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SCHOOL OF AGRICULTURE

EFFECT OF NONI FRUIT (*Morinda citrifolia*) EXTRACT ON PRE-LAY SEXUAL DEVELOPMENT, EGG PRODUCTION PERFORMANCE, AND BLOOD METABOLITE STATUS OF LAYING BIRDS IN GHANA

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BY

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DEPARTMENT OF ANIMAL SCIENCE

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DECLARATION

1, KOFI AARON ABBOA-OFFEI AGYEI-HENAKU, hereby declare that except for references to other people's work, the work in this thesis submitted for the award of Doctor of Philosophy in Animal Science is entirely my own produced from research under supervision and has neither been submitted nor presented in whole or in part (Or any degree in this University or elsewhere.

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ABSTRACT

The 2020 per capita egg consumption of Ghana was 1.07 kg. The demand for poultry products also exceeds local production, and the shortfall in animal protein intake is more of a supply problem rather than a demand problem. Commercial laying birds are popular in Ghana due to their high egg-production capabilities amongst others thus enhancing the growth and egg production of layers can have a considerable impact on the livelihoods of poultry farmers and the overall agricultural economy of the country. Disease conditions in livestock and poultry production caused stunting and delays in reaching market weight resulting in economic losses. Anti-microbials and anti-parasitic drugs or pesticides were used on farms as interventions to enhance growth and productivity but contaminated the poultry products. Ingestion of these antimicrobials in the contaminated poultry products by humans caused anti-microbial-resistant bacteria to develop in them. Therefore, those interventions using antimicrobials as therapeutic and prophylactic agents for improving health, growth and production performance in poultry are being gradually discontinued and being replaced by phytogenic feed additives, due to the increased public awareness of the risk of developing cross-resistance of pathogens to antibiotics. The method of administering feed additives to the diet of poultry could influence their performance and immune competence an indication that administering through drinking water could be superior to the more conventional in-feed supplementation. In addition, the time of administration of the additive has also not received attention, especially in Ghana, with most scientists in literature administering additives both during early lay or late lying periods and not during the entire growth and production cycle of layer-type birds.

The objective of this study was to investigate the effects of noni fruit extract and physiological status on pre-lay sexual development performance, egg production performance from early to late lay, and on the overall health of layers. Four experiments were conducted involving 600 layer-type birds in two groups of 300 birds. The two groups of 300 layer-type birds were fed a regular layer ration. The first Group of 300 birds was allocated to the first three experiments, and the second group of 300 layer-type birds was used for the fourth experiment. The maximum yield and concentration of noni fruit extract used for the four experiments were determined in the laboratory. Experiment 1 had three treatments (T_1, T_2, and T_3) with 20 pullets per treatment, respectively, that were replicated five times in a completely randomised design. Treatment one (T_1) served as the control with 0 mg/ml noni fruit extract. Treatment two (T_2) was composed of 20 mg/ml noni fruit extract and treatment three (T_3) 40 mg/ml noni fruit extract administered through drinking water. This treatment structure was repeated for experiments 2 and 3, respectively. The treatments administered to the birds began at 16 weeks of age for experiment 1 and were run for 6 weeks. Daily feed and water consumption, as well as weight gain, were recorded. The trial for the first experiment ended at week 22 and 10 birds per treatment (2 birds per replicate) were randomly selected and euthanised. The right tibia bone (representing medullary bone), abdominal fat and reproductive tract (uterus) tissues to assess sexual development were collected at pre-lay. The age at first egg (a proxy measure of sexual maturity) was also recorded. Blood samples were collected from 10 randomly selected birds per treatment (2 birds per replicate) and analysed for their haematological and serum biochemistry. The results of the extraction and analysis of the noni fruit extract yielded 580ml/kg of fruit and an antioxidant capacity (concentration) of approximately 4.0 mg/ml at 8 weeks. The final

body weight, daily weight gain, egg weight, and egg mass increased significantly in the birds that received 40 mg/ml noni fruit juice. Feed intake decreased ($p < 0.05$) with increasing concentration of noni fruit extract; however, water intake was similar ($p > 0.05$) among treatment groups. The noni fruit extract delayed the mean age at first egg, with the 20 mg/ml concentration causing the longest delay of 3.4 days compared to the 40 mg/ml concentration and the control groups. Significant reduction ($p < 0.05$) of abdominal fat, demonstrating anti-obesity properties with dose effect of noni fruit extract was obtained. Additionally, the weight of the uterus and right tibia bone growth and mineralisation characteristics increased ($p < 0.05$) with increasing dose of noni fruit extract. Experiment 2 continued from week 22 using 270 layer-type birds randomly assigned to three treatments of 18 birds per treatment replicated five times in a completely randomised design. The second experiment lasted for 26 weeks and ended when the birds attained the age of 48 weeks. Eggs were collected at week 22; week 30 and week 48 representing the physiological stages (early-lay, peak-lay and late-lay) and used for internal egg quality assessments for the three physiological stages. Ten eggs per treatment were sampled to evaluate the effect of storage duration (5 different evaluation durations) on egg quality. The results of the second experiment showed that adding noni fruit extract up to 40 mg/ml to drinking water improved ($p < 0.05$) egg production performance indices (egg mass, %HDEP, FCR and NFEI) and the overall quality of eggs (AH, HU, yolk index, yolk colour and estimated eggshell thickness) with a dose effect. Over time, stored egg quality decreased, but noni fruit extract administered up to 40 mg/ml aided in slowing down the degradation process and preserved the yolk colour under ambient temperature. In experiment three, the application of noni fruit extract on the health status of the layers was evaluated using haematological and serum biochemical parameters.

White blood cells (WBC), heterophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells (RBC), haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and thrombocytes (Trb) and packed cell volume (PCV) The following biochemical profile measurements were analysed: total protein (TP), albumin (ALB), creatinine (CRE), urea, uric acid (UA), total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT). Others were total cholesterol (TCHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides (TG), glucose (GLU), calcium (Ca^{2+}), sodium (Na^{+}), chloride (CL^{-}), and potassium (K^{+}) ions, albumin/globulin ratio, AST/ALT ratio and globulin (GLB). Administration of noni fruit extract up to 40 mg/ml had considerable influence on the haematological and serum biochemical profile of laying hens with a dose effect. Physiological stage played a significant role in influencing these effects that were within normal reference range for chicken, demonstrating that noni fruit extract had no adverse effects on the physiology and health of the layer-type birds. In experiment four, 300 layer-type birds were used to evaluate egg production performance and quality characteristics, and health status with the administration of 40 mg/ml noni fruit extract. There were 3 treatments, each comprising of 5 replicates and 20 birds per treatment. Pullets in treatment 1 (control) received 0 mg/ml of noni extract from 16 weeks of age. Treatment 2 comprised 16-week-old pullets receiving 40 mg/ml of noni extracts in their drinking water. In treatment 3, the pullets were started on 0 mg/ml of noni fruit extract at 16 weeks of age. At 20 weeks of age, the layers were provided with 40 mg/ml of noni fruit extracts in their drinking water. At 16 weeks of age, the average body weight in

all the 3 treatment groups was 1.3 ± 0.233 kg. Weekly body weights were measured in all the treatment groups from week 16 until week 22. The days at first egg (sexual maturity) were recorded. Other measurements (egg quality and haematological and serum biochemistry) were taken at 22, 30, and 48 weeks of age using the procedures outlined in experiments 2 and 3. The parameters measured were pre-lay growth and egg production performance characteristics, egg quality and blood metabolite indices as listed in experiments 1, 2, and 3. The sexual maturity of the birds that received noni fruit extract at 16 weeks was approximately 129 days compared to approximately 126 for birds that receive noni fruit extract at 20 weeks and the control group. At 22, 30 and 40 weeks of age the birds that received 40 mg/ml noni fruit extract at weeks 16 or 20 had similar ($p > 0.05$) body weights but higher ($p < 0.05$) compared to the control group. The significantly ($p < 0.05$) better pre-lay sexual development, egg production and performance, and egg freshness indices, as well as, reduced abdominal fat weight, and egg weight loss during storage, blood haematological indices and serum biochemical profile indicated a better health status of the birds that received 40 mg/ml noni fruit extract at 16 weeks compared to those of week 20 and control. Overall, the results from this study demonstrate the potential of noni fruit extract as a beneficial feed supplement that could enhance the productivity and health of poultry when administered at the onset of sexual development in the layer-type birds.

DEDICATION

I dedicate my work to my late parents, Mr. David Kwame and Mrs. Felicia Maate Kaale Agyei-Henaku, my wife, Mrs. Mishael Agyei-Henaku, and my children, Joel-Luke Kwadwo Agyei and Jenissi-Justine Asantewaa Nyameye, for their patience, endurance, and unwavering support.

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BMSC - Bone marrow mesenchymal stem cells

EBPs - Enhancer-binding-proteins

HFD - High-fat diet

kDa - Kilo Dalton (1000 Daltons - atomic mass)

LPL - Lipoprotein Lipase

NEFAs - Non-esterified fatty acids

NID (0, σ^2e) - Normally and independently distributed with mean zero and variance σ^2

PFAs - Phytogenic Feed Additives

PPAR-γ - Peroxisome proliferator-activated receptor gamma (glitazone reverse insulin resistance receptor)

Runx2 - Runt-related transcription factor-2

WAT - White adipose Tissues

ZO - Zonula occludens proteins

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background

Poultry farming contributes to the Ghanaian economy, accounting for 14% of the country's total gross domestic product (Biovet, 2022) and contributing significantly to food security, income generation, and job creation (Nti, 2018). The production and sale of eggs constitute a major driver of the overall poultry sector in Ghana (College of Agriculture and Natural Resources, 2016; Onumah and Ayeduvor, 2023). Therefore, optimising egg production and quality is crucial for the industry's success (Nti, 2018). Layer-type birds are popular due to their high egg-production capabilities amongst others and hence, enhancing the growth and egg production of layer-type birds can have a considerable impact on the livelihoods of poultry farmers and the overall agricultural economy of the country (Hagan and Apori, 2013; Nti, 2018).

The per capita egg consumption in Ghana is low compared to its neighbouring countries. The per capita egg consumption of Nigeria, La Cote D'Ivoire, Togo and Burkina Faso is 2.8 kg, 2.18 kg, 1.84 kg, and 1.13 kg respectively (Shane, 2024). According to the FAO (2023) per capita egg consumption is increasing and reached 1.07 kg in Ghana in 2020, which was 1.90% more than the previous year. The demand for poultry products in Ghana exceeds local production, and the shortfall in animal protein intake is seen as a supply problem rather than a demand problem (Pomaah *et al.*, 2023). The impact of feed and feed intake on the growth rate and egg production performance of laying birds is an important area of study (Hagan and Apori, 2013). Enhancing these parameters not only benefits poultry farmers economically but also positively affects food availability for consumers (Nti, 2018).

Feed additives, often antimicrobials, have hitherto been used as therapeutic and prophylactic agents for improving health, growth and performance in poultry. However, the use of traditional antimicrobials are gradually being discontinued due to the increased public awareness of the risk of developing cross-resistance of pathogens to antibiotics (Ricke *et al.*, 2020; Selaledi *et al.*, 2020). Therefore, in the last two decades, research scientists have investigated, and continue to explore, the use of health and performance-enhancing properties of plant-derived additives in poultry diets as these are natural, and to shift away from antibiotic supplementation (Mian-Ying *et al.*, 2002; Sunder *et al.*, 2011a; 2011b; Sunder, 2014; Sunder *et al.*, 2015a; 2015b; Sunder *et al.*, 2016). This has resulted in tremendous growth in research focusing on the implementation of effective alternative control methods, management, and dietary amendments aiming to improve animal health, welfare, and productivity (Abdelli *et al.*, 2021). These plant-derived additives are termed phytogenics (Noonan, 2018).

The plant *Morinda citrifolia L.*, commonly known as noni, is one plant with phytogenic properties that have received increased attention. It is native to Southeast Asia and tropical Northern Australia, with a distribution spanning from Asia and the Pacific to the Caribbean region (Nelson and Elevitch, 2006; Abou Assi *et al.*, 2017). In Thailand, the young leaves are prepared and consumed as a vegetable, while the fruits are fermented and enjoyed as a traditional beverage (Newton, 2002). During the 1990s, fermented noni fruit extract gained popularity as a medicinal product and was widely consumed in North America, Europe, and Asia, with the belief that the fermentation process could enhance the phytochemical content, thus increasing its potential health benefits (Puva *et al.*, 2013; Bhatia *et al.*, 2015). Noni thrives prevalently in tropical regions (Nelson, 2006) including Ghana, where the fruit has

recently received enormous attention from scientists and medical professionals due to its pharmaceutical properties. The fruit of the noni plant (*Morinda citrifolia*) is phytogenic and has been used for centuries in almost every culture for its health benefits and remedies (Singh *et al.*, 1984; Whistler, 1985). Noni's therapeutic properties include anti-viral, anti-bacterial, anti-fungal, anthelminthic, anti-tumour, anti-cancer, anti-epileptic, analgesic, anti-inflammatory, anti-lipogenic, immune-enhancing effects, and digestion-enhancing effects among others (Mian-ying *et al.*, 2002; Muralidharan and Srikanth, 2010; Puva *et al*., 2013; Inada *et al.*, 2017; Aroche *et al.*, 2018; Ayunda *et al.*, 2020; Abdelli *et al.*, 2021). Data from clinical studies, toxicity tests, and chemical tests have substantiated the use of noni fruit extract as a safe food. A more detailed examination of the few reported cases of adverse health effects revealed that these were likely due to factors other than the noni fruit (West *et al.*, 2006). Noni fruit extract was accepted in the European Union as a unique food in 2002 (Dussossoy *et al.*, 2011).

There are no reports on the extent to which the noni tree is distributed in Ghana, but the current study made observations on the noni tree along roadsides and backyards in the Greater Accra Region of Ghana. Traditional medicine, particularly herbal medicine, is an important component of the Ghanaian healthcare system and has great economic benefits (Mintah *et al.*, 2022). It is widely acknowledged that the use of medicinal plants for primary healthcare is prevalent in the country, with an estimated 60% to 70% of the population depending on it directly in 2001 (Roberts, 2001). Even though medicinal plants are all over the country and are of great benefit, knowledge of their usage and benefits are not fully documented or known to many Ghanaians, especially the youth and livestock farmers. This information is locked up with mostly herbalists and a few elderly people (Abel and Busia,

2005; Verma, 2014). Tabong *et al.* (2018) also observed that diabetic patients widely use noni and other phytogenic plants as local remedies (alongside taking their orthodox medications) as part of the home-based management of diabetes. According to Aroche *et al.* (2018), phytogenics are classified based on the part of the plant they are harvested from.

They may be:

- Herbs (products from flowering, non-woody, and non-persistent plants from which leaves and flowers are used).
- Spices (non-leafy parts of plants such as seeds, fruit, bark, or root with intense taste or smell).
- Essential Oils (EOs: volatile lipophilic substances obtained by cold extraction, steam, or alcohol distillation).
- Oleoresins (extracts derived by non-aqueous solvents).

Several plant-derived products described as phytogenic feed additives (PFAs) are substances of plant origin added to animal diets at recommended levels to improve performance (Noonan, 2018). The main bioactive components of PFAs are secondary metabolites like polyphenols. Other bioactive compounds include terpenoids (monoterpenes, steroids), phenolics (tannins), glycosides, and alkaloids (Yeoman and White, 2014).

Phenolic compounds are plant secondary metabolites known for their anti-oxidative and anti-inflammatory properties (Mian-Ying *et al.*, 2002; 2008; 2009; Almeida *et al.*, 2019). Several factors influence the composition and concentration of these bioactive substances such as the parts of the plant they are harvested from, their geographical origin, harvesting season,

climatic conditions, processing techniques such as extraction, distillation, and stabilisation as well as storage conditions (Windisch *et al.*, 2008; Applegate *et al.*, 2010).

There is no documentation on the use of noni in animal production in Ghana although extensive research has been reported in Asia (Nelson, 2006; Sunder *et al.*, 2011a; 2011b). However, anecdotal reports suggest that livestock farmers supplement poultry diets with ripe whole noni fruits under extensive production systems in Ghana. Poultry production is challenged to develop management strategies to optimise the chickens' efficiency while limiting food safety concerns. This has led to interest in investigating the influence of noni on production and reproductive performance of chickens in this study.

1.2 Justification

The method of administering additives to the diet of poultry could influence their performance and immune competence (Torshizi *et al.*, 2010), and it has been shown that the administration of additives through drinking water could be superior to the more conventional in-feed supplementation (Torshizi *et al.*, 2010; Agyarko, 2013). The time of administration of the additive has not received attention, with most scientists administering additives either during early lay or late laying periods and not during the entire growth and production cycle of layer birds (Más-Toro *et al.*, 2015; Sunder *et al.*, 2016; Asmara *et al.*, 2019). Investigating the role of noni fruit extract in this aspect can lead to practical recommendations for poultry management practices.

This research will add to the existing body of literature regarding the use of noni fruit extract in animal husbandry, particularly in the context of poultry production. By focusing on

specific metrics such as pre-lay sexual development performance, egg production, and blood metabolites, the study will clarify some of the potential benefits (health and physiology) and mechanisms of action of noni fruit extract. While there is substantial anecdotal evidence regarding the health benefits of noni fruit extract, there is limited scientific research exploring its effects on poultry, especially in the context of Ghanaian agriculture. This study will aid in filling this gap by providing empirical data and analysis, thereby contributing to informed decision-making among poultry farmers and stakeholders. Also, if proven effective, incorporating noni fruit extract into poultry diets could serve as a natural and cost-effective strategy for enhancing productivity and health in laying birds. This could lead to increased profitability for farmers and improved welfare for the birds, bringing them in line with sustainable agricultural practices and its broader implications beyond Ghana to catalyse further research into the application of noni and other natural supplements in livestock management globally.

1.3 Objectives

1.3.1 Main objective

The main objective of this study was to determine the effects of Noni fruit extract on pre-lay sexual development performance, egg production from early to late lay, and the overall health and physiological status of layers reared in Ghana.

1.3.2 Specific objectives

The study was conducted with the following specific objectives:

1. To determine the effect of duration of fermentation on yield and antioxidant capacity of Noni fruit extract.

2. To assess the effect of noni fruit extract on feed and water intake, weight gain, egg weight, egg mass and selected internal organs of pullets administered with two different levels of noni fruit extract in drinking water.
3. To evaluate the effect of varying concentrations of noni fruit extract, and physiological stage (early-lay, peak-lay and late-lay) on egg laying performance and egg quality characteristics in layer-type hens.
4. To assess the effect of noni fruit extract on the extension of shelf life in table eggs.
5. To investigate the effect of varying concentrations of noni fruit extract, and physiological stage on liver and kidney function, lipid profile and overall health in layers.
6. To evaluate the best age of administering noni fruit extract on the laying performance, selected internal organs, egg quality indicators, and haematological and serum biochemical profiles at the physiological stages (early, peak, and late lay).

1.4 Hypothesis

The alternate hypotheses that were tested are as follows:

1. Administering noni fruit extract in drinking water would improve the pre-lay sexual development, egg quality, production performance and blood metabolite status of layers.
2. Administration of noni fruit extract would have dose-dependent and age (physiological stage) effects in layers.

1.5 Importance of Study

This study aims to bridge the knowledge gap in noni-related research by investigating the effect of noni fruit extract on the pre-lay sexual development, health, and production performance of layer- in Ghana. Additionally, it will contribute to the existing literature on the use of noni in the development of the poultry industry.

1.6 Scope of the study

The research focused on the influence of noni fruit extract on various performance indicators (e.g. pre-lay sexual development performance, egg production and quality, physiology and health performance) on the productive lifespan of layers, from the pre-lay period (week 16) to the late lay period (week 48). This study is divided into 9 main chapters. Chapter 1 of the study outlines its background, justification, objectives, hypothesis and scope. Chapter 2 reviews the literature of studies on Noni and blood metabolite indicators. It provides perspectives on the use of noni fruit extract and phytogenics to enhance performance in poultry production. Chapter 3 provides a general description of the research methodology and design of the study. Chapter 4 presents the results, discussion and conclusion of the experiment to determine the yield, antioxidant concentration and effect of noni fruit extract on the body weight and pre-lay characteristics of pullets fed varying levels of noni fruit extract in drinking water. Chapter 5 provides the results, discussion and conclusion on the effect of noni fruit extract, and physiological stage on egg production performance and egg quality characteristics. Chapter 6 presents the results, discussion and conclusion of the experiment that examines the effect of noni fruit extract, and physiological stage on the

haematological and blood biochemical profiles of layers. In Chapter 7, the physiological stage (weeks 16 or 20) of administration of noni fruit extract to achieve the best effect was investigated. Chapter 8 provides the general discussions and Chapter 9 the general conclusions and recommendations of the whole study.

1.7 Limitations of the study

The research was conducted in a controlled environment, which may not accurately represent real-world situations, such as variable climates, housing systems, or feed availability. The study focused exclusively on specific dosages of noni fruit extract (0 mg/mL, 20 mg/mL, and 40 mg/mL), potentially overlooking a broader spectrum of effects. Additionally, the examination was limited to a single production cycle.

While several parameters were assessed, others, such as gut microbiota, and behavioural changes, were not investigated. The study also did not compare noni fruit extract with other feed additives, which limits insights into its relative efficacy. Furthermore, it did not assess the cost-effectiveness of using noni fruit extract, an essential aspect for practical adoption. Egg quality was evaluated under ambient storage conditions, while the effects of refrigeration or controlled environments were not examined.

Hence, addressing these limitations in future research could significantly enhance the applicability and robustness of the findings.

CHAPTER 2

2.0 GENERAL LITERATURE REVIEW

2.1 Poultry Production

2.1.1 Importance of poultry production

Poultry farming is essential to global agriculture and food systems. Processed poultry products significantly contribute to national economies through export revenues and domestic sales. Additionally, poultry production supports millions of jobs across various sectors, including farming, feed production, processing, logistics, and retail. For many, smallholder commercial poultry farming provides a pathway to improved livelihoods, as it offers low-capital entry points and quick returns on investment (Adei and Asante, 2012; Mottet and Tempio, 2017).

In Ghana, a closer look at commercially produced layer poultry reveals its vital role in enhancing food security and nutrition by providing table eggs and meat. These products serve as versatile sources of affordable, high-quality protein, vitamins, and minerals for households. Poultry farming acts as a major catalyst for economic growth and employment, creating opportunities in both urban and rural settings (Adei and Asante, 2012). It bolsters rural livelihoods, generating year-round income for feed mills and retail sectors, particularly in developing countries (Adei and Asante, 2012).

The demand for eggs remains relatively stable, although seasonal gluts can occur occasionally. Moreover, poultry farming promotes sustainable agriculture by utilizing resources efficiently and supplying manure to enhance soil health, all while contributing to the nutritional well-being of families (Kumar *et al.*, 2019).

2.1.2 Major constraints in poultry production

The prices of corn and soybean meal significantly affect production costs, as feed constitutes about 70% of total expenses. Smallholders often have limited resources to implement strict biosecurity measures, leading to gaps that can result in bird flu, Newcastle disease, and other respiratory infections, which cause significant losses. In intensive systems, water and energy costs (utilities) also impact operating expenses (Sonaiya, 2007; Adei and Asante, 2012). Underdeveloped infrastructure, such as limited cold chain facilities and poor egg handling, along with unreliable transportation in some areas (Antwi *et al.*, 2025), affects product quality. Fragmented supply chains lead to gluts and price fluctuations, reducing profit margins.

2.2 Useful phytogenics in poultry production

Some useful phytogenic feed additive used in poultry production includes; Thyme (*Thymbra spicata*), Rosemary (*Rosemarinus officinalis*), Cumin (*Cuminum cyminum* L.) seed oil, eucalyptus leaves, fennel seeds, black cumin seeds, green tea, essential oils, *Aerva lanata, Cynodon dactylon, Piper betle, Pulicaria gnaphalodes* powder, turmeric extract, *Nigella sativa* seeds, ginger (Zingiber officiale) rhizome powder or oil extracts (Abdelli *et al*., 2021). Researchers in Asia have used parts of the Noni plant such as the leaves and fruits to improve poultry production (Más-Toro *et al.*, 2015; Sunder *et al.*, 2016; Asmara *et al.*, 2019).

2.3 Biology of Noni (*Morinda citrifolia*)

2.3.1 Morphology

Generally, young noni plants (Plate 1) grow at the rate of 30 cm to 60 cm per year in height, depending on the environment to attain a height of 1 m to 3 m although at maturity it attain heights up to 6 m and yield up to 80,000 kg of fruit per hectare annually (Nelson, 2006). The stem is straight and smooth and turns brown at maturity. The leaves are 10 to 30 cm in length, large, glossy, and broadly elliptic arranged in an alternate pattern on the stem and often have a wavy margin. The upper side of the leaf is dark green while the underside is lighter (Nelson and Elevitch, 2006). It has small, fragrant flowers borne in clusters, typically appearing in axillary or terminal racemes, each flower has five petals that are white or creamy and feature a tubular corolla. Noni plants have a relatively shallow but extensive root system, aiding in their ability to thrive in poor soils. The roots are thick and fleshy, aiding in storing water and nutrients. Noni plants grow in mixed cropping systems throughout the Pacific as a tradition and have naturalised outside their native range in many locations throughout the tropics. However, it is rarely considered a pest (Nelson, 2006; Nelson and Elevitch, 2006).

Plate 2.1. Noni tree (www.Wikipedia.com)

2.3.2 Distribution and climate

The noni plant is widely distributed in both tropical and subtropical regions. It bears fruits throughout the year and is widely adapted to the tropics at altitudes of 1 to 800 m above sea level depending on the latitude. It tolerates mean annual temperatures of 20 – 35 ºC and annual rainfall of 250 - 4000 mm. It grows in association with a wide range of common littoral forest shrubs, as well as numerous cultivated plants. Noni grows in an extremely wide range of soils. It can grow in infertile, acidic, and alkaline soils and is accustomed to very dry to very wet areas. The noni plant grows naturally in relatively dry to mesic sites or lowland areas or as an important forest undergrowth species in Pacific Island forests and rainforests. Noni's extensive range of environmental tolerances also includes wind, fire, flooding, and saline exposure (Nelson, 2006; Heryanto *et al.*, 2023).

2.3.3 Description of the fruit

Morinda, the genus of the botanical name of noni was derived from the two Latin words *morus* (reference to the similarity of the fruit to true mulberry '*Morus alba*'), and *indicus* (meaning Indian). The species name indicates the resemblance of the plant foliage to that of some citrus species; the colour of the mature fruit is yellow and the shape is distinctively ovoid 'grenade-like' with a lumpy polygonal-shaped sectional surface (Plate 2.2). The fruit ranges between 4 – 12 cm in length, 3 – 8 cm in circumference, and 50 – 300 g in weight and contains numerous small drupes fused to its rough surface (Plate 2.3). Ripe noni fruit is soft and translucent–greyish and contains approximately 90 % water (Plate 2.4). The seeds are triangular-shaped, reddish-brown with an air sac for buoyancy (Plate 2.4; Nelson, 2006; Almeida *et al.*, 2019; Heryanto *et al.*, 2023).

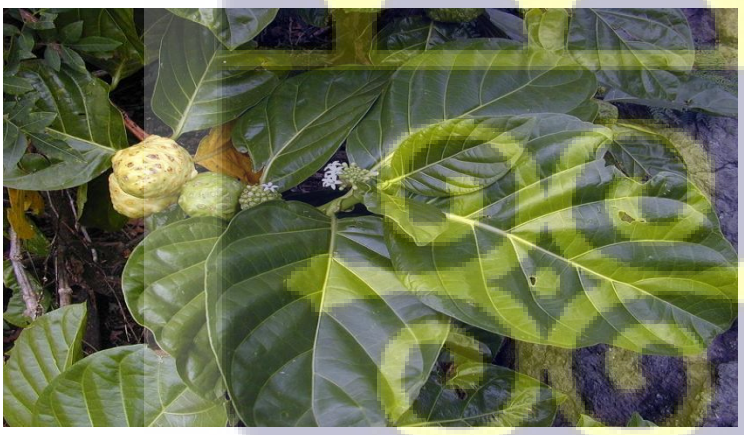


Plate 2.2. Mature fruit with flowers and leaves of noni (Ayunda *et al.*, 2020)

Plate 2.3. Cross section of noni fruit

(NCCIH, 2015)

Plate 2.4. Ripe noni fruit (NCCIH, 2015)

Plate 2.5. Noni fruit seeds (www.onszaden.com)

2.3.4 Composition of Noni fruit

According to Singh and Sharma (2020), noni contains almost 200 phytochemicals, although the complete phytochemical composition has not been reported to date. The chemical composition and their concentrations are related significantly to the plant parts and country of origin (Deng *et al.*, 2011). Heinicke (1985) reported that the active ingredient in the noni fruit, xeronine (an alkaloid) is not present in the fruit, but is formed after ingestion of the fruit or extract by the action of the enzyme proxeroninase on a natural precursor present in the noni fruit called proxeronine. Xeronine modulates the conformation and stability of specific proteins and restores their function.

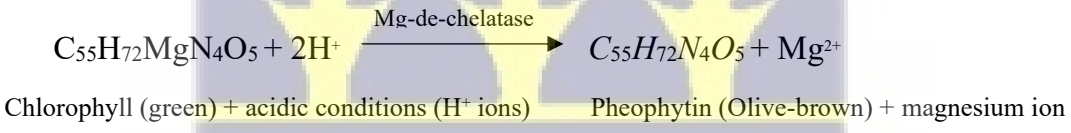
The nutritional and phytochemical composition of the fresh noni fruit is shown in Table 2.1

Table 2.1 Nutritional and phytochemical composition of fresh Noni fruit

Components	Composition	Author
Nutritional		
Water	90 %	Chunhieng *et al.* (2005)
Dry Matter (DM; 10%)	Soluble Solids: Fructose (5 %) Sucrose (1.3 %) Fibre (3.7 %)	Motshakeri and Ghazali (2015)
Protein (11.3% DM)	Mainly Aspartic acid and other amino acids	Singh and Sharma (2020)
Minerals (10 – 12% DM)	Mainly Potassium K^+ (30 – 150 ppm) Calcium, Sulphur, Magnesium, Sodium, Phosphorus, and traces of Selenium	Singh nd Sharma (2020)
Vitamins	Vitamin C (25 mg – 158 mg/100g DM) Pro-vitamin A, and Niacin	Yang *et al.* (2010) Luján *et al.* (2014)
Fatty acid	Mainly malic acid, Caprylic acids, and Caproic acids.	Luján *et al.* (2014)
Esters	Octanoic and Hexanoic acids, Methyl octanoate, Methyl decanoate	Deng *et al.* (2012)
Ketones and Lactones	2-heptanone and E-6-dodeceno-γ-lactone	
Phytochemicals		
Flavonoid glucosides	Acacetin 7-O-β-D- glucopyranoside, 9-epi-6α-methoxy geniposidic acid	Singh and Sharma (2020)
Anthraquinones	Morindafurone, 2,4-dimethoxy-9-anthrone, 1,8-dihydroxy-6-methoxy- 3-methyl-9-anthrone, Morinaphthalenