiple types of land, such as sup-optimal land (swamps), making Indonesia a country that has great potential in finding numerous kinds of probiotics that can be isolated from various types of green vegetation. The probiotics that are being developed and come from forages or plants in swamps are probiotics from Kumpai grass silage (*Hymenachne amplexicaulis*). The type of probiotic produced is a type of lactic acid bacteria. Swamp grass silage can be used as a probiotic because the lactic acid bacteria produced have characteristics such as gram-positive, non-spore, catalase-negative, non-motile, and not form spores (Sandi et al., 2018). (Jannah, 2017) reported that probiotics from copper Kumpai grass silage significantly affected the total lactic acid bacteria needed to accelerate the decrease in pH. The total lactic acid bacteria produced from the manufacture of probiotics was 8.24 (107 CFU / ml), and the resulting isolates had high resistance to acids, which could survive at pH 2.5 and pH 7. According to Fauziah et al. (2013), the use of probiotics containing 3.6 ml of lactic acid bacteria can work well in the digestive tract by increasing ration consumption. According to an *in vitro* study by Sandi et al. (2019), that Lactic acid bacteria (LAB) isolated from Kumpai grass silage as a probiotic showed resistance and can survive and thrive at different pH levels.

The use of probiotics both in feed and drinking water can help improve enzyme Activity. Based on Zhang et al. (2012) research, the addition of probiotics can increase egg production, which will affect the physical quality of the eggs. Based on this description, a study was conducted on the effect of providing probiotic Kumpai grass on the egg quality characteristics of Pegagan ducks.

MATERIALS AND METHODS

Ethical approval

An animal feeding experiment was conducted at the experimental station, Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya. The ducks were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The approval of the experiment was granted from Universitas Sriwijaya.

Study design

The study used a completely randomized design (CRD) with five probiotic treatments, which included a diet without probiotics (Con), diet + Probiotics 0.2% (P2), diet + Probiotics 0.4% (P4), diet + Probiotics 0.6% (P6), and diet + Probiotics 0.8% (P8). The feed used in the study was a formulation diet made from corn, rice bran, concentrate, meat and bone meal, premix, methionine, and lysine. Meanwhile, The used probiotics were collected from lactic acid bacteria isolated from copper Kumpai grass silage. Lactic acid bacteria isolates were cultured in MRSB (deMannRogosa Sharpe Agar in liquid/broth form) and then incubated for 48 hours. The bacterial culture was centrifuged at 3000 rpm for 15 minutes to obtain the substrate from the supernatant. The substrate was mixed with skim milk and 5% (w/w) maltodextrin. The next step is to spray dry at a temperature of 160-180℃ to produce a dry powder product which can then be added to the diet according to the treatment (Bregni et al., 2000).

Management and sample collection

In the current study, the pegagan ducks used came from the Kotodaro village community farm, Tanjung Raja district, Ogan Ilir regency (OI), South Sumatra Province, Indonesia. As for the selected female ducks, they were already in the laying phase and had physical characteristics of blackish-brown fur color and shiny blue wings black. A total of 400 female Pegagan ducks aged five months were randomly assigned to 5 treatment groups, each consisting of 4 replications (20 ducks per replication, 80 ducks per treatment). For each replication, ducks were housed separately in cage size of 2000 m^2. In accordance with recommendations for good management of poultry raising, ducks were subjected to the same humidity, temperature, feeding regime, drinking water, and lighting (Cherry and Morris, 2008).

The study was conducted in 16 weeks, from May to September 2020. During the trial period, chickens were provided with feed and drinking water *ad-libitum*, while the compartment temperature measurement was ranged from 15 to 28℃. The basal diet used is presented in Table 1. In this experiment, the observed variables consisted of the observation of performance and egg quality. Observation of performance data included consumption, conversion, egg production, and egg weight. Meanwhile, egg quality analysis included Haugh units, egg size, albumen index, and egg yolk. At the beginning and end of the experiment, body weight was measured. which is then Based on the difference between the times, the weight gain is calculated. The feed consumed was chopped at a three-day interval. Feed consumption was recorded at the beginning and end of the trial period, then calculated as gram/hen/day. The feed conversion ratio was calculated as kilograms of feed consumed per kilogram of egg produced.

Table 1. Nutrient composition of diets of Pegagan Ducks

Ingredients	Amount (g/kg)
Maize (Corn)	484
Rice bran	185
Meat Bone Meal	64
Konsentrat	245
Premix	10
Metionin	8
Lysin	4
Calculated energy and Chemical analysis	
Metabolisme Energy (MJ/kg)	2750.80
Dry matter, (%)	89.09
Crude Fiber, (%)	3.78
Ether Extract, (%)	7.36
Crude Protein, (%)	20.94
Calcium (%)	3.31
Phosphorus, (%)	1.08
Ash (%)	2.66

Furthermore, all eggs collected and weighed based on the treatment were then determined as egg weight Based on these observations, egg production, egg weight, and daily egg yield were calculated. Egg quality was selected for three consecutive days at the 30-day trial and at the end of the test. A total of 20 eggs were randomly collected from each replication on the third and sixth day of the experiment. Each egg was weighed, and the shape index was calculated as a percentage according to the formula (egg length) / (egg width) with the instrument (shape index instrument, 75135/2, BV. Apparatenfabreik Van Doorm, De Bilt, Netherlands). Eggshell thickness was measured using a micrometer and the yolk color was determined using the Roche Yolk Fan. Haugh unit was calculated according to the formula of Nesheim et al. (1979):

$$\text{Unit Haugh } (\%) = 100 \times \log(H + 7.57 - 1.7W^{0.37})$$

where H is the albumen height, and W is the egg weight.

Data analysis

The data obtained were analyzed by variance analysis (ANOVA). If the treatment significantly affected the observed variables ($p < 0.05$), the analysis was continued with Duncan New Multiple Range Test (DNMRT) test using the SPSS program (version 20).

RESULTS AND DISCUSSION

Performance

The results of the analysis can be seen in Table 2. Overall daily egg production, egg weight, feed consumption, and FCR showed significant results ($p < 0.05$). Observation data on P8 has established a considerable effect on egg weight, compared to other treatments ($p < 0.05$). The same results were also obtained for daily egg production and FCR, where there was a significant effect on P8 probiotic treatment, compared to P2 and P4 probiotic treatments ($p < 0.05$). However, it was not significantly different from the control treatment and P6 ($p > 0.05$). Regarding feed consumption, the overall treatment diet showed significant results, compared to the control treatment ($p < 0.05$), however, there were no significant differences among the probiotic treatments ($p > 0.05$).

Analysis of performance data, including egg weight and daily egg production, is often tested on laying chickens. The increase in egg weight followed by an increase in the level of treatment during the study could occur probably due to the high concentration of probiotic bacteria lactic acid in the Kumpai grass silage given, which led to optimal absorption of nutrients in the digestive tract. Furthermore, an increase in the value of daily egg production was also shown in treatment P8. This occurs presumably because of the close relationship between consumption value and the conversion of the treated diet. Consumption and feed conversion have an essential role in measuring livestock performance because the amount of consumption value can be used as a benchmark for determining nutrient intake obtained by livestock.

In contrast, the conversion was used as a benchmark to determine absorbed nutrients and was employed for livestock to meet their maintenance and production needs. Hajiaghapor et al. (2018) and Yu et al. (2020) reported that prebiotic or probiotic supplementation in the ration of laying hens could improve the health of the digestive system of these animals as evidenced by the high activity of lactic acid bacteria and an increase in the length and width of villi in the jejunum and ileum. In another study, Mikulski et al. (2020) reported that probiotics in rations with low and medium energy composition in laying poultry showing the probiotic supplementation on low-energy rations led to an increase in consumption value and a decrease in conversion value, thus affecting the performance.

Based on the result of this study, strong suspicions were set against lactic acid bacteria in the form of *lactobacillus plantarum* as the main factor causing the increase in Pegagan ducks' performance, which included egg weight, daily egg production, consumption, and feed conversion. Lactic acid bacteria is a type of bacteria that is widely used as a probiotic in livestock in general because of its ability to reduce or inhibit the growth of pathogenic bacteria, such as *Escherichia coli* in the digestive tract (Patterson and Burkholder, 2003; Khan and Naz, 2013; Al-Khalaifa et al., 2019). These results correlate with previous studies that show that giving probiotics isolated from Kumpai grass silage tends to affect carcass weight gain, which is thought to be due to increasing nutrient absorption efficiency (Sari et al., 2019).

According to Sandi et al. (2018), the types of lactic acid bacterial strains in the Kumpai grass silage are *Lactobacillus plantarum* strains. Qiao et al. (2019) showed that *Lactobacillus plantarum* has the potential as a feed supplement in the laying hen industry because it has a good influence at the genus level on intestinal development digestibility of laying hens. *Lactobacillus plantarum* can produce lactic acid, which contains bacteriocin bioactive compounds in the digestive tract and have antibacterial activity so that they can kill or inhibit the growth of pathogenic bacteria in the digestive tract (Choe et al., 2012; Ahmed et al., 2014; Bali et al., 2016). However, Sjofjan et al. (2020) reported that 0.8% *Lactobacillus plantarum* concentration did not show significant differences at concentrations of 0.2%, 0.4%, and 0.6% on egg weight but was significantly different from the control treatment.

Table 2. Effect of dietary treatments on performance of Pegagan Ducks

Variable	Treatment					SEM	p-value
	Con	P2	P4	P6	P8		
Egg weight (g)	56.96^{a}	59.09^{b}	62.32^{c}	64.30^{d}	68.36^{e}	0.279	< 0.05
Daily egg yield (g/hen/day)	56.96^{ab}	52.59^{a}	52.32^{a}	63.55^{b}	62.87^{b}	1,252	< 0.05
Feed consumption (g/hen/day)	367.51^{a}	385.68^{b}	400.06^{b}	399.03^{b}	394.79^{b}	2,274	< 0.05
Feed conversion ratio (g/g)	6.45^{a}	7.37^{ab}	7.78^{b}	6.28^{a}	6.35^{a}	0,159	< 0.05

Con: Diet without probiotics, P2: Diet + Probiotics 0.2%, P4: Diet + Probiotics 0.4%, P6: Diet + Probiotics 0.6%, P8: Diet + Probiotics 0.8%, SEM: Standart error means. [abc] Means in the same row without common letter are different at $p < 0.05$

Table 3. Effect of dietary treatments on the egg traits of Pegagan Ducks

Variable	Treatment					SEM	p-value
	Con	P2	P4	P6	P8		
Egg Shape Index (%)	77.09	80.20	78.03	77.46	79.85	0.545	0.30
Albumen Index (%)	0.063^{a}	0.088^{b}	0.090^{b}	0.085^{b}	0.098^{b}	0.002	< 0.05
Yolk Index (%)	0.338	0.370	0.393	0.420	0.408	0.018	0.64
Albumen Weight (%)	26.41^{a}	29.31^{b}	30.41^{bc}	30.48^{bc}	32.02^{c}	0.313	< 0.05
Yolk Weight (%)	21.31^{a}	21.53^{a}	23.81^{ab}	25.27^{b}	26.50^{b}	0.404	< 0.05
Eggshell Weight (g)	78.33	84.35	81.20	89.95	102.50	0.257	0.07
Eggshell Thickness (mm)	0.543	0.543	0.600	0.538	0.538	0.017	0.71
Haugh Units	61.31^{a}	71.36^{b}	73.56^{b}	73.88^{b}	73.75^{b}	0.906	< 0.05

Con: Diet without probiotics. P2: Diet + Probiotics 0.2%. P4: Diet + Probiotics 0.4%. P6: Diet + Probiotics 0.6%. P8: Diet + Probiotics 0.8%. SEM: Standart error means. [abc] Means in the same row without common letter are different at $p < 0.05$

Egg quality

The effect of probiotic-enriched feed on egg properties is given in Table 3. In particular, several variables show a significant effect, namely albumen index, albumen weight, yolk weight, and Haugh unit; the value of each observed variable has increased along with increasing probiotic treatment levels. The best results were found in treatment P8, namely providing a diet with 0.8% probiotics for each variable. The provision of probiotics did not affect these variables such as egg index, egg yolk index, shell weight, and thickness.

The high and low egg index, which includes the albumen index and the yolk index, is strongly influenced by the albumen and yolk weights. In this study, the observation of the albumen index showed that the probiotic treatment at each level was significantly different compared to the control. These results have a positive correlation with the increase in albumen weight in eggs treated with probiotics. However, different results were shown on the egg yolk index, which did not show a significant difference, although yolk weight showed an increase with increasing dose or level of probiotics in the feed. Furthermore, the increase in the observed variables carried out was thought to have a strong relationship with ducks' high-performance data shown in Table 2. Due to the high value of consumption and conversion of treatment rations, the high absorption of nutrients into the body of the livestock will affect the productivity of the eggs produced, including egg weight and egg quality parameters. Zhang et al. (2012) reported that probiotics in lactic acid bacteria could increase daily egg production, egg weight, and feed conversion value even though the resulting consumption values are not significantly different.

Furthermore, previous studies also revealed that probiotic supplementation had a significant effect on increasing egg production and egg quality (Zhang and Kim, 2013; Bidura et al., 2019; Mikulski et al., 2020). The egg index value, which is inversely proportional to the resulting yolk weight, is thought to be closely related to a decrease in fat and cholesterol content in eggs because of lactic acid probiotics (Li et al., 2011). However, Selim et al. (2020), in their report, stated that antioxidant compounds and bio-active compounds contained in feed could result in a high percentage of albumen and yolk weight in laying hens.

Table 3 shows that there is an increase in the Haugh unit value of eggs given probiotic treatment compared to control. Haugh unit value is generally used as an indicator of albumen in eggs. The high Haugh unit value is directly proportional to the increase in albumen weight. Besides, this increase strengthens the notion that developing lactic acid bacteria causes an increase in the digestive health system, resulting in increased nutrient absorption in the livestock body. Similar research results regarding the use of probiotics in livestock rations that affect Haugh units have been found in the last 10 years (Zhang and Kim, 2013; Bidura et al., 2019; Mikulski et al., 2020; Selim and Hussein, 2020).

CONCLUSION

Based on the current research results, it can be concluded that probiotics at the level of 0.8% produced from the Kumpai grass silage process can be used and contribute as a growth promoter for laying ducks to reduce using commercial antibiotic products. In further studies, it is recommended to test the combination of treatments with macro and micro minerals

DECLARATIONS

Competing interests

The authors declare no conflict of interest

Authors' contributions

All authors contributed to the design and implementation of the research, the analysis of the results, and the writing of the manuscript.

Ethical considerations

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.

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Subchronic Toxicity of Ivermectin and Butaphosphan in Layer Chickens

Evgenia Indyuhova[1*], Mikhail Arisov[1], Vladimir Maximov[2], and Tatiana Azarnova[2]

[1]*Federal State Budget Scientific Institution "Federal Scientific Centre VIEV", 28, Bolshaya Cheremushkinskaya st., Moscow, 117218, Russia*
[2]*Federal State Budgetary Educational Institution of Higher Education "Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after K.I. Skryabin", 23, Academician Scriabin st., Moscow, 109472, Russia*

*Corresponding author's Email: indyuhova@vniigis.ru; ORCID: 0000-0003-3294-6119

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ABSTRACT

The development of new veterinary drugs to treat and prevent poultry parasitic infections, as well as the study of their safety is a hot topic for modern parasitology. The purpose of this research was to study the subchronic toxicity of the ivermectin and butaphosphan-based drugs at a therapeutic and threefold therapeutic dose during a seven-day oral administration to the Hisex White chickens. The provisional name of the drug is Iverbutan. The chickens from the first experimental group were given the drug at a threefold therapeutic dose of 3 mL of the drug per one liter of drinking water. The chickens from the second experimental group were given the drug at a therapeutic dose of 1 mL of Iverbutan per one liter of drinking water. The chickens from the control group received water without the drug. The chickens were weighed, and then the body temperature and blood samples from the axillary vein were measured on days 1, 8, and 17 of the experiment before the morning feeding. On day 8 of the study, the chickens from the first experimental group showed a 7.4% decrease in body weight and increase in body temperature by 0.8%, an increase in alanine aminotransferase activity by 2.1 times, aspartate aminotransferase activity by 1.6 times, and bile acids by 1.4 times. Moreover, there was a 4.6% decrease in glucose concentration, a 3.5% increase in lactate dehydrogenase activity, a 7.3% decrease in triglycerides, as well as a decrease in hemoglobin by 3.2% and erythrocytes by 10.6% in the first experimental group, compared to the control group. On day 17 of the experiment, the above blood parameters in the chickens from the first experimental group did not significantly differ from the control group, indicating the reversibility of the hepatotoxic effect. In this regard, a three-fold therapeutic dose can be considered a threshold. The chickens from the second experimental group showed no changes in their physiological status as compared to the control. Thus, the study results confirm the safety of the drug in the recommended dosage regimen.

Keywords: Blood, Butaphosphan, Chickens, Ivermectin, Metabolism, Subchronic Toxicity

INTRODUCTION

The development of new combined veterinary drugs to treat and prevent poultry parasitic infections, as well as the study of their safety is a hot topic for modern parasitology. Combined and safe drugs introduced into veterinary practice minimize the number of injections of various drugs and the time spent by veterinarians for additional treatments (Arisov et al., 2020a). This is especially important in the industrial sector.

Various pharmacological class drugs affect physiological functions and physiological processes in the animal's body. The presented work has studied the tolerability of combined Iverbutan (the provisional name of the drug), which contains 0.4% ivermectin and 10% butaphosphan as active ingredients. Iverbutan is administered to broiler chickens, breeding poultry, replacement of chickens or poultry during the molt period. One of the means to control chicken parasites is broad-spectrum antiparasitic drugs including those based on ivermectin (Moreno et al., 2015; Mestorino et al., 2017). This compound is a semi-synthetic derivative of avermectins (Campbell and Benz, 1984).

Causative agents of parasitic diseases are known to cause pronounced permanent changes in the body of animals, particularly, metabolism, morphophysiology of blood, hormonal status, etc. Furthermore, parasitic diseases are accompanied by stress reactions that develop in the body of animals (Samadieh et al., 2017; Indyuhova et al., 2021a; Indyuhova et al., 2021b). Therefore, biostimulants are necessary to improve animals' physiological status in the course of treating parasitosis. Thus, butaphosphan is an organic source of phosphorus, which plays a major role in phosphorylation processes and gluconeogenesis processes in the liver. This organophosphorus compound is well tolerated (Fisinin et al., 2016) and does not accumulate in the body; it supports carbohydrate, energy, protein, and lipid metabolisms in the body, activates the immune system, and fastens recovery after pathologies of various origins (Rollin et al., 2010; Lima et al., 2017).

This work continues a series of studies to investigate the pharmaco-toxicological properties of new antiparasitic Iverbutan (Arisov et al., 2018; Indyuhova et al., 2021c). This work evaluates the study results of subchronic toxicity of the combined antiparasitic drug in poultry. It should be noted that these studies are necessary to assess a dose range, a route of drug delivery to the body, and its safety (Arisov et al., 2020b)

Based on the foregoing, the work aimed to study the subchronic toxicity of the drug based on ivermectin and butaphosphan in therapeutic and three-fold therapeutic oral dosages for seven days in layer chickens of Hisex White Cross.

MATERIALS AND METHODS

Ethical approval

The study of subchronic toxicity in chickens was approved at the meeting of the Scientific Council of the Federal Science Center (No. 2019/05/FSC VIEV; Russia). All manipulations were carried out in compliance with international requirements (Anonymous, 1986; Anonymous, 2010).

Feeding and housing conditions

The experimental part of the work was conducted at the Podolsk Base of the VNIIP-FSC VIEV (All-Russian Scientific Research Institute for Fundamental and Applied Parasitology of Animals and Plant, a branch of the Federal State Budget Scientific Institution “Federal Scientific Center VIEV”, Russia). To study the subchronic toxicity, 15 replacement layer chickens of Hisex White Cross were selected. The chickens were selected according to the principle of analogs taking into account age, body weight, feeding, and housing conditions. The average weight of the chickens was 190 g before the experiment. The chickens were 20 days old at the beginning of the experiment. The chickens were then divided into three equal groups (first experimental, second experimental, and control), five chickens each. The chickens were kept in groups, in double-deck cages (5 chickens each). The first and second experimental groups were kept on the upper tier. Each group was in a separate cage module. The chickens from the control group were kept on the lower tier. Overall dimensions of one cage module were 0.7 m^2 which contained 5 chickens. Each cage was equipped with an autonomous drinking system. The access to water was unlimited. The feeding and housing conditions corresponded to the zootechnical requirements.

One day old chickens were vaccinated with a polyvalent vaccine (Kursk Biofactory, Russia) against Marek's disease. The chickens were vaccinated with a live vaccine (ARRIAH, Russia) against Gumboro disease on day 10 and also were vaccinated with a live vaccine (Kursk Biofactory, Russia) against virulent Newcastle disease on day 15. Prior to the experiment, the chickens received no other veterinary medicinal products.

Experiment design

The dosage and the frequency of administration were selected to identify potential negative effects on the chickens when the drug is used for a long time or when it is overdosed (GPSMP, 2012).

According to the draft instruction, the veterinary drug Iverbutan is administered orally at a dose of 1 mL/L of drinking water. The prepared solution is given once to treat against nematodes of the poultry and three times in case of arachnoentomiasis (treat twice with a 24-hour interval and then once after 14 days). The tolerance was studied on the layer chickens of Hisex White Cross which were treated with the combined Iverbutan (the provisional name of the drug) containing 0.4% ivermectin and 10% butaphosphan. The chickens were given Iverbutan by a group method with drinking water for seven days. The research was carried out for 17 days. The chickens from the first experimental group were given the drug at a threefold therapeutic dose of 3 mL of the drug per one liter of drinking water. The chickens from the second experimental group were given the drug at a therapeutic dose of 1 mL of Iverbutan per one liter of drinking water. The chickens from the control group received water without the drug.

Iverbutan was diluted at ¼ of the daily intake of drinking water. To ensure that the chicken received the required dose of the drug, the water supply was stopped 2 hours before the solution was given. A new solution was prepared daily.

Sampling collection

During the experiment, the physiological status of the chickens was monitored in each group and their ethologic status was determined. Methods for determining the ethologic status in the chickens include follow-up with a recording of symptomatic motor activity, and assessment of chickens' responses to various stimuli (Maximov and Lysov, 2006). The chickens were weighed, and then the body temperature and blood samples from the axillary vein were measured on days 1, 8, and 17 of the experiment before the morning feeding.

Hematological and biochemical parameters

The following hematological parameters of chicken's blood are presented in the study: hemoglobin concentration, red blood cell count and white blood cell count, and white blood cell differential count (pseudoeosinophiles, basophiles, eosinophiles, monocytes, lymphocytes). The studied blood biochemical parameters in the chickens included aspartate aminotransferase, alanine aminotransferase, bile acids, creatinine, total protein, alkaline phosphatase, α-amylase, glucose, lactate dehydrogenase, triglycerides, lipase.

Hematological and biochemical parameters of the blood were determined according to the generally accepted method of Kondrakhin (2004) and Nebyltsova et al. (2011).

Erythrocytes and leukocytes were counted in a Goryaev chamber by a generally accepted method. Hemoglobin was determined by the colorimetric method using sodium lauryl sulfate. A visual microscopic assessment of Romanovsky-Giemsa-stained blood smears was carried out with differential leukocyte count.

A biochemical blood assay was performed on a Cobas 6000 analyzer (c 501 module, Roche Diagnostics GmbH, Germany); test systems were Roche Diagnostics (Switzerland). A colorimetric method was used to determine total protein, bile acids, and alkaline phosphatase. An enzymatic colorimetric method was used to determine triglycerides, alpha-amylase, and lipase. A kinetic method was used to determine alanine aminotransferase, aspartate aminotransferase, creatinine, and lactate dehydrogenase. A hexokinase method was used to determine glucose.

Statistical analysis

The obtained digital data were processed statistically using the Student's t-test. The Microsoft Excel 2016 software was utilized for the statistical analysis. The following calculations were carried out using it. To compare the average values of hematological and biochemical parameters, the arithmetic mean values with a quadratic deviation were first calculated for each group. Also in the software, the formula calculated a substantial difference t score, that is, a number showing how many times the difference between the arithmetic means is greater than the square root of the sum of mean squared errors. Further, the significance of the obtained results was manually assessed in the Student's table using the t score and the joint degree of freedom. The results were considered significant at $p \leq 0.05$ (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

RESULTS AND DISCUSSION

The chickens from the experimental groups actively moved in their cages for seven days, and their behavior did not differ from the controls. The assessment results of the chickens' weight during the experiment are summarized in Table 1.

The chickens from the first experimental group were found to have a significant decrease in body weight. Thus, the values of their body weight were 0.25 ± 0.004 kg versus 0.27 ± 0.001 kg ($p < 0.01$) in the control group after the drug administration on day 8 of the experiment. In the chickens from the second experimental group, a 2.9% increase in the body weight was recorded with Iverbutan in the therapeutic dose on the seventeenth day of the study compared with the control data. The chickens' temperature status is shown in Table 2.

Table 1. The body weight of the Hisex White cross chickens on days 1, 8, and 17 of the experiment at the VNIIP – FSC VIEV Podolsk Base, Russia

Group	Study period (Day)		
	Day 1 (kg)	Day 8 (kg)	Day 17 (kg)
First Experimental Group	0.19±0.001	0.25±0.004**	0.35±0.003
Second Experimental Group	0.19±0.001	0.27±0.002	0.36±0.002
Control Group	0.19±0.001	0.27±0.001	0.35±0.002

**: Means in a column differ significantly from the controls ($p < 0.01$)

Table 2. The temperature status of the Hisex White cross chickens on days 1, 8, and 17 of the experiment at the VNIIP – FSC VIEV Podolsk Base, Russia

Group	Study period (Day)		
	1 (℃)	8 (℃)	17 (℃)
First Experimental Group	41.52±0.73	41.76±0.38	41.48±0.38
Second Experimental Group	41.10±0.65	41.10±0.50	41.24±0.53
Control Group	41.46±0.52	41.44±0.59	41.26±0.55

The body temperature of the chickens from two experimental and one control groups corresponded to the physiological standard for this animal species. On the eighth day of the study, the chickens from the first experimental group showed a 0.8% increase in the body temperature (41.76 ± 0.38°C versus 41.44 ± 0.59°C control). This value was comparable with the upper threshold of the physiological standard for chickens. The stated above is obviously associated with hepatotoxicity (Altuntas et al., 2003) during the drug administration in an increased dose (Table 3). The chickens from the second experimental group showed no rising tendency in body temperature on the eighth day of the study compared to the control.

The biochemical and hematological parameters of the Hisex White chickens are summarized in tables 3 and 4.

Table 3. Biochemical parameters of the Hisex White cross chickens' blood on days 1, 8, and 17 of the experiment at the VNIIP - FSC VIEV Podolsk Base, Russia

Parameter	Control group	First experimental group	Second experimental group
Day 1			
Aspartate Aminotransferase (U/L)	258.20 ± 12.39	260.20 ± 29.52	243.60 ± 19.28
Alanine Aminotransferase (U/L)	11.20 ± 3.66	10.80 ± 5.07	11.40 ± 3.68
Bile Acids (μmol/L)	50.40 ± 4.69	49.60 ± 2.86	51.20 ± 5.15
Creatinine (μmol/L)	24.00 ± 3.16	26.00 ± 3.62	24.60 ± 4.78
Total Protein (g/L)	31.40 ± 2.08	31.60 ± 2.08	33.20 ± 3.33
Alkaline Phosphatase (U/L)	1030.0 ± 31.73	1071.4 ± 40.36	1070.2 ± 26.83
α-Amylase (U/L)	394.60 ± 81.05	450.80 ± 111.53	421.80 ± 129.03
Glucose (mmol/L)	12.80 ± 0.14	12.64 ± 0.14	12.84 ± 0.16
Lactate Dehydrogenase (U/L)	508.20 ± 7.68	518.20 ± 10.84	515.60 ± 11.27
Triglycerides (mmol/L)	4.68 ± 0.18	4.82 ± 0.22	4.98 ± 0.17
Lipase (U/L)	8.50 ± 0.32	8.68 ± 0.29	8.58 ± 0.32
Day 8			
Aspartate Aminotransferase (U/L)	271.60 ± 12.05	427.60 ± 54.73*	261.20 ± 30.77
Alanine Aminotransferase (U/L)	12.20 ± 1.84	25.80 ± 4.76*	11.60 ± 2.99
Bile Acids (μmol/L)	48.40 ± 4.17	67.00 ± 3.62*	49.20 ± 1.62
Creatinine (μmol/L)	25.00 ± 4.56	22.60 ± 2.57	24.60 ± 4.69
Total Protein (g/L)	31.40 ± 3.79	32.00 ± 1.96	31.80 ± 2.22
Alkaline Phosphatase (U/L)	1062.2 ± 41.24	1089.8 ± 41.04	1072.8 ± 26.12
α-Amylase (U/L)	441.40 ± 84.87	465.80 ± 118.81	484.60 ± 53.86
Glucose (mmol/L)	12.66 ± 0.16	12.08 ± 0.22	12.96 ± 0.12
Lactate Dehydrogenase (U/L)	526.0 ± 8.60	544.60 ± 5.09	519.60 ± 7.19
Triglycerides (mmol/L)	4.66 ± 0.18	4.32 ± 0.06	4.54 ± 0.13
Lipase (U/L)	8.58 ± 0.21	8.84 ± 0.16	8.64 ± 0.23
Day 17			
Aspartate Aminotransferase (U/L)	250.60 ± 37.80	259.80 ± 32.87	250.40 ± 33.30
Alanine Aminotransferase (U/L)	13.60 ± 2.57	12.00 ± 3.16	12.80 ± 3.87
Bile Acids (μmol/L)	50.60 ± 3.68	49.20 ± 3.09	49.40 ± 3.24
Creatinine (μmol/L)	24.20 ± 3.76	23.00 ± 4.89	27.00 ± 2.63
Total Protein (g/L)	30.20 ± 2.83	31.40 ± 3.35	32.20 ± 2.04
Alkaline Phosphatase (U/L)	1087.0 ± 40.28	1063.8 ± 40.57	1097.8 ± 28.36
α-Amylase (U/L)	410.40 ± 69.44	441.20 ± 97.07	465.60 ± 35.77
Glucose (mmol/L)	12.94 ± 0.16	12.84 ± 0.16	13.04 ± 0.17
Lactate Dehydrogenase (U/L)	517.20 ± 7.28	522.60 ± 7.70	524.60 ± 5.67
Triglycerides (mmol/L)	4.84 ± 0.17	4.96 ± 0.21	4.82 ± 0.20
Lipase (U/L)	8.56 ± 0.28	8.58 ± 0.24	8.66 ± 0.25

*: Means in a row differ significantly from the controls ($p < 0.05$)

Table 4. Hematological parameters of the Hisex White cross chickens on days 1, 8, and 17 of the experiment at the VNIIP - FSC VIEV Podolsk Base, Russia

Parameter	Control group	First experimental group	Second experimental group
Day 1			
Hemoglobin (g/L)	149.2 ± 1.93	148.8 ± 2.29	149.4 ± 2.25
Erythrocytes ($\times 10^{12}$/L)	3.04 ± 0.11	2.96 ± 0.11	3.0 ± 0.11
Leukocytes ($\times 10^{9}$/L)	21.08 ± 0.56	20.78 ± 0.58	21.28 ± 0.54
Pseudoeosinophiles (%)	27.8 ± 0.37	28.4 ± 0.24	28.2 ± 0.58
Basophiles (%)	2.2 ± 0.2	1.6 ± 0.24	1.8 ± 0.37
Eosinophiles (%)	6.0 ± 0.32	6.6 ± 0.24	6.6 ± 0.24
Monocytes (%)	4.6 ± 0.4	5.4 ± 0.51	5.6 ± 0.51
Lymphocytes (%)	59.4 ± 0.4	58.0 ± 0.63	57.8 ± 0.58
Day 8			
Hemoglobin (g/L)	146.0 ± 1.3	141.4 ± 1.44	150.4 ± 1.69
Erythrocytes ($\times 10^{12}$/L)	3.02 ± 0.07	2.7 ± 0.11	3.14 ± 0.09
Leukocytes ($\times 10^{9}$/L)	21.42 ± 0.35	20.40 ± 0.34	21.08 ± 0.37
Pseudoeosinophiles (%)	27.0 ± 0.55	28.6 ± 0.51	27.8 ± 0.37
Basophiles (%)	1.6 ± 0.24	1.8 ± 0.37	1.6 ± 0.24
Eosinophiles (%)	6.8 ± 0.37	6.8 ± 0.37	6.8 ± 0.37
Monocytes (%)	5.8 ± 0.58	5.6 ± 0.4	6.6 ± 0.51
Lymphocytes (%)	58.8 ± 0.58	57.2 ± 0.73	57.2 ± 1.2
Day 17			
Hemoglobin (g/L)	148.0 ± 1.67	148.8 ±2.35	149.8 ± 2.13
Erythrocytes ($\times 10^{12}$/L)	3.06 ± 0.13	3.1 ± 0.10	3.18 ± 0.10
Leukocytes ($\times 10^{9}$/L)	21.4 ± 0.29	21.56 ± 0.35	21.36 ± 0.39
Pseudoeosinophiles (%)	28.6 ± 0.4	28.8 ± 0.37	28.4 ± 0.51
Basophiles (%)	1.8 ± 0.2	2.0 ± 0.32	1.8 ± 0.37
Eosinophiles (%)	7.4 ± 0.24	7.2 ± 0.37	7.2 ± 0.37
Monocytes (%)	6.6 ± 0.51	6.4 ± 0.68	5.8 ± 0.58
Lymphocytes (%)	55.6 ± 0.93	55.6 ± 1.21	56.8 ± 1.07

The main mechanism of the cytotoxic action of various drugs is damage to cell plasmalemma and its cytoskeleton. The described process is accompanied by the release of cytosolic enzymes into the blood, namely, alanine aminotransferase and aspartate aminotransferase (Floyd et al., 2006; Yin et al., 2020). The liver function in chickens was assessed by a combination of biochemical parameters (Vinogradova et al., 1989; Altuntas et al., 2003; Varga et al., 2017). Thus, on the eighth day, the blood of the chickens from the first experimental group showed a significant increase in the activity of alanine aminotransferase by 2.1 ($p < 0.05$), aspartate aminotransferase by 1.6 ($p < 0.05$), and bile acids by 1.4 ($p < 0.05$) compared to the control. This work detected an increase predominantly in the alanine aminotransferase activity, a marker of hepatocyte cytolysis. (Donkova, 2003).

The AST to ALT ratio is known to decrease with toxic liver damage (Ushakova et al., 2021), which was also found in the chickens from the first experimental group on day 8 of the study. According to Donkova (2003), the stated ratio ranges in chickens from 20 up to 24 units. Thus, on day 8, the de Ritis ratio in chickens from the first experimental group was 16.6 units (22.3 units in the control). The chickens from the second experimental group had the de Ritis ratio value of 22.5 units (22.3 units in the control). The toxic liver damage developed in chickens was also observed in the pharmaco-toxicological studies during the administration of various drugs in high therapeutic doses (Niyogi and Bhowmik, 2003; Kamel et al., 2010).

The chickens from the first experimental group also showed a number of physiological and biochemical changes which indicate a liver function abnormality during the drug administration in the three-fold therapeutic dose. Thus, on the eighth day of the study, a 4.6% decrease in glucose and a 3.5% increase in lactate dehydrogenase activity were found in the chickens from the first experimental group compared to the control. It is evident that the described changes cause a decrease in carbohydrate metabolism and energy metabolism in the chickens from the first experimental group. This is possibly associated with abnormality in hepatic glycogen synthesis (Chudov and Ismagilova, 2012). The increased lactate dehydrogenase activity may indicate the increased proportion of oxygen-free glycolysis amid decreased hemoglobin by 3.2% and erythrocytes by 10.6% in the chickens from the first experimental group, which contributes to a potential decrease in oxygen delivery to their cells and tissues. These hematological parameters were within the reference constant values (Buyko et al., 2014). This may in part have influenced a decrease in carbohydrate and energy metabolisms in the study chickens. It should be noted that the erythrocyte system is impaired in hepatopathies of various origins (Sysueva, 2008). The stated above causes changes in red blood cells. Moreover, a 7.3% decrease in triglycerides in the chickens from the first experimental group was detected on the eighth day of the study, compared to the control. On the seventeenth day of the study, the above blood parameters in chickens from the first experimental group did not significantly differ statistically from the control group, which attests to the reversibility of hepatotoxic effects (Janakat and Al-Merie, 2002). At the same time, on the eighth day of the study, the chickens from the first experimental group showed no changes in hepatic protein synthesis, which may be due to the stable rate of protein metabolism with butaphosphan (Rollin et al., 2010), which is part of the drug.

The study by Arkhipov et al. (2014) demonstrated a significant increase in the alanine aminotransferase activity in the blood. These data were obtained in the study on the tolerability of an antiparasitic drug containing ivermectin for oral administration, which was given to broilers at doses of 1.2 and 2 mg/kg (by active ingredient). Moreover, they reported no significant changes in the values of the alanine aminotransferase activity in the chickens' blood after 10 days, compared to values of the control group. The stated above is consistent with the results presented in the current study on reversible hepatotoxic effect.

The tendency for an increase in the percentage of eosinophils in the chickens from the first experimental group was observed. Thus, the percentage of their eosinophils ranged from 6.6 ± 0.24% to 7.2 ± 0.37% during the experiment. At this time, a decrease was determined in the percentage of lymphocytes from 58.0 ± 0.63% to 55.6 ± 1.21%. On this basis, it can be assumed that there is a tendency for possible initiation of allergic processes and immunosuppression (Wakenell, 2010). However, all hematological parameters in the chickens from the first experimental group were within the reference values.

The chickens from the second experimental group showed an increase in the α-amylase activity by 9.8 and 13.5% on the eighth and seventeenth days of the study, respectively, compared with the control. On the eighth day of the study, a 2.4% increase in glucose was found in the blood of the chickens from the second experimental group related to the control. Obviously, the chickens from the second experimental group showed a rising tendency in carbohydrate and energy metabolisms during the administration of the combined drug in the therapeutic dose. On day 17 of the study, a 6.6% increase should be noted in total protein in chickens from the second experimental group related to the control. At the same time, they showed an increase in hemoglobin by 3.0 and 1.2%, as well as erythrocytes by 4.0 and 3.9% on the eighth and seventeenth days of the study, respectively, compared to the control values. Biochemical and hematological parameters of the chickens (Tables 3 and 4) show that the drug in the therapeutic dose does not have a negative effect on the physiological state of the chickens. All blood parameters were within the reference values (Buyko et al., 2014). Furthermore, the increased body weight of the chickens on the seventeenth day of the study confirms the previously stated.

CONCLUSION

Iverbutan administered for seven days in the therapeutic dose and three-fold therapeutic dose did not cause any change in the ethologic status of the Hisex White chickens. There were no negative changes in the physiological or biochemical status of the chickens after the drug was orally administered in the therapeutic dose for seven days, compared to the control chickens. The three-fold therapeutic dose can be considered as a threshold due to the rendered reversible hepatotoxic effect. Thus, the obtained study results confirm the safety of the drug in the recommended dosage. The obtained data on studying the Iverbutan tolerability on target animals (chicken breeds for producing eggs) allow us to study its therapeutic efficacy in the industrial sector, which requires further research in this field.

DECLARATIONS

Authors' contribution

Evgenia Indyuhova developed the study design, conducted research work, collected and analyzed the data, and prepared the Article. Mikhail Arisov participated in the development of the study design and analyzed the study results. Vladimir Maximov analyzed the obtained data. Tatiana Azarnova analyzed and interpreted the obtained data.

Consent to publish

All authors agree to publish this manuscript.

Competing interests

The authors have declared that no competing interest exists.

Ethical considerations

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.

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The Effect of Kepok Banana (*Musa Paradisiaca* L.) on Immunoglobulin, Vitamins, and Cholesterol Content of Eggs in Laying Hens

Jein Rinny Leke[1*], Erwin Wantasen[1], Ratna Siahaan[2], Florencia Sompie[1], Charles Kaunang[1], Tuti Widjastuti[3], and Muhammad Halim Natsir[4]

[1]*Faculty of Animal Husbandary, University of Sam Ratulangi, Manado, North Sulawesi, Indonesia*
[2]*Faculty of Mathematics and Natural Sciences, University of Sam Ratulangi, Manado, North Sulawesi, Indonesia*
[3]*Faculty of Animal Husbandry, University of Padjadjaran, West Java, Indonesia*
[4]*Faculty of Animal Husbandry, University of Brawijaya, Malang, Middle Java, Indonesia*

*Corresponding author's E-mail: rinileke@unsrat.ac.id; ORCID: 0000-0002-7093-3872

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ABSTRACT

Eggs contain all the proteins, lipids, vitamins, minerals, and growth factors necessary for the development of the embryo. Egg white and yolk proteins are considered functional food substances since they have biological activities, such as metal-chelating, antimicrobial, anticancer, antioxidant, and immunomodulatory activities. The current study aimed to determine the effect of banana kepok on the production levels of immunoglobulin Y (IgY), vitamins, and cholesterol of eggs in laying hens. A total of 200 laying hens (medium brown 402) were used at 80 weeks of age with 5 treatments and 5 replications and each entailed 8 chickens. The treatment groups included the use of kepok banana flour (KBF) as R0 (no KBF), R1 (95 Basal feed + 5% KBF), R2 (90 basal feed + 10% KBF), R3 (85 basal feed + 15% KBF), and R4 (80 basal feed + 20% KBF). A total of 50 eggs were used in egg yolk sampling. The investigated variables were egg IgY, vitamins (A, B1, B6, D2, D3), and cholesterol content. The results of the study indicated that the administration of kepok bananas at different levels could provide a significant difference in IgY, vitamins (A, B1, D2, D3), and cholesterol of eggs. However, it did not significantly affect Vitamin B6. The study concluded that KBF can positively affect IgY and vitamins in eggs. Moreover, it could decrease the cholesterol in eggs.

Keywords: Egg cholesterol, Egg IgY, Egg vitamins, Kepok banana flour

INTRODUCTION

Egg production is an integral part of the livestock industry and has increased by 119% (35.5 vs 76.8 million tons) from 2018 to 2019 (FAO, 2020). Global egg production is going to increase by 24% from 7.8 tons in 2020 to 9.7 tons in 2050 (Macelline et al., 2021). Eggs are one of the primary nutrient sources comprising of protein, vitamins A, D, E, and B12, and amino acids. Chicken meat and eggs are rich sources of nutrients, including proteins, vitamins meaning that it includes all nutrients (Leke et al., 2019). Eggs are also a source of macro and micronutrients that meet all the requirements to support food needs.

Complete and balanced nutrition as well as high digestibility and affordable price are contributing factors to eggs being a staple of human beings (Réhault-Godbert et al., 2019). Eggs are one of the most significant antibody producers with low production costs. Immunoglobulin Y (IgY) is an important therapeutic source of resistance to antibodies against several viral diseases for which there is no treatment (Pereira et al., 2019). Egg quality is closely related to the feed and age of laying hens (Leke et al., 2019). Passive antibody production is common in chicken egg yolk.

In laying hens, IgG in the blood is efficiently transferred across the follicular epithelium for storage in the yolk during oogenesis. The IgG is the dominant class of Ig transferred to the yolk called IgY (Pereira et al., 2019). Egg yolk is a relevant alternative source of antibodies. The main immunoglobulin in poultry blood is hereditary and accumulates in the egg yolk, allowing large amounts of antibodies to be uptake. Immunoglobulins are formed in the blood due to the transfer of antigens into the yolk and are called IgY, which has two heavy (H) and two light (L) chains.

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Vitamins are essential nutrients in specific foods and are very important for health because a lack of vitamins can cause disease. Vitamin A deficiency can cause blindness, especially in children, and miscarriage in women. Folate deficiency causes an increase in plasma homocysteine concentrations associated with plasma homocysteine concentrations and an increase in cardiovascular disease (Zang et al., 2011). Antioxidants are widely used for poultry production, especially in the form of vitamins (Poungpong et al., 2019; Sharma et al., 2020).

Low cholesterol and high levels of vitamins and IgY in egg production can be achieved by formulating feeds with a high level of beta-carotene and antioxidants. One source of beta-carotene and antioxidants is banana kepok (*Musa paradisiaca*), which is a type of processed banana. It also contains pyridoxine compounds or Vitamin B6 and can be used as an alternative to the main ingredient due to high levels of carbohydrates and vitamins. Bananas are rich in B6 (pyridoxine) content, which acts as a coenzyme for metabolism reactions as well as a facilitator of protein synthesis and metabolism. Several studies have confirmed that kepok bananas in the form of juice effectively reduce LDL cholesterol, total cholesterol, and blood serum triglycerides, and increase blood serum HDL cholesterol. The performance of laying hens and egg quality is influenced by genetics, environmental factors, and nutrition. Feed ingredients are required to contain Vitamin D3 and Vitamin A as a source of beta-carotene as well as minerals, calcium, phosphorus, sodium, and chlorine (Çalişlar, 2020). Beta-carotene source effectively increases egg yolk, eggshell, laying hens immunity, and the chicken endocrine system. Production can be improved in case the nutritional quality of feed ingredients is high (Ilona et al., 2020).

The purpose of the study was to determine the effect of laying hens' diet supplemented by pyridoxine compounds from kepok banana on IgY, vitamins, and cholesterol of eggs.

MATERIALS AND METHODS

Materials

Prydoxine compound was obtained from dried kepok bananas. The dried kepok bananas were processed from fresh banana kepok ingredients obtained from Bunaken Island, Tongkaina Village, North Sulawesi, Indonesia. Fresh kepok bananas were cut into small pieces and dried in the sun for 3-4 days. Proximate analysis of kepok bananas was carried out in the PAU laboratory of the Gadjah Mada University Faculty of Animal Husbandry, Indonesia. The results of the analysis indicated that dry matter content, ash, crude protein, crude fat, crude fiber were 89.51%, 23.80%, 4.15%, 3.77%, 5.11%, respectively. The metabolism energy was estimated as 3407.54 MJ/Kg.

Treatment diet

The kepok banana flour (KBF) was used as R0 (no KBF), R1 (95 Basal feed + 5% KBF), R2 (90 basal feed + 10% KBF), R3 (85 basal feed + 15% KBF), and R4 (80 basal feed + 20% KBF). The diets were mixed with 41% corn, 15% rice bran flour, 2% Ca CO3, and 42% layer concentrate. The diets were given for 8 weeks, including 100 g of feed per day. Drinking water was given *ad libitum* and the chickens were fed in the morning (8 am) and afternoon (2 pm). The environmental factors included a 110 cm × 35 cm × 56 cm cage size at the temperature of 32-35°C and a humidity of 60-70%. The Newcastle disease vaccine was given at 3 days of age and repeated at 2 weeks of age. The chemical composition of the feed is given in Table 1.

Table 1. Nutritional content of treatment diet

Nutritional content / Diets	R0	R1	R2	R3	R4
Crude protein (%)	17.94	17.71	17.48	17.24	17.01
Crude Fat (%)	7.91	7.90	7.89	7.89	7.88
Crude Fiber (%)	5.64	5.80	5.95	6.11	6.26
Calcium	1.88	1.89	1.91	1.93	1.94
Phosphorus	1.04	1.03	1.02	1.01	1.01
Metabolism energy (Kcal/kg)	2844	2825.55	2807.10	2788.65	2770.20
Vitamin A (mg/100 g)*	69.69	118.93	104.71	98.43	87.39
Vitamin C (mg/100 g)*	1.2	1.85	1.73	1.38	1.71
Vitamin B6 pyridoxine (mg/100 g)*	0.47	0.37	0.40	0.41	0.43
Beta-carotene (ppm)	0.36	0.48	0.44	0.41	0.41

R0: No kepok banana flour (KBF), R1: 95 Basal feed + 5% KBF, R2: 90 basal feed + 10% KBF, R3: 85 basal feed + 15% KBF, R4: 80 basal feed + 20% KBF

A total of 200 medium brown 402 were used in the current experiment. The research design used 5 treatments and 5 replications and each replicate consisted of 8 laying hens. A total of 50 eggs were sampled for examination of IgY, vitamins, and cholesterol in eggs.

Determination of immunoglobulin concentration in egg yolk

A total of 50 eggs were analyzed for IgY content at the Integrated Laboratory, Department of Nutrition Science and Feed Technology, Bogor Agricultural University, Indonesia. The ELISA method was used to isolate IgY in eggs and determine the concentration of IgY in egg yolk (Selvaraj and Cherian, 2004). As much as 150-200 mg of egg yolks were diluted 1:6 (v/v) with acidified deionized water (pH 2.5), vortexed well, and stored at 4°C. After overnight cooling, the samples were centrifuged at 10.62 g at 4°C for 15 minutes and the supernatant was collected and the egg IgY content was measured by the Elisa method.

Egg vitamin content analysis

Egg yolk vitamin analysis determined the vitamins in egg yolk using the high-performance liquid chromatography method (Staffas and Nyman, 2003). Five samples were used for the analysis in the current study (each treatment was used as a composite of four replications). The analytical procedure was as follows, the sample weighed 0.5 g, then put into a flask and 30 ml of 95% ethanol was added. The flask was shaken for having an even mixture, then heated for 30 minutes at 80°C using a water bath and reverse cooling. After the completion of heating, the condenser was rinsed with 20 ml of water. The sample was extracted with diethyl ether. The sample was then filtered using folded filter paper to remove the remaining water present. The solvent extract of the vitamin was evaporated using a rotary evaporator. The residue was dissolved with ethanol and then injected into the HPLC apparatus. The results of egg yolk vitamins were analyzed descriptively with the analysis of vitamins A, B1, B6, D2, D3 in the Integrated Laboratory of the Department of Nutrition Science and Feed Technology, Bogor Agricultural University, Indonesia.

Egg cholesterol analysis

Measurement of egg cholesterol was based on the Liebarmann Burchard method (Kenny, 1952). Egg samples obtained were analyzed at week 6 of the study. Each sample treatment had 2 replications so 50 samples were obtained. The cholesterol content of egg yolks was determined using the Liberman Burchard method using a spectrophotometer at a wavelength of 530 µm.

Statistical analysis

A completely randomized design was applied in this experiment. Data were analyzed by Analysis of variance (ANOVA) using the Minitab 16 software program. If there was a significant difference ($p \leq 0.05$), then Duncan's test was further used with multiple distance tests.

RESULTS AND DISCUSSION

Egg immunoglobulin

The average egg immunoglobulin was 19.24-23.26 mg/100 g/egg (Tabel 2). The kepok banana flour (KBF) led to a significant effect on increasing egg immunoglobulin ($p < 0.05$). The highest effect was in R4 treatment followed by R3, R1, and R2 treatments. These results were related to the previous research indicating that egg IgY level produced by chickens with active virus antigens was 10-100 mg IgY (Rollier et al., 2000). The IgY antibodies in an egg were 10-20 mg/ml egg yolk (Carlander, 2002). Chicken egg yolk can be used for the production of IgY in large quantities. The reason is that chickens have a high sensitivity to foreign antigen exposure and their immune system is also very responsive so that it persists to produce IgY (Zang et al., 2011). The high level of IgY in the blood is not always the same as the level of IgY in the egg yolk because the transfer of IgY into the yolk is known to occur in 2 stages. Each stage takes a certain amount of time. In the early stages, IgY is transferred from the serum to the yolk in a process analogous to the transfer of antibody (IgY) in the fetus across the placenta in mammals. The next stage is the transfer of antibodies (IgY) from the embryo sac to the developing embryo in chicken eggs (Kim et al., 2004; Senggagau and Bond, 2020). A previous study indicated that IgY level with pyridoxine supplementation treatment in laying hens via drinking water was 2.15 g/100 ml, it was 2.13 g/100 ml for a treatment mixed with feed ingredients was, and 2.18 g/100 ml for treatment by intravenous injection (Silitonga et al., 2013).

These results showed that different pyridoxine supplementation methods (via drinking water, mixing in rations, or intravenous injections) do not significantly affect the obtained IgY levels. Pyridoxine compounds contain vitamins that are used as a defense in the body of laying hens. This vitamin plays a role in forming the body's defense system against invading microorganisms (Senggagau and Bond, 2020).

From various research findings, it is known that pyridoxine deficiency conditions in humans and various animal species lead to abnormalities in the body's defense system, such as fewer antibody-producing cells, fewer lymphocytes, and decreased immune system function, compared to normal conditions (Kumar and Axelrod, 1968; Debes and Kirksey, 1979). Efforts to increase the production of IgY in egg yolk have been made and it was demonstrated that oral/feeding pyridoxine supplementation at a dose of 3.0 mg/kg ration could result in the production of IgY of 106.1 mg/egg. This means that it has undergone an increase of about 6%, compared to the IgY content of another study (Li et al, 1998). Table 2 tabulates the results of the levels of egg IgY, vitamins, and cholesterol.

The effect of giving kepok bananas up to 20% in laying hens diets on IgY was significant ($p < 0.05$). This showed that adding 20% KBF could increase egg IgY because the nutrient content in the diet is protein, fat, and vitamins. The nutritional content of the feed affects the yolk and as a result the IgY of the egg. Setiani (2016) reported that the IgY concentrations of 7.89, 10.07, and 26.31 mg/ml in layer eggs, native chicken eggs, and duck eggs, respectively. Nutrition treatment feed contains protein, vitamins, essential amino acids that affect egg yolks. The quality of the vitelline membrane and egg yolk is influenced by diet protein. The IgY is transferred from maternal blood to egg yolk via oocyte cytoplasmic membrane receptors from development to maturity (Nimmerjahn and Ravetch, 2007).

Table 2. Egg immunoglobulin, vitamins, and cholesterol contents of laying hens in Indonesia

Nutritional content \ Diets	R0	R1	R2	R3	R4	SEM	P Value
Immunoglobulin (IgY; mg/100 g)	19.24 ± 0.09^{d}	20.28 ± 0.47^{c}	20.20 ± 0.1^{c}	22.38 ± 0.26^{b}	23.26 ± 0.13^{a}	0.113	0.00
Egg Vitamin A (µg/100g)	0.47 ± 0.04^{c}	0.54 ± 0.05^{b}	0.63 ± 0.01^{a}	0.61 ± 0.01^{a}	0.62 ± 0.01^{a}	0.014	0.00
Egg Vitamin B1 (µg/100g)	123.8 ± 4.76^{c}	138.6 ± 6.87^{b}	149.8 ± 1.64^{a}	132.6 ± 9.28^{bc}	150.8 ± 1.10^{a}	2.53	0.00
Egg Vitamin B6 Pyridoxine (µg/100g)	295.2 ± 4.81	294 ± 12.25	299 ± 7.44	296 ± 4.47	302.2 ± 4.91	3.306	0.347
Egg Vitamin D2(µg/100g)	4.88 ± 0.09^{c}	5.38 ± 0.61^{bc}	5.82 ± 0.52^{ab}	5.92 ± 0.16^{ab}	6.06 ± 0.21^{a}	0.170	0.00
Egg Vitamin D3(µg/100g)	2.88 ± 0.19^{b}	2.98 ± 0.24^{ab}	3.38 ± 0.22^{ab}	3.40 ± 0.51^{ac}	3.56 ± 0.47^{a}	0.158	0.027
Cholesterol (mg/100g)	199.52 ± 0.43^{a}	193.04 ± 5.53^{ab}	188.42 ± 6.93^{b}	188.78 ± 0.57^{b}	188.90 ± 0.41^{b}	1.783	0.001

R0: No kepok banana flour, R1: 95 basal feed /bf+ 5% KBF, R2: 90 bf + 10% KBF, R3: 85 bf + 15% KBF, R4: 80 bf + 20% KBF; Significant differences amongst the treatments were indicated by different superscript letters in a row ($p < 0.05$)

Egg vitamin

The average yields of egg vitamins A, B1, B6, D2, D3 were 0.47-0.63,123.8-150.8, 294.0-302.2, 4.88-6.06, 2.88-3.56 µg/100g, respectively. In another study, the contents of egg vitamins were reported as vitamin A precursor or beta-carotene of 88 g/100g, Vitamin B1 (Thiamin) of 17688 g/100g, Vitamin B2 (Riboflavin) of 528 g/100g, Vitamin B6 350 of g/100g, and Vitamin D of 5.4 g/100g (Réhault-Godbert et al., 2019).

As can be seen in Table 2, supplementation of chickens' diet by KBF led to significant differences ($p < 0.05$) in the levels of vitamins A, B1, D2, and D3, compared to the control. The results showed that the addition of 20% KBF could increase egg vitamins. The average contents of vitamins A, B1, B6, D2, and D3 in eggs were highest at R4. This difference is possible because the vitamins used by reproduction organs require the same amount of vitamins so that the vitamin deposition in egg yolks is different. Vitamins A and D are fat-soluble vitamins, which are stored in the liver or adipose tissue in poultry. These vitamins are excreted in the bile and then feces.

According to the results, the kepok banana contains vitamins and beta-carotene, which can increase egg vitamins. The beta-carotene content is one of the major sources of vitamins in the feed. Eggs contain almost all vitamins except vitamin C. The vitamins are grouped into fat-soluble vitamins (A, D, E, and K) and water-soluble vitamins (thiamin, riboflavin, pantothenic acid, niacin, folic acid, and Vitamin B12, Çalişlar, 2020).

The Vitamin A content of egg yolks is influenced by beta-carotene consumption (Suci et al., 2020). Consumption of beta-carotene was higher in the KBF diets than in the control diets (Table 2). Beta-carotene is a carotenoid that can act as pro-vitamin A which will be converted into vitamin A in the intestinal mucosa and absorbed in the form of vitamin A. Beta-carotene consumed is catabolized through an oxidation reaction by the enzyme beta-carotene dioxygenase which produces Vitamin A. The higher beta-carotene in the ration increases the levels of Vitamin A in eggs (Wiradimadja et al., 2010).

Egg yolk is a food that is rich in most vitamins (Réhault-Godbert et al., 2019). Chickens transfer vitamin D from feed to egg yolks so that they are able to produce high levels of 25-hydroxy vitamin D3 (Macelline et al., 2021).

Egg cholesterol

The average egg cholesterol of chickens fed with KBF is 188.42-193.04 mg/100 g The papaya leaf flour treatment ration to medium brown 402 laying hens on egg yolk reduced egg cholesterol level to 118-212 mg/100g (Leke et al., 2019). The treatment of KBF had a highly significant effect on egg cholesterol ($p < 0.05$). The results showed that the higher the level of KBF, the lower the egg cholesterol. This is due to the fiber content in the kepok banana. The small intestine of hens can not digest the fiber. One of the properties of dietary fiber is that it can bind cholesterol from food and is excreted with feces so that the absorbed cholesterol is reduced. Kepok bananas contain resistant starch, inulin, calcium, phenolic compounds, beta-carotene, vitamins A, B1, B2, B3, B6, C, and minerals. The increase in beta-carotene and crude fiber content due to the addition of KBF levels affects a decrease in egg cholesterol. Bananas that can reduce the concentration of cholesterol, free fatty acids, and triglycerides in serum and tissue are found in flavonoid and phenol compounds (Rusdaina and Syauqy, 2015). The cholesterol related to the hydroxymethyl glutaryl-CoA (HMG) enzyme in cholesterol can be reduced by Beta-carotene (Bidura et al., 2017). This enzyme has an important role in the formation of mevalonic in cholesterol biosynthesis. The cholesterol and beta-carotene synthesis are derived from acetyl CoA. According to the obtained results of the current study, supplementation of diet with up to 20% KBF can lower egg cholesterol.

CONCLUSION

In conclusion, the obtained results of the current study indicated that supplementation of poultry diet with kepok banana flour at different levels (5%, 10%, 15%, and 20%) can enhance immunity and vitamin (A, B1, D2, D3) contents. It can also decrease the level of cholesterol in eggs. Therefore, kepok banana flour can be used in the laying chickens' feed although the obtained results should be also confirmed by future studies.

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Effects of Group Sizing on Behavior, Welfare, and Productivity of Poultry

Ali Kiani

Justus Liebig University, Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management, Department of Animal Breeding and Genetics, Giessen, Germany

Corresponding author's E-mail: alikianee@gmail.com; ORCID: 0000-0001-7271-3446

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ABSTRACT

The excessive intensive production of poultry meat and egg caused significant changes in poultry husbandry, behavior, and welfare. Therefore, animal welfare and behavior have become an important issue in poultry production and arises the necessity to reconsider all husbandry practices including group size and density. This review aims to investigate the association of group size with growth performance, detrimental behaviors, and welfare by reviewing current norms and regulations, as well as scientific literature in industrial poultry farms, including chicken, turkey, and quail. It has been found that group size can affect production performance, especially growth rates, feed efficiency, and number of competitors, which can lead to damaging behavior and consequently injuries in poultry. Due to the intensification of the poultry production systems, many natural behaviors of domesticated poultry, including food search strategies, hierarchy formation, and aggressiveness, are changed or modified, compared to their ancestors. Therefore, challenging behaviors in commercialized conditions and large groups of poultry must be investigated. The current recommendations and regulations of the industry for commercial poultry on group size and space requirements differ from scientifically investigated trials. On the other hand, available scientific research about the impact of flock size on poultry welfare, behavior, and production, has been carried out in experimental settings with flock sizes that are varied considerably from those used in the commercial settings. In conclusion, results from studies on optimum group size have indicated some degree of confounding and interactions between enclosure size and density. Furthermore, the social and physical environment can have a significant impact on a variety of welfare-related aspects and behavioral indicators. It is important to note that this evaluation focused on studies conducted in experimental settings, making it difficult to extrapolate the findings to commercial settings where thousands of birds are reared at once.

Keywords: Chicken, Group size, Quail, Turkey, Welfare

INTRODUCTION

Poultry has been considered the most important and most efficient alternative to red meat for sustainable food supply with regard to population growth and high demands of animal protein, notably meat and eggs (Speedy, 2003; Bonnet et al., 2020). This approach has resulted in more intense poultry farming than in any other area of animal agriculture, and consequently significant changes in poultry husbandry and some serious behavioral and welfare changes (Wolfson, 1996). The poultry industry has been adopting density and group size as a common procedure to change the welfare, behavior, and production performance of birds in high-density and commercial conditions (Estevez et al., 2003). Turkeys and quails, alongside chickens, have been progressively used as industrial poultry in recent years. The welfare of turkeys, like that of other poultry, can be influenced by various husbandry management approaches.

While implementing beak trimming or low light intensities as a frequent method to prevent cannibalism generated concerns about animal welfare, additional research on other husbandry strategies, such as the appropriate group size, is needed. There is limited research on the effects of group size and density on welfare indicators in turkeys, and also the available results are inconsistent (Martrenchar, 1999).

Small body size and simple handling are benefits of quail for use in genetic studies and embryological investigations, as well as hardiness, high laying turnover, rapid generation turnover, and being an oviparous species (Tsudzuki, 1994). However, quails are used frequently for

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industrial and scientific applications since their products are generally believed to be healthier, which heightens the need to investigate the welfare issues in Japanese quail (Minvielle, 2007). Although the breeding programs of this commercial poultry are similar in several aspects, physiological variations, such as moving ability, thermal comfort zones, and body size as well as different breeding objectives (egg or meat) and local priorities in the prevalence of poultry derived products consumption have resulted in various husbandry strategies.

Several studies have shown that group size influences blood parameters, mating system, social dynamics, feather pecking, laying rate, and other factors in chickens (Campo and Davila, 2002; Estevez et al., 2007; Bovera et al., 2014). As a result, changing group size can have a considerable impact on the major welfare indicators in poultry, such as hip joint lesions, metabolic and skeletal disorders, and painful leg disorders, including tibial dyschondroplasia, angular bone deformity, and contact dermatitis, such as hock burns, breast burns, and foot pad dermatitis (Vits et al., 2005).

In the wild, poultry creates social groups of various sizes; for example, multiple females are linked to one male in chickens, turkeys form either mixed-sex groups or same-sex, and ostriches form mixed-sex groups. To commercialize poultry production, selective breeding programs have been used to increase the physiological efficiency of organisms in commercial strains, such as the digestive system, respiratory system, skeletal system, and nutritional needs (Rauw et al., 1998). Several husbandry and farming strategies, including density, feeding systems, and ventilation, were altered as a result of these changes. Therefore, it appears that other parameters in commercial rearings, such as group size, must be reconsidered and justified in the same way.

Furthermore, as there are no possibilities for hens to engage in natural behavior, conventional cages have been banned in Sweden since 1999 (SFS, Swedish Ministry of Agriculture, 1988), and furnished cages (including, cages furnished with litter, perches, and nests) have been substituted for conventional cages (Wall and Tauson, 2007).

In addition, in recent years, poultry husbandry strategies for laying hens have experienced considerable adjustments in Germany, resulting in major overall improvements. This is mainly due to the fact that in Germany, poultry housing in traditional battery cages has been prohibited since January 1, 2010, and only organic production systems, small-group housing systems, or barn and free-range systems, have been authorized since then (federal ministry of food and agriculture, Germany). Furthermore, the German animal welfare law states that no more than 6,000 laying hens may be housed together without being separated spatially (Council Regulation 834/2007 for animal production; KAT, 2013).

In recent years, the number of animal product labeling programs based on welfare assessment standards has expanded, in addition to increasing regulations. For example, the European Commission's Directorate-General for Health and Consumers (2010) determined that a reconsideration of overall risk assessment techniques is required to guarantee continuing consumer, animal welfare, and environmental protection (Egeberg, 2010).

Even though group size has been recognized as one of the key determinants affecting poultry welfare (Wechsler and Schmid, 1998; Estevez et al., 2003; Buchwalder and Huber-Eicher, 2005), particular group size recommendations for commercial poultry production vary greatly among certifying programs and industrial guidelines. Furthermore, since group size is frequently linked to behavioral parameters and welfare (Estevez et al., 2007), a better understanding of the elements that influence or are influenced by group size can improve individuals' understanding of behavioral parameters and welfare issues in poultry. This research aimed to examine the scientific literature on the impact of group size on poultry welfare, behavior, and production, with a focus on chicken, turkey, and quail. Understanding the importance and effects of group size can assist the optimization of guidelines for commercially housed poultry flock size.

Chickens

Domesticated poultry species show different forms of social structures (Mench and Keeling, 2001). While jungle fowl live in small and stable groups, the typical pairings are several females with one male, with other males alone or in small groups (Collias and Collias, 1996). In domesticated poultry species, each group has a designated foraging and roosting space and establishes different social groups with their own home ranges (Wood-Gush et al., 1978). Small farmyard flocks face similar conditions, and their social behavior is likely to be similar to that of wild birds, however, commercial poultry has undergone significant modifications as a result of domestication and subsequent breeding efforts. Domesticated animals converted to less frightened and fearful animals, and their food-searching techniques changed. Hierarchy decreased aggression when multiple birds were reared in the same house, and birds with higher egg production rates were selected for breeding purposes.

As a result, there is a stronger demand for food and energy, as well as more time to look for new food sources in domesticated poultry (Eklund and Jensen, 2011). Schütz et al. (2001) found that there was a decreasing tendency in the incidence of energy-demanding behaviors, such as exploratory behaviors and foraging, as well as social contact in selected leghorn hens for high production. These researchers indicated that breeding programs based on genetic selection for production traits could lead to a decrease in social behaviors. In addition, Pagel and Dawkins (1997) found that hens in small groups can pay high costs of establishing dominance relationships, while cost of establishing dominance increases as group size decreases.

Furthermore, it has been demonstrated that the formation of a hierarchy breaks down at greater group sizes mainly because group members do not benefit from using recognition by all group mates (Pagel and Dawkins, 1997; Pizzari and McDonald, 2019). Commercial chickens can largely be split into two main categories of broilers and laying hens.

Table 1. Summary of published literature examining group size effects on laying hen's welfare and productivity

Reference	Age (week)	Experimental design	Evaluated parameters	Group size	Density	Results
Lindberg and Nicol (1996) Experiment 1	-	T-maze preference tests	Preference for being in a larger or a smaller group of familiar flockmates	5, 120	Constant for both groups	When space was constant and large, a strong preference for the smaller group in a large space emerged.
Experiment 2	-	5 different group size/space options were tested using a T-maze	Pecking behaviors in different group sizes	4, 70		There were preferences for a larger group (70 over 4 or 0 hens), a larger space (9 m^2 over 1 m^2), and 4 hens rather than an empty space.
Hughes (1977) Experiment 1	1-18	Battery brooder subdivided into groups of 50 at 2 weeks, 25 at 6 weeks, and 12 at 10 weeks of age	Selection of cages containing different numbers of birds	1, 2, 3, 4, 5	-	Cages became progressively less attractive as the number of birds in them increased.
Experiment 2	-	Large battery was divided into 8 cages with a central runway	Selection of empty cage versus occupied cage	1, 2, 3, 4, 5	-	Hens reared singly chose empty cages rather than cages occupied by one other unfamiliar bird, whereas group reared hens selected the occupied cages.
Hughes and Wood-Gush (1977)	1-72	Factorial design with two housing methods (battery cage and deep-litter pen)	Aggressive head pecking, threats, pecks, and pulls	3, 6	0.76, 0.81 m^2/bird	Aggressive head pecking occurred more often in groups of six than in groups of three
Abrahamsson and Tauson (1997)	1-79	Two housing systems were used, a modified furnished cage in two blocks and a conventional battery cage in one block	performance, health, and space usage	5, 6, 7, 8	600 cm^2/bird	The rolling out efficiency from nests was best in the larger group sizes but hens in the larger group sizes had the dirtiest feet
Bilčík et al. (1998)	1-40	Randomized block design	Tonic immobility	15, 30, 60, 120	5 m^2/bird	Duration of tonic immobility increased with group size
Nicol et al. (1999)	14 -30	Four identical percheries, each perchery was treated as one independent unit	Production performance, feather pecking, and aggression	72, 168, 264, 368	6, 14, 22, 30 m^2/bird	Aggressive pecking was most common in the smaller flocks at the lowest stocking densities.
Bilčík and Keeling (2000)	1-37	Randomized block design	Feather pecking and ground pecking	15, 30, 60, 120	5 m^2/bird	Higher rate of feather pecking in the largest group size
Campo and Davila (2002)		Different mating ratios with two group sizes	Blood indicators of fearfulness and stress	12, 60, 120, 240		The heterophil to lymphocyte ratio was significantly higher when the group size was 60 birds than when it was 12 birds
Estevez et al. (2003)	3-18	4 groups (12 focal birds per pen were used for tests)	Aggressive behaviors	5, 30, 60, 120	5 m^2/bird	Linear reduction in the frequency of pecks and threats given per focal bird with increasing group size but the frequency of pecks and threats received per focal bird was higher in larger than smaller groups
D'Eath and Keeling (2003)		Two large pens and four small pens	Social discrimination and aggression	10, 120	6.67 m^2/bird	Hens in small groups discriminated between familiar and unfamiliar subjects by more aggression towards

						unfamiliar hens. In large groups, the overall level of aggression towards subjects was reduced in that attempted fights were rare
Vits et al. (2005)		Three different furnished cage systems with different group sizes	Production, performance, Bone and egg parameters	10, 20, 40, 60	Constant for all groups	The highest egg production was found in the groups of 20 hens in the Aviplus system and the highest proportion of dirty eggs was found in the groups of 10 hens in the Eurovent 625A system.
Fahey and Cheng (2008)	17-60	2 genetic strains of White Leghorn hens were used in response to group size and density	Blood samples, body weight, adrenal weight, and hematological parameters	4, 6	542, 434 cm^2/bird	The genetic basis of variations in immunity may correlate with the line-unique ability of birds to cope in social environments and their survivability
Guo et al. (2012)	1-36	Three housing systems: a standard battery cage system two furnished systems	Performance, Nesting, perching, and walking behavior and blood parameters	4, 21, 48	398, 543, and 586 cm^2/bird	The furnished cage systems with small group sizes were favorable for hen welfare without markedly affecting performance
Bovera et al. (2014)	20 -36	2 groups	Performance and egg quality	25, 40	749 cm^2/bird	Hens raised from a group of 40 hens had a lower percentage of egg production and higher feed conversion ratio than a group of 25 hens
Marin et al. (2014)	1-44	Randomly assigned to 45 pens provided with nests and perching	Production performance, first egg laid, and morphometric measures of the eggs	10, 20, 40	8 m^2/bird	Groups of 40 individuals showed a reduction in BW gain and weekly hen-day-egg production after 30% phenotypic appearance changes
Mohammed and Rehan (2018)	50	360 birds (180 Lohmann brown and 180 Lohmann selected leghorn) in 6 cages (60 layers/cage "5 m^2"), 198 birds (99 Lohmann brown and 99 Lohmann selected leghorn) (33 layers/cage "2.8 m^2")	Immunological indicators and welfare status in two strains of Lohmann layers	33, 60	same floor space relatively	In large group sizes, the scores of plumage condition were referred to the best, especially in Lohmann brown. In large group feet condition in Lohmann brown was better than Lohmann selected leghorn

Broiler chickens

Since reducing group size has a large economic and husbandry impact on broiler farms, it is critical to figure out the association between group size and welfare as precisely as possible. When a more precise assessment of the interplay between group size and welfare characteristics is available, decisions on what group size is appropriate from the standpoint of animal welfare can be made. However, unlike the association between group size and profitability, identifying the relationship between group size and welfare can be a complex issue. Studies on commercial and rural breeds, on the other hand, have led to distinct findings addressing the effect of group sizing behavior on welfare and productivity in various environments (Parveen et al., 2017; Sohsuebngarm et al., 2019). However, the assessment of rural poultry populations and the optimization of breeding goals have received insufficient attention (FAO, 2011).

Some researchers have investigated the effects of stocking density and group size on behavior and welfare indicators in broilers (Leone et al., 2007; Leone et al., 2010; Kiani and von Borstel, 2019), and found that detrimental behaviors, such as cannibalism, feather pecking, fear, aggression, stress, and behavioral disturbances are all affected. On the other hand, studies that evaluated the effects of group size, density, and enclosure size show some discrepancies (Christman and Leone, 2007). Reiter and Bessei (2000) used a two-factorial design to separate the effects of group size and density, as well as interactions between the two. They used four different group sizes (10, 20, 40, and 60 birds) and three different stocking densities (5, 10, and 20 birds/m or 9, 18, and 36 kg/m^2 floor area) to measure performance and behavioral parameters for a 5-week rearing period. This study showed that increasing group size caused a significant increase in feeding activity in the second week of the rearing period, and scratching activity increased significantly in the fifth week. Reiter and Bessei (2000) indicated that feeding activity at the fifth week was highest when broiler chickens were kept in a group size of 20 birds. Also, there was a short-time periodicity of activity and resting with a cycle length of 20 minutes, however, this rhythm was not observed in large group sizes with high stocking density. Reiter and Bessei (2000) concluded that in the tested area of their experiment, both group size and stocking density had only a minor impact on the birds' performance and behavior. They found that litter conditions, ambient temperature, and social stimulation, rather than physical space restriction, explained the effects of both parameters on scratching and wandering behaviors. Reiter and Bessei (2000) also concluded that a lack of behavioral synchronization among group members could lead to the elimination of short-term activity and resting rhythms. Preliminary research showed that there are different types of hysterical or nervous behavior experienced by chickens of different ages, caused by different factors involved, for example, by disturbance during operations, such as feeding, or spontaneously with no observable stimulus (Hansen, 1976). Moreover, several studies investigated associations among age, husbandry management practices (such as light period and litter quality), animal welfare indicators (such as foot pad dermatitis and lameness), and behaviors (such as fear, scratching, and wandering) in broiler chickens (Bassler et al., 2013; Riber et al., 2018, Phibbs et al., 2021). However, to the best of the researcher’s knowledge, systematic research into different nutritional or environmental treatments in relation to group size in broiler production is very limited and would be valuable for making clear recommendations to the industry.

While several researchers have revealed the impact of group size on broiler production, contradictory outcomes have been reported. Some of these outcomes may have been influenced by differences in experimental study design (controlled versus on-farm, modifying group size by changing pen size or density), while others may have been influenced by using different welfare metrics which makes it difficult to compare the results of various investigations. As a result, no precise range of group size that influences welfare has yet been found. Some researchers have demonstrated that describing a relevant factor for broiler welfare as a whole is easier to assess when key components of the multidimensional concept of welfare respond simultaneously to the same factor, such as response to variable group size or density (Buijs et al., 2009; Kiani and von Borstel, 2019). For example, Buijs et al. (2009), used physiological (leg health and postmortem measurements) and behavioral indicators (corticosteroid metabolites and tonic immobility) to assess the welfare of four replicates of broiler chickens kept at 8, 19, 29, 40, 45, 51, 61, and 72 broilers per pen (or 6, 15, 23, 33, 35, 41, 47, and 56 kg achieved BW/m^2). The 72 broiler chicken group exhibited a longer tonic immobility duration than the 8, 19, 29, 45, and 51 broiler chickens and they tended to deviate from the 61 broiler chicken group. There was also a substantial difference in latency to lie between the 8 broiler chicken group and all groups ≥ 40 broiler chickens per pen. A shortcoming of the strategy by Buijs et al. (2009) was that a high score on one indicator could cover a low score on another indication. The effects of different group sizes include small (100 broiler chickens, 10 m^2), medium (300 broiler chickens, 30 m^2), large (1000 broiler chickens, 100 m^2), and very large (5000 broiler chickens, 500 m^2) with a constant density (10 broiler chickens/m^2) on leg disorders and plumage cleanliness in broiler chickens were explored in another study by Kiani and von Borstel (2019). Gait scores, plumage cleanliness, and hock burn were found to predict improved welfare in small groups in this study. According to Kiani and von Borstel (2019), the general assumption that large group sizes have negative impacts should be reconsidered, especially for new commercial broilers with commercially relevant group sizes.

Perching is considered a highly natural and driven habit for chickens and undisturbed napping is critical to the chickens’ welfare. In laying hens, a link between group size and welfare indicators, such as perching behaviors has been established (Abrahamsson and Tauson, 1997; Wall and Tauson, 2007), however, few researchers have investigated this possibility in broilers. Martrenchar et al. (2000) evaluated broiler perching behavior between two groups of broiler chickens (1020 versus 4590 broiler chickens, 17 broiler chickens/m^2 with no replicates). These researchers demonstrated that the perching behavior of broilers in the large group size during weeks 5 and 6 was slightly lower, compared to the small group size, (6.8% versus 7.9% respectively in week 6). Since the difference in the absolute value of incidence of perching birds between the 1020 and 4590 group sizes was 1.1% at week 6, these researchers concluded that group size has no significant effect.

Several studies have been reported on the effects of group size, density, as well as the dimensions and shape of the pens (Christman and Leone, 2007; Leone et al., 2010; Kiani and von Borstel, 2019). In these investigations, some researchers combined the effects of group sizes with other parameters to study interaction effects, in addition to diverse experimental settings, such as age, breed, and husbandry conditions (Christman and Leone, 2007; Leone et al., 2010). As a result, it may be difficult to separate and divide the outcomes of each effective component, particularly group size. Some researchers have attempted to adopt a different experimental design that allows them to manipulate one component at a time and control important effects by several contrasts to separate and compare the main effects of experimental treatments (Newberry and Hall, 1990; Leone et al., 2010). For example, Leone et al. (2010), included several factors, such as group size, density, and enclosure size in their study and hypothesized that these factors could have a distinct effect on movement and space usage in broiler chickens. They built square enclosures with three group sizes of 10, 20, and 30 broiler chickens as small (S., 1.5m^2), medium (M., 3.0m^2), and large (L., 4.5m^2). When the group size was kept constant (10S, 10M, 10L), they found no variations in movement activity among enclosures of different sizes, however when the density was kept constant (10S, 20M, 30L) and comparisons were made across consistent enclosure sizes, differences between enclosure sizes were significant (10M, 20M and 10L, 30L). It could beconcluded from studies that group size, density, and enclosure size have different effects on space usage and movement of broilers. Furthermore, it has been demonstrated that broiler chickens in small pens use less space than broiler chickens in large pens. This limitation is likely due to the fact that broiler chickens could move a shorter distance before hitting an end wall and moving back to areas where they had already spent time (Newberry and Hall, 1990). As a result, changing group size, which is linked to modifying pen size, can vary the space use and movement of chickens.

Sohsuebngarm et al. (2019) evaluated the fluctuations of microclimate variables (relative humidity, ambient temperature, heat index, air velocity, effective temperature, and ammonia) over the length of commercial broiler houses and found that the microclimate variables had different trends. Specifically, regardless of the social hierarchy structure of the group, it is impossible to determine the size of the group. Several elements, including sex, breeds, husbandry practices, and environmental conditions, have been confirmed to influence hierarchy formation (Siegel and Hurst, 1962; Hocking, 1993). In addition, several studies have shown that age has an impact on hierarchy formation in poultry flocks (Newberry and Hall, 1990; Hocking, 1993; Anderson et al., 2004). In another study, Newberry and Hall (1990) have studied the impact of pen size and age on space used by male broiler chickens. The broilers were divided into two groups of large pens (407 m^2) with 3040 broiler chickens and small pens (203.5 m^2) with 1520 broiler chickens. At hourly intervals, the positions of 18 marked chickens in a large group and 10 marked chickens in each of two small groups were recorded. The results of their investigation revealed that broilers in small groups consumed less space, compared to broilers in large groups over 6 weeks. They hypothesized that broiler chickens in small groups move within a shorter distance before colliding with an end wall and being reflected back to regions where they had previously spent time. Newberry and Hall (1990) also mentioned that chicken's tendency for staying close to the walls is the reason for a larger proportion of the available pen area, which is not used by broilers. According to these researchers, the distance moved by male broilers at a commercial stocking density can be altered by group size and age. Newberry and Hall (1990) showed that chickens in the large pens spent more time near their home brooder, compared to chickens in the smaller pens.

Broiler slaughter age has been reduced as a result of selection for production qualities, which has also influenced broiler behavior (Schutz and Jensen, 2001). Several studies have found that with increasing age in broiler chickens, they restrict their mobility due to social pressure (McBride and Foenander, 1962; Craig et al., 1969; Craig and Bhagwat, 1974). It has been shown that pecking and threatening behavior in broilers fed *ad libitum* remained extremely low between 4 and 9 weeks of age (Mench, 1988). The availability of food, the movements of the chickens, and strategies to escape predators during the night have all been found to influence the movements of young domestic chickens living in the wild (McBride et al., 1969; Wood-Gush et al., 1978). Similarly, it has been shown that with increasing age, walking time and distance moved per hour decrease, which is usually linked with increased difficulty in walking leading to a decrease in home range (Newberry et al., 1986). Yang et al. (2020) concluded that broiler activity index at different ages, at the feeder and open area generally decreased from week one to week seven. In domestic fowl, developing and maintaining social bonds in groups with more than 100 groupmates is not possible (Guhl, 1953). As a result, for broiler chickens reared in large flocks with several thousand birds, confronting strangers during normal activities within the pen during the mating time is unavoidable. Adrenal hypertrophy occurs when broilers are exposed to stranger flock mates which increases the chance of aggressive behavior and has a negative impact on broiler welfare (Siegel and Siegel, 1961). On the other hand, some studies indicated that in commercial poultry farming, large group chickens would be restricted to narrow regions, allowing chickens to become acquainted with other birds in the area and prevent confrontations with outsiders (McBride and Foenander, 1962).

Laying hens

Currently, there are three main types of housing systems for laying hen, namely standard cages, furnished cages, and barn systems with and without outdoor access (Philippe et al., 2020). Corresponding to the growth of laying hen husbandry and egg production in the 1960s and the use of cage batteries, animal welfare organizations, scientists, and political activists began criticizing this method of animal farming in Europe, which eventually expanded to Northern America. The ability for hens to engage in species-specific behaviors, such as foraging, dustbathing, perching, and building or selecting a suitable nest varies depending on the housing system. If hens cannot accomplish such high-priority behaviors, they may experience substantial frustration, deprivation, or damage, which consequently deteriorate their welfare condition (Molnár and Szőllősi, 2020). On the other hand, there is relatively limited information about how hens react to varied numbers of cage-mates, and these responses are not evaluated in alternative housing systems with different group sizes.

Many behavioral interactions within a group are linked to reproduction traits, which has an impact on group size. In reproduction behaviors that are based on increased available energy profits at reproduction season, two main parameters are involved: first, increased available energy for aggressive behaviors in the population during the reproductive season, and second, a range of behavior including production and care of young appears in the breeding season. In poultry, social interaction among group members during reproduction activities has not been extensively explored (Brown and Brown, 1981; Brown, 1982). Those wild birds that defend territories, but do not breed in them during the non-breeding season, do not defend their territories and mate in groups of more than two birds (Davies and Houston, 1981; Faaborg and Arendt, 1984). Vocalization behavior, as a behavioral interaction within a group in chickens, is also considered a welfare indicator (Manteuffel et al., 2004). Distress vocalization can be found in chickens when they are exposed to a conflict or lose group interaction to call for help (Andrew, 1964). It also raises the question of whether the birds are aware when their groupmates are removed from the herd in a commercial environment (Jones and Harvey, 1987).

A preliminary study on the effects of group size on laying hen's welfare suggested that hens should not be housed either separately or in groups of four or more, but that their welfare would be best served in groups of two or three (Brambell, 1965). However, these recommendations were modified based on additional studies and sufficient data to support the association between welfare and group size, which was primarily based on two indicators of egg production and mortality. Guo et al. (2012) investigated the way group size and stocking density affected the welfare and production performance of chickens housed in furnished cage systems during the summer. Three different housing systems were used, namely a standard battery cage system (control, 4 hens/cage, 398 cm^2 /hen) and two furnished systems (including nest and perches), one with small group size (21 hens/cage; 586 cm^2/hen) and one with large group size (48 hens/cage; 543 cm^2/hen). The furnished cage with small group size hens showed a higher rate of egg breakage in comparison to the control group. In addition, hens reared in the furnished cage with a small group size cage had a lower rectal temperature, compared to the control group. Guo et al. (2012) concluded that using furnished cage systems with small group sizes (about 20 hens) was more effective in maintaining thermal balance during the summer. The findings imply that furnished cage systems with small group sizes are more desirable for hen welfare while having no negative impact on performance. In another study, Vits et al. (2005) assessed the classification of furnished cages under practical situations. There were three different furnished cage systems in their experiment (Aviplus, Eurovent 625a, and Eurovent 625A), each with four tiers of double-decker cages. In the Aviplus and Eurovent 625A systems, hens were kept in groups of 10 and 20 per cage, respectively, and in groups of 40 and 60 per cage in the Eurovent 625a system. These researchers have found that the size of a group inside a housing system had a significant impact on all production traits and Haugh units (the measure of albumen quality used by the poultry industry). The Aviplus system had the highest egg production per average hen housed (89.4%), however, the proportion of cracked eggs was greater (0.7%) in groups of 60 hens, compared to other group sizes. The Aviplus system groups of 10 hens had the strongest humerus bones (198.2 N), whereas the Eurovent 625A system's groups of 20 hens had the strongest tibias (146.7 N). More cracked eggs in bigger groups (60 hens) in their research may be due to more eggs in the nest box and/or on the conveyor belt at the same time.

Injurious pecking is a major issue in the production of laying hens, and it is particularly difficult to control in large group furnished cages and non-cage systems (Singh and Groves, 2020). Appropriate housing and management, as well as genetic selection, can help the alleviation of this problem. With increasing group size, it is more likely for laying hens to be disturbed by other group mates and increase aggressive behavior in cage mates. Although feather pecking is more common in caged layers than in pens with deep litter (Hughes and Duncan, 1972), it has been demonstrated that feather pecking and aggressive pecking are two distinct behavior patterns (Hughes, 1973; Blokhuis and Arkes, 1984).

Considering recent regulations on poultry welfare, minimizing feather pecking and cannibalistic pecking by beak trimming is a questionable issue because some pieces of evidence indicated that painful trimmingscan increase sensitivity that persists for at least a few weeks or a month (Kaukonen and Valros, 2019). Selection for behavioral traits in poultry breeding programs is considered as an alternative for modifying aggressive behaviors in poultry. It was indicated that when cannibalism among intact-beak hens is a significant problem for a genetic stock, selection of hens based on family averages improves both survival and hen-housed egg production when sisters are housed together but separately from hens of other families (Craig and Muir, 1996).

Furthermore, reports on the effective determinants of group sizing behavior, welfare, and production differ across commercial and rural broiler breeds. For example, Parveen et al. (2017) compared the growth performance of Desi and Fayoumi (Pakistani rural poultry breeds) and Rhode Island Red breeds (commercial poultry breeds) under local environmental conditions and found that Rhode Island Red breeds outperformed rural chicken breeds.

Savory et al. (1999) have studied the influence of certain environmental and dietary parameters on the development of feather pecking damage in groups of 10 to 20 growing bantams in multi-unit brooders up to 6 weeks of age. They used two group sizes (10 and 20 hens) and three stocking densities (744, 372, and 186 cm^2/hen) in their study. Results of their study showed that the mean pecking damage score was considerably higher in the larger group (20 hens) and maximum density (186 cm^2 floor space per hen), compared to the smaller group/density. According to Savory et al. (1999), the number of birds in large groups, as in alternative layer housing systems, may be less essential than stocking density. Bilčík and Keeling (2000) conducted another experiment with four different group sizes of 15, 30, 60, and 120 hens at four different ages to determine the rate of feather pecks and aggressive pecks, both given and received. The findings of this investigation revealed that groups with 120 hens differed from groups of 15 hens and 60 hens in terms of severe pecks. The group of 120 hens differed significantly from groups 15, 30, and 60 hens in terms of soft feather pecks received. Groups of 120 hens were significantly different from the other groups in terms of the number of severe feather pecks they received. They concluded that increasing group size provides grounds for increasing the frequency of aggressive pecks.

Large group size can impair laying hen performance, including feed intake, feed efficiency, and laying rate, in addition to behavior. In current poultry production systems, more freedom of movement and behavioral options may increase the occurrence of undesired behaviors, negatively impacting animal health, welfare, and production performance of laying hens (Sossidou and Elson, 2009). Marin et al. (2014) investigated whether variations or changes in the phenotype of Hy-line Brown laying hens can change egg production body and weight; The hens were divided into groups of 10, 20, or 40 (8 hens/m^2). They altered the phenotypic appearance of hens to maintain constant proportions of hens throughout the various group size treatments in their study; therefore, in a small group, 30% consisted of 3 whereas the 30% in groups of 20 and 40 consisted of 6 and 12 hens, respectively. At the end of the first phase of the study, there were no impacts of initial phenotype or group size on first egg laid, cumulative 25% egg production, or cumulative 50% egg production, and no effects on cumulative hen-day egg production (34 weeks of age). These findings imply that early life factors influenced the adaptation capacity of layers. When certain social conditions (group sizes and phenotypic appearance combinations) were imposed from a very early breeding period and age, egg production was not affected.

By definition, tonic immobility is a state of motor inhibition and reduced response to external stimuli caused by a brief duration of physical restriction (Gallup, 1977; Jones, 1990). Some studies have considered tonic immobility as a criterion for assessing fearfulness. For example, Bilčík and Keeling (2000) used tonic immobility to assess the influence of group size on fearfulness in laying hens kept in floor pens in groups of 15, 30, 60, and 120. When the hens were evaluated in their home pens, they discovered that group size had a significant impact on tonic immobility duration. The findings of this study disprove the theory that smaller groups of hens are more scared than larger ones due to a theoretically higher chance of predation.

Because of scaling effects, the results of trials conducted on small flocks cannot always be applied to commercial flocks where birds are kept in flocks of thousands. For instance, aggressive behaviors, such as feather pecking are more common in small groups, compared to large flocks because birds can adapt to avoid harmful social interactions (Hughes et al., 1997; Nicol et al., 1999). Zimmerman et al. (2006) investigated the behavior of laying hens under commercial stocking densities (low: 7 hens/m^2, medium: 9 hens/m^2, high: 12 hens/m^2), flock sizes (small: 2450/3150 birds, large: 4200 birds), and management settings (standard and modified). They discovered that the connection between flock size and age affects feather pecking and aggression levels. Nicol et al. (2006) also assessed the physiological and physical responses of chickens in non-cage commercial setups and showed that welfare indicators were not affected by flock size. A summary of published studies on laying hens is provided in Table1.

The general assumption is that a larger group size is associated with a higher prevalence of disease (Nunn et al., 2015). Otte et al (2021) carried out a partial budget analysis of the breakeven cost of biosecurity investments for free-range poultry flocks of 1-20 and 21-50 birds. In their study, average parameter values (initial and final inventory, number of birds lost to disease, and number of eggs produced) and prices of the flock size groups of 1-20 and 21-50 birds were used for the analysis. These researchers indicated breakeven costs of biosecurity measures above which their cost would be higher than the returns. However, Otte et al (2021) reported an average loss (deaths/initial flock plus entries) of 22% for small group size (1-20 birds), compared to 13% of birds in large group size (21-50 birds).

On the other hand, social network analysis (SNA), also known as network analysis or contact analysis, has recently gained popularity in the field of animal behavior concerning group size and infectious disease to evaluate animal social networks and to compare social networks within and between groups. It has been indicated that poultry, the contact structure is heterogeneous, however instead of being tied to social systems, contacts are typically dependent on group size, spatial structure, and animal movements, which are commonly controlled by husbandry management systems (Craft, 2015).

All management practices, including group size and density, can contribute to the health of the flocks and the transmission of infectious disease, and the efficiency of reproduction organs in layers (Edwards and Hemsworth, 2021). Since complex environmental factors are usually associated with difficulties in cleaning and persistency of parasites and infectious diseases, the disease agents can spread in a larger group size easily (Lay et al., 2011). Therefore, there is a need for more studies on the association of different infectious and respiratory diseases, such as infectious bronchitis (IB) or egg drop syndrome, caused by a viral infection in laying hens, and group size in layers.

Turkey

Turkeys, like chickens, can form different group sizes in the wild and live in small mixed-sex groups during the non-breeding season. Separating males from females and all-female or all-male flocks are more common in commercial conditions (Schorger, 1966; Brant, 2007). Male and female flocks are divided due to varying growth rates and dietary requirements. Commercial turkeys are sometimes found in groups similar to wild turkeys, particularly during the breeding season, with several females and one male, but male sibling groups are more typically kept together in mixed-sex production (Appleby et al., 2004).

Fast-growing turkey broiler strains are typically housed in vast buildings that can hold 1000-25000 birds at stocking rates of up to 60 kg/m^2 (FAWC, 2009), or around 3 adult males/m^2. Turkeys raised for commercial purposes have a high level of aggression. Some researchers believe that this intensive activity is caused by exogenous variables such as genetic disposition or endogenous factors such as housing, management, and food (Sherwin and Kelland, 1998; Hafez, 1999). Increased aggressive behavior in turkeys could be due to domestication-related causes because wild and commercial fattening breeds have different fighting behavior patterns (Healy, 1992).

A limited number of studies have been conducted on the effect of flock size on welfare or behavioral indicators in commercial turkeys, and available studies do not reflect the flock sizes in a commercial situation. There are no simple methods to separate the influence of group size and density for turkey flocks or laying hens. Different housing systems, climates, and husbandry practices make it difficult to draw conclusions from the few published studies on the effect of group size, however, some studies address the main subject more directly than others, which are intermixed with density effect. For example, Buchwalder and Huber-Eicher (2005) conducted a study to evaluate how adding individual birds into small or large test groups of turkey toms affected the incidence of hostile encounters. These researchers expected that in small groups, the reaction to an introduced turkey tom would be more hostile than in large groups. These researchers used six groups of six animals (small groups) and six groups of 30 animals (large groups) to count and time hostile behavior such as pecks, fights, and leaps between locals and introduced or reintroduced turkey toms. Their findings revealed that turkeys in small groups were more hostile with the imported species than turkeys in large groups. In comparison to the large group (6×13 m), the small group (2×3 m) received more pecks toward newly introduced unknown toms. Some other researchers have reported similar behaviors when unfamiliar conspecifics are introduced to wild turkey flocks in order to drive them out (Watts and Stokes, 1971; Williams, 1981). According to Buchwalder and Huber-Eicher (2005), turkeys in large groups are barely able to distinguish between resident group members and introduced birds, but domesticated and wild turkeys can distinguish between group members and non-group members, and non-group members would exhibit aggressive behaviors significantly more than group members, at least in groups of four.

Several studies have assessed the welfare of turkey broilers housed at high stocking densities, particularly during the final fattening period when the body weight of turkey broilers per space is high (Coleman and Leighton, 1969; Zuidhof et al., 1993; Martrenchar et al., 1999). There was a discrepancy between group size and density in some studies because experimental pen size was not changed across treatments. For example, Martrenchar et al. (1999) provided floor space of 24 dm^2, 18.5 dm^2, and 15 dm^2 until week 12 and 40 dm^2, 31 dm^2, and 25 dm^2 from week 12 for the males and 16 dm^2, 12.3 dm^2, and 10 dm^2 for the females, but because the size of the pens was the same in all experimental treatments, treatments differed in both stocking density and group size. As a result, it is impossible to determine the relative effect of each variable. These researchers stated that this experimental design was chosen intentionally in their experiment because, despite new regulations regarding stocking density, farmers are unlikely to change the size of their houses and pens; instead, they prefer to house fewer birds while simultaneously changing stocking density and group size.

Sherwin and Kelland (1998) examined the frequency of comfort behaviors and the incidence of injurious pecking for varied group sizes and stocking densities when male turkeys were housed as pairs in pens. These researchers, in contrast to Buchwalder and Huber-Eicher (2005), found that the degree of injuries and the frequency of fighting were reduced in small groups, implying that small group size and/or low stocking density may mitigate or lessen the effects of harmful pecking in turkeys.

Although observations of relatively lower aggression in large groups of domestic fowl, some researchers support the hypotheses that aggressive behaviors decrease with increasing group size (Carmichael et al., 1999; Nicol et al., 1999; Estevez et al., 2002). Estevez et al. (2003) hypothesized that domestic fowl in small groups create a dominance hierarchy through violent interactions, whereas domestic fowl in large groups adopt a low-aggression (tolerant) social strategy. These researchers tested aggressive interactions among group members as group size increased using groups of 15, 30, 60, and 120 female White Leghorn hens housed in a constant density. Estevez et al. (2003) indicated that while the majority of birds in larger groups can adopt a tolerant strategy, a minority may exhibit more aggressive behaviors and be despotic, directing aggression indiscriminately toward other members of the group. A dominating member of a group's optimal group size may differ from that of new or junior members. Many facets of conduct in social interactions can be influenced by dominance. When food resources are scarce or improperly distributed within a group, a dominant has easier access to food and will often take food discovered by subordinates (Baker et al., 1981; Rohwer and Ewald, 1981, Lindenwald et al., 2021). As a result, the effects of resource depletion may be less for a dominant than for a subordinate (Rohwer and Ewald, 1981; Brown, 1982; Lacher et al., 1982). Different social interactions among group members, such as competing for resources and space, might have an impact on new member acceptance (Brown, 1982).

It is not straightforward to classify acceptable group sizes for commercial conditions. Most behavioral tests and observations are conducted on much smaller groups than possible group sizes under commercial situations. Large pens are divided into smaller ones in behavioral experiments, and each little pen is treated as an independent replicate. Because of technical constraints, most studies only have a few replications (typically less than six), making it challenging to extrapolate the outcomes of these tests to commercial flocks (Denbow et al., 1984; Cunningham, 1992; Classen et al., 1994).

Quails

In comparison to chicken and turkey farming, quail farming is a relatively new addition to industrialized poultry production. During the nonbreeding season, the Northern Bobwhite, *Colinus virginianus*, forms social groups (coveys) of around a dozen birds of various ages and sexes (Johnsgard and Jones, 1988). Northern bobwhites congregate in small groups of no more than 30 quails, with an average group size of 12 individuals (Wing, 1941). These coveys change in the spring as males and females team up for breeding, and new coveys arise.

The rate of migration affects social interaction in Japanese quail, and the composition of groups varies as a result of migration. Overall, group size and social organization in the wild are influenced by a variety of factors, including the current availability of resources in the habitat and the risk of predation, and can fluctuate as these conditions change (Wilson and Bermant, 1972).

In a breeding cage of quails, stocking density and group size are effective environmental characteristics that can alter performance parameters and welfare indicators (Seker et al., 2009; El Sabry et al., 2022). Seker et al. (2009) have studied the effects of group sizes of 3 and 10 quails with a constant density of 125 cm^2/quails on the performance of Japanese quails. The results of this study revealed that with a constant density (125 cm^2/quails), a group size of 10 may yield better results in terms of live weight, feed intake, and feed conversion ratio than a group size of 3 quails/cage. Waheda et al. (1999) reared 90 Japanese quails from 50 to 125 days of age in two group sizes (6 and 9 birds/cage) and three stocking densities (150, 175, and 200 cm^2/bird) and reported a higher egg production in the smaller group size with intermediate stocking density, compared to the larger group sizes and higher stocking density. The size of the group can have a significant impact on the association between group mates in domesticated fowl, social interactions, and modifying their adaptation to new situations (Jones, 1996; Bilčík et al., 1998; Estevez et al., 2003), consequently, the quality of group-mated interactions can have an impact on performance, health, and welfare metrics.

Despite the fact that Japanese quail have been widely utilized as a model animal to study social behavior in large groups of domestic birds (Schweitzer et al., 2009), there are few studies on social connections in Japanese quail.

Several characteristics of social interactions, such as cohesiveness, affiliation, and aggression, are likely to be influenced by social motivation (Launay et al., 1991; François et al., 2000; Williams et al., 2003). Breeding groups of 15-20 birds are kept in battery cages with a floor space of 1 m × 0.5 m and a height of 16-20 cm in industrial cage production (Gerken and Mills, 1993). Quail are typically distressed in industrial settings, with issues like head-banging as a result of escape responses, aggressive pecking, leg weakness, feather damage, and foot disorders. Aggressive pecking is one of the most common causes of skin or eyelid lesions, quail head injuries, and eye loss, all of which can negatively impact quail welfare in commercial quail farming. Wechsler and Schmid (1998) investigated the effect of breeding groups on the aggressive behavior of Japanese quail. They used a 2×2 factorial design with four groups of 5 males and 15 hens and 4 groups of 5 males and 35 hens, introducing 2 groups of each composition into the pens at the ages of 4 and 6 weeks, respectively. These researchers showed that the effect of group size or age of introduction into the experimental pen on pecking rate was not significant, and there was no significant interaction between the two factors.

Environmental enrichment or alterations can be utilized to promote animal welfare, reduce aggression and fear, and change social behavior in poultry husbandry because environmental enrichment can boost behavioral possibilities and lead to improvements in biological functioning (Gvaryahu et al., 1994; Newberry, 1995). Environmental enrichment has been observed to promote aggressive interactions among caged laying hens (Reed et al., 1993), hence it is hypothesized that environmental enrichment will alter social behaviors in poultry, particularly grouping behaviors. Japanese quail has been the subject of numerous environmental enrichment and social behavior studies as a common laboratory and production species. Miller and Mench (2005) found that social housing versus singleton dwelling had an effect on social proximity choice in Japanese quail.

Since strong social motivation is the primary criterion for quail selection, they prefer to spend more time with conspecifics (Launay et al., 1991; Carmichael et al., 1998; Formanek et al., 2008) and show more social isolation in comparison to quails selected for low social motivation (Launay et al., 1993; Mills et al., 1993). Low social motivation encourages social bonding between cage mates in quail chicks housed in pairs, but high social motivation chicks display a social attraction for any conspecific, whether they are familiar or not (Schweitzer et al., 2010). Schweitzer et al. (2011) conducted an experiment in Japanese quail with varying levels of social incentive to see how group size impacts the strength of social connections between familiar conspecifics. Quails with high or low social reinstatement behavior were selected and housed in various group sizes. Quails that demonstrated high or poor social reinstatement behavior were chosen and housed in groups of 6, 15, or 30; Increasing group size improved the calming index only in high social reinstatement quail chicks which point to a lower calming effect of the return of a conspecific with increasing group size. In all lines, the number of nonaggressive pecks and the time spent in contact decreased as group size increased. The findings of this study show that social bonds exist in both high and low social reinstatement quail chicks, contrary to the findings of Schweitzer et al. (2010), who found that social bonds between familiar conspecifics exist in low social reinstatement but not in high social reinstatement quail chicks housed in pairs. In addition, Craig et al. (1969) discovered that strangeness and crowding in female chickens are connected with higher rates of social interaction compared to socially undisturbed and uncrowned flocks.

Precocial bird mothers can modify their chicks' emotional and social behavior through non-genetic postnatal mechanisms, causing both long-term and transgenerational effects (Houdelier et al., 2013). The few studies that investigated the impact of brood size on precocial birds were more concerned with offspring survival than with mother care (Pittet et al., 2014; Aigueperse et al., 2017). Aigueperse et al. (2017) evaluated the impact of brood size on Japanese quail maternal behavior and its interactions with chicks. In their study, two types of broods were compared: small broods of three chicks (N = 9) and large broods of six chicks (N = 9), and also assessed mother behavior by using two methods. Aigueperse et al. (2017) showed that mothers in the large group produced more maternal vocalization (cooing and food calls) one day after maternal induction than mothers in the small group. These researchers also observed that brood size had no effect on the time spent warming chicks and that mothers in the large group had fewer covering postures, compared to mothers in the small group. The authors find that brood size influences mother behaviors such as warmth, vocalization, and huddling.

Parasites are one of the most common worries in the poultry industry (chicken, turkey, and quail), and they can be found practically anywhere poultry is produced and cause serious economic and production consequences. It has been established that a mix of interconnected elements such as stress, management, and diet resulted in parasite exposure (Lynch Ianniello et al., 2014). Moore et al. (1988) hypothesized that parasite transmission is easier and larger in stable social groupings and conducted an experiment with varying covey sizes in bobwhite quail over different seasons to test the theory. Their findings revealed that the number of monoxenous parasites is related to the size of the group. Moore et al. (1988) also discovered that *T. tenuis* and *R. cesticillus* intensities were higher in large coveys than in small coveys. These researchers concluded that for parasitism evaluation, the immune system of the host animal, variation caused by the biology of intermediate hosts, or a longer generation time must all be taken into account.

CONCLUSION

Many recent studies reveal that optimum group size on an industrial scale needs to be thoroughly studied, preferably in commercial trials, with consideration to recent specific legalization in the poultry industry in various regions. However, it is clear that studies focusing on the optimum group size have some confounding and interactions between density and enclosure size. It is important to note that this evaluation focused on studies conducted in experimental settings, making it difficult to extrapolate the findings to commercial settings where thousands of chickens are bred at once. On the other hand, many of the scientific findings can be implemented in the industry. According to the findings of the current review, future research in poultry welfare and behavior should concentrate on the effect of group size on more specific responses and the separation of the effect of group size from other correlated factors. In addition, in comparison to small group sizes, more specialized factors, including parameters with more economical use, such as leg diseases, growth performance, and laying rate, should be evaluated in different types and breeds of poultry in commercial-scale group sizes.

DECLARATION

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Ethical consideration

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

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Synergistic Effects of Phytogenic Compounds on Early Growth Parameters of Native Chickens

Albino Taer[1*] and Erma Taer[2]

[1]*Lecturer, Department of Poultry Science, Surigao State College of Technology-Mainit Campus, Mainit 8407, Surigao, Philippines*
[2]*Faculty of Agriculture, Department of Crop Science, Surigao State College of Technology-Mainit Campus, Mainit 8407, Surigao, Philippines*

*Corresponding author's Email: albinotaer74@gmail.com; ORCID 0000-0002-2958-4682

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ABSTRACT

The survival of native chickens during the first 6 weeks of growth is less than 50%. Hence, this study proposes the introduction of prophylactic antibiotics for poultry diseases prevention and treatment. However, the ban on antibiotics has prompted the search for plant-based biomedicines. Therefore, this trial aimed to determine the effect of phytogenic compounds (PC) of five herbs as water additives on the survival and growth responses of native chickens. A total of 204 Bisaya chickens (unsexed) were randomly assigned to 4 treatments replicated three times with 17 chickens in each replicate. Chickens were fed *ad libitum* and received water with 1.5 g antibiotics/1000ml water (control), 20 ml PC/1000ml water (T2), 40 ml PC/1000ml water (T3), and 60 ml PC/1000ml water (T4) for 35 days. No significant differences were observed on feed intake for chickens in antibiotics and PC treatments, however, the 60-PC group consumed slightly higher feed intake, compared to chickens under antibiotics and the other level of PC supplementation. Chickens in 60-PC ate 4-12% more feed than the others at the end of the trial period. Cumulative water used per kg feed did not differ among the experimental groups. The control and the PC supplemented chickens shared homogenous body weight and weight gains patterns, averaging 403.79 to 415.20g and 85.49 to 86.85g, respectively. Supplementation of 40-PC and 60-PC in drinking water for native chickens reduced the mortality rate and comparable feed conversion ratio with antibiotics. The 60-PC as a phytogenic water additive could enhance the growth performance, increase the survival rate, reduce mortality, and improve feed conversion ratio correlative to antibiotics.

Keywords: Antibiotic, Mortality, Native chicken, Phytogenic compounds, Synergistic effect, Survival rate

INTRODUCTION

The higher mortality rate for native chickens at the growing stage is a common problem mostly encountered by backyard and smallholder native chicken farmers. Mortality during the first week of life in chickens is an essential production criterion widely used in poultry production. These first days of life, many things happen to chicks in a relatively short period due to adjustments from a controlled environment in the hatchery to a more independent life on the farm and chicks' digestive systems are immature (Yassin et al., 2009; Yerpes et al., 2020). During this period of adjustment, some factors can negatively affect the chick morpho-physiological welfare.

In broiler chicken farming, housing factors and management routines are important for reducing the first-week mortality rate, rearing the chickens, and constructing new broiler houses (Yerpes et al., 2020). It requires reliable access to inputs, commercial stock, feed, labor, and health services, as well as efficient marketing channels (Mack et al., 2005; Thieme et al., 2014; Abebe and Tesfaye, 2017). Native chicken farming is a small-scale poultry production, mainly involving chickens of the poultry population in rural areas of low and medium-income countries (Gilbert et al., 2015). They engaged in free-ranging systems where chickens are scavenging for feed with minimal supplement and inadequate housing (Sonaiya, 2004; Thieme et al., 2014). The free-range chickens are commonly mixed with crops and other livestock and are vulnerable to environmental risks (Alders et al., 2014; Thieme et al., 2014).

Alfred et al. (2012) reported that the total chick deaths in the initial six weeks of age were 53%. The primary cause of chick deaths was predation (55%), diseases other than ectoparasites (30%), ectoparasites (5%), management factors (6%), and unknown causes (5%) for free-range chickens. Although free-ranging chick predation causes higher death rates than other problems, chicken keepers were more concerned about the diseases than predation. Moreover, predation losses can be addressed by the appropriate shelter of chicks from day-old to 6 weeks before free-range production. In diseases, prevention is worth better than treatment. However, rural areas are typically distant from markets and veterinary assistance (Thieme et al., 2014). The widespread rural areas and a scarcity of supplies and infrastructure can restrict veterinary and extension services (FAO, 2014). In most cases, services focused on crop or ruminant production, with little attention to the small-scale poultry raisers (Bagnol, 2009). Hence, they resorted to prophylactic treatment by antibiotics as means of preventing diseases in chickens. Antibiotics can prevent, control, and treat infectious diseases in humans and animals (Hao et al., 2014). In 1996, the use of antibiotic growth promoters (AGPs) in poultry diets was banned by the European Union (Saeed et al., 2017). The use of antibiotics in feed has improved growth performance and feed efficiency in livestock (Lin, 2014). The global emergency of antibiotic resistance has led to the prohibition of antibiotics worldwide (Marshall and Levy, 2011; Tang et al., 2017). A number of alternatives, including prebiotics, probiotics, symbiotics, and plant extracts have been proposed in this regard (Nikpiran et al., 2013; Vahdatpour and Babazadeh, 2016; Saeed et al., 2018; Vieco-Saiz et al., 2019).

Due to customers' choices, preferences, values, and desire for natural products, phytogenics (phytobiotics or botanicals) have drawn significant recognition from livestock, and poultry has shifted the fastest-growing segment of animal feed additives. However, adding extracts from single herbs (Chamomile, Lemon balm, and St John's wort) to water did not affect feed conversion and water use by broiler chickens (Skomorucha and Sosnówka-Czajka, 2013). Better effects are achieved when using a mixture of well-chosen herbs than incorporating the same herbs individually into the diet (Schleicher et al., 1996; Dahal and Farran, 2011). Cho et al. (2014) revealed that the combined extracts of four medicinal herbs (*Galla rhois*, *Achyranthes japonica* Nakai, *Terminalia chebula* Retz, and *Glycyrrhiza uralensis*) were effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli* and exhibited the highest DPPH radical-scavenging activity synergistic actions.

Therefore, the current trial aimed at determining the effect of five herbal extracts as phytogenic compounds in water on growth responses and the survival rate of native chickens under controlled growing conditions.

MATERIALS AND METHODS

Ethical approval

The study complied with the rules and regulations on the scientific procedures of using animals under the Philippines Republic No. 8485, otherwise known as the "Animal Welfare Act of 1998", and ethical standards at Surigao State College of Technology, Surigao City, Philippines.

Animals, experimental design, and location

A total of 204 one-day-old unsexed native chicks (Bisaya) with an average body weight of 53.05 ± 1.54g, were utilized from the college chicken hatchery unit and were randomly allocated to one of the four treatment groups with three replicates containing antibiotic or levels of phytogenic compounds (PC) in drinking water, such as T1 (Antibiotics 1.5 g/lit Water), T2 (20 ml PC/1000ml water), T3 (40 ml PC/1000 ml water), and T4 (60 ml PC/1000ml water). Doxycycline (PBAC Doxy 500 mg/g powder by Life Biopharma SDN BHD, Malaysia) was the most commonly available veterinary drug in the locality during the trial time. Basal diets were commercial ration and kept constant in all treatments throughout the 35 days trial period divided into two phases, including 1-14 days chick booster and 15-35 days chick starter period. The study was carried out at Surigao State College of Technology-Mainit Campus, Philippines from December 21, 2020, to January 25, 2021.

Twelve cages measuring 60 inches Wide × 70 inches Long × 20 inches Hight made of local materials were constructed and stationed in open-sided poultry housing. A total of 17 chicks occupied each cage provided with 24-hour light using 25 watts electric lamp as the heater and provide light during the night. The temperature was kept at 35°C in the first week of brooding and reduced gradually by reducing the wattage of the heated lamp until reached a normal room temperature of 32°C.

Preparation of phytogenic compounds

Phytogenic compounds consisted of five fresh herbs namely, ginger (*Zingiber officinale*), garlic (*Allium sativum*), red onions (*Allium cepa*), turmeric (*Curcuma longa*), and black pepper (Piper nigrum) sourced from the public market in the community. Each herb was prepared separately, then the preparations were combined to produce PC. The preparation of PC was the modification of Chang et al. (2014). Briefly, fresh herbs were sliced or crushed, weighed, and each herb was placed separately in a clean glass jar to fill 2/3 full. An equal amount of molasses was added to each jar and then the jars were covered tightly, sealed, labeled, and then sat for 7 days at room temperature. After 7 days, each jar was filled with pure coconut vinegar (1:1 solution), sat at room temperature, and stirred clockwise every morning for 14 days. The solutions from each jar were strained and labeled according to preparations. The phytogenic compounds (PC) were comprised of several herbal extracts (1 part from ginger, garlic, red onions, turmeric, and black pepper) and then were mixed in one jar before applying as treatments

Feed and water management

Chickens had unrestricted access to commercial maize-soybean basal diets and drinking water during the whole trial. The feed crumbles were offered in plastic feeders (60 cm) size. Plastic gravity-type drinkers (1000 ml round-shaped) were washed daily and refilled with potable water. The drinkers were apart from the feeders to avoid contamination. The feed and water intakes were calculated daily at 16:00 as the difference between the weight given and the remaining. The daily feed intake per bird (FI/bird) was the quotient between the feed intake per cage by the number of animals present in the cage: FI/bird = FI/cage/number of animals per cage. The water used per kg feed (Lit./kg feed) was the quotient between the water intake per pen by the total feed received on weekly basis. The BW was recorded on days 1, 7, 14, 21, 28, and 35.

Statistical analysis

All data gathered were subjected to analysis of variance (ANOVA) for a completely randomized design (CRD) using IBM SPSS (version 26) for windows. Treatment means were compared using Tukey's HSD test and differences were considered significant at 5% level of probability.

RESULTS

The data on the performance and survival of native chicken growers supplemented with levels PC is presented in tables 1-5. There was no significant change in the feed intake of chickens in antibiotics and PC-treated groups during 1 to 35 days of feeding ($p > 0.05$). However, the 60-PC treatments consumed slightly higher feed intake than chickens under control (antibiotics) and the rest of PC supplementation. Chickens in the 60-PC consumed 4-12% more feed than the others at the end of the trial period (Table 1). Throughout the experiment, cumulative water used per kg feed did not differ among the experimental groups (Table 2). The control and the PC supplemented chickens shared homogenous body weight and weight gains patterns ($p > 0.05$), averaging 403.79 to 415.20 g and 85.49 to 86.85 g, respectively, at the end of the experiments (Tables 3 and 4). Supplementation of 40-PC and 60-PC in water for native chicken growers reduced mortality rate (1.96%) with the higher percentage of survival (98.04%) apiece and shared a statistically similar feed conversion ratio with antibiotics treatment (Table 5).

Table 1. Feed intake of native chickens treated with different levels of phytogenic compounds as water additive

Treatment	Booster		Starter		
	1-7 days	**8-14 days**	**15-21 days**	**22-28 days**	**29-35 days**
Antibiotic (g/l^{-1})	1941.67	2653.33	3397.03	4187.43	5045.20
20ml PC/1000ml water	1948.67	2779.33	3637.70	4536.10	5493.20
40ml PC/1000ml water	1937.00	2764.67	3621.47	4520.73	5482.47
60ml PC/1000ml water	1955.67	2841.33	3754.27	4706.47	5715.60

(gl^{-1}): Grams per liter, PC: Phytogenic compound

Table 2. Water use of native chickens treated with different levels of phytogenic compounds as water additives

Treatment	Booster		Starter		
	1-7 days	**8-14 days**	**15-21 days**	**22-28 days**	**29-35 days**
Antibiotic (gl^{-1})	1.95	2.02	2.06	2.16	2.27
20ml PC/1000ml water	1.94	1.98	2.08	2.10	2.19
40ml PC/1000ml water	1.93	2.00	2.07	2.17	2.24
60ml PC/1000ml water	1.95	2.01	2.10	2.14	2.22

(gl^{-1}): Grams per liter, PC: Phytogenic compound

Table 3. Body weight of native chickens treated with different levels of phytogenic compounds as water additives

Treatment	Booster			Starter		
	Initial	1-7 days	8-14 days	15-21 days	22-28 days	29-35 days
Antibiotic (gl^{-1})	53.98	102.34	175.83	243.89	318.30	403.79
20ml PC/1000ml water	53.05	100.18	171.32	240.22	315.81	398.87
40ml PC/1000ml water	54.59	100.79	170.19	240.11	313.16	397.72
60ml PC/1000ml water	54.45	101.61	178.47	254.11	328.36	415.20

(gl^{-1}): Grams per liter, PC: Phytogenic compound

Table 4. Weight gain of native chickens treated with different levels of phytogenic compounds as water additives

Treatment	Booster		Starter		
	1-7 days	8-14 days	15-21 days	22-28 days	29-35 days
Antibiotic (gl^{-1})	48.36	73.49	68.06	74.41	85.49
20ml PC/1000ml water	47.13	71.13	68.90	75.59	83.06
40ml PC/1000ml water	46.19	69.40	69.92	73.05	84.56
60ml PC/1000ml water	47.17	76.86	75.64	80.24	86.85

(gl^{-1}): Grams per liter, PC: Phytogenic compound

Table 5. Feed conversion ratio, mortality rate, and the survival rate of native chickens treated with different levels of phytogenic compounds as water additives

Treatment	Feed conversion ratio	Mortality (%)	Survival (%)
Antibiotic (gl^{-1})	2.90	3.92	96.08
20ml PC/1000ml water	3.02	3.92	96.08
40ml PC/1000ml water	3.03	1.96	98.04
60ml PC/1000ml water	3.09	1.96	98.04

(gl^{-1}): Grams per liter, PC: Phytogenic compound

DISCUSSION

The antibiotic and PC treatments did not show any differences in feed intake and water used per kg feed at different ages of growing chickens. The 60-PC had the highest FI value among other PC treatments indicating that PC as water additives induce intake of feeds. The improved consumption of feed by chickens under 60-PC was probably due to concentrations of phytogens derived from various herbs used. The phytogens help the discharge of digestive enzymes in chickens, as the PC-enriched drinking water flows through their digestive tracts and thus enhanced the integrity of the intestinal mucosa (Herkel' et al., 2014). The phytogens stimulate the olfactory receptors and taste buds, which subsequently encourage intake, endogenous enzyme production and digestive fluids, and better nutrient digestibility of feed (Panda et al., 2009). The natural-growth promoting actions of feed additives phytogens are feed hygiene maintenance, including the beneficial effect on the gastrointestinal microbiota through regulatory pathogens (Roth and Kirchgessner, 1998), appetite, feed intake, endogenous digestive enzyme secretion, immune responses, antibacterial, antiviral, and antioxidant improvement and stimulations (Dorman and Deans, 2000; Hosseini-Vashan et al., 2012). The result of the current study was in line with other findings on the same herbs. For instance, garlic extract use of 0.75% in the diet was a more efficient growth enhancer of broiler chicks (Islam et al., 2018), dietary supplementation of ginger at 2 g/kg feed was beneficial on overall performance of broilers (Rafiee et al., 2014), and turmeric powder improved the performance of broiler using 7.5 g/kg concentration in the diet (Shohe et al., 2019).

The final mean values for water used per kg feed averaged 2.19 to 2.27 liters at the end of the experiment. Water use was 2-3 ml higher per gram feed intake for poultry. This finding indicated regular water consumption by chickens despite the varying levels of herbal extracts. A two to three times higher water intake per gram diet was also observed by broilers that received varied levels of aqueous *Moringa Oleifera* leaf extract, compared to those who received water with antibiotics and tap water (Alabi et al., 2017).

Varied PC in water did not significantly differ from antibiotics treatment in terms of body weight and body weight gain of native chicken growers across the trial period. However, the 60-PC-treated chickens had higher body weight and weight gain, compared to antibiotic treatments during the late booster phase until the entire starter phase feeding period. On days 8-35 of the experiment, chickens from the 60-PC groups showed a tendency for greater body weight compared to chickens not supplemented with herb extracts. The addition of herbal extracts to a poultry diet positively affects the body weight of chickens as confirmed by many studies (Buğdaycı et al., 2018; Abd El-Hack et al., 2020; AL-Sagan et al., 2020). According to Hippenstiel et al. (2011), the antibacterial behaviors of volatile oils improved the bacterial flora of the digestive tract which increased digestive enzymes and enhanced digestion. the increased digestive enzymes and enhanced digestion resulted from the antibacterial actions of volatile oils that help improve the bacterial flora of the digestive tract. However, other studies indicated contradictory effects of herbal medicines on the performance of chickens (Lee et al., 2003; Khalaji et al., 2011). A mixture of well-chosen herbs led to better results, compared to incorporating the same herbs individually into the diet (Schleicher et al., 1996; Dahal and Farran, 2011). The tendency for a greater body weight of chickens in 60-PC in this study was that antimicrobial combination involved in the ingredient of herb mixtures such as red onions, garlic, red onions, turmeric, and black pepper controlled the microflora in the gastrointestinal tract. Active ingredients are gingerol and shogaol for ginger (Rahmani et al., 2014), allicin, diallyl disulfide, diallyl trisulfide, and alliin for garlic (Shang et al., 2019), quercetin, cycloalliin, S-methyl-L-cysteine, S-propyl-L-cysteine sulfoxide, Dimethyl trisulfide, S-methyl-L-cysteine sulfoxide, N-acetylcysteine, alliuocide for red onions (Marrelli et al., 2018). Turmeric has three curcuminoids; curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Dasgupta, 2019), and black pepper has the active ingredient piperine (Butt et al., 2013) that probably involved in enhancing the digestibility of feed and growth performance in native chickens.

Phytogenic compounds at 40 to 60 ml per liter in water reduced the mortality rate and improved the survival of growing chickens (Table 5). Polyphenolic and carotenoids content of the herbal extract activate lymphocyte proliferation and enhances cytoprotection against free radicals is higher (Geetha et al., 2002; Kalia et al., 2017). Though phenolics, flavonoids, and carotenoids content of the tested five herbs (ginger, garlic, red onions, turmeric, and black pepper) were not quantified as part of the limitations in this trial, it was presumed higher in amount considering the levels of supplementation and the number of utilized herbs extract might cause the synergistic effect those phytomolecules could have been stimulated the generation of T lymphocytes and support the immune system (Geetha et al., 2002; Dorhoi et al., 2006; Kalia et al., 2017). It also showed that the mitogenic activity of *Hippophae rhamnoides* in bird lymphocytic cells helps maintain feed intake under challenging conditions and modulates gut microbiota to decrease pathogenic bacteria pressure. Moreover, it minimizes intestinal stress, makes more nutrients available for production, enhances gut integrity, and improves immune resilience against stressors (Kalia et al., 2017). The improved feed conversion ratio of chickens receiving antibiotics in water was not significantly different from PC-treated chickens. The synergistic therapeutic mechanism of herbal medicines suggests that mixed drugs affect either the same or distinct targets in diverse pathways and interact in an agonistic, synergistic way (Yang et al., 2014). More specifically, enzymes and transporters involved in hepatic and intestinal metabolism that boost oral medication bioavailability also suppress microbial and cancer cell drug resistance mechanisms, reduce side effects, and increase pharmacological effectiveness (Yang et al., 2014).

CONCLUSION

Based on the results, it can be concluded that the 60 ml of phytogenic compounds as a water additive offered better growth and immune responses of the early growing Bisaya chickens on par with the antibiotic treatments. It increased the survival rate, reduced mortality, and improved the feed conversion ratio. Therefore, this dose is recommended as an alternative to Doxycycline. However, there is a need to conduct comparative studies on the effects of phytogenic compounds and other antibiotics.

DECLARATIONS

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Author's contribution

Taer A, contributed in the study design, gathering data, statistical analysis, and interpretation of data. Taer E contributed in conceptualization, writing the draft of manuscript, and revising the article. All authors confirmed the analyzed data and final proof of article.

Competing interests

The authors have not declared any conflict of interest.

Ethical consideration

The authors carefully checked all ethical issues concerning plagiarism, consent to publish, misconduct, data fabrication, falsification, double publication or submission, and redundancy of the manuscript.

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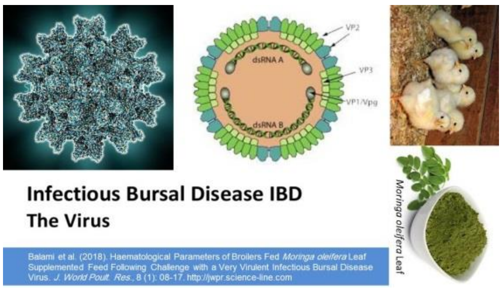

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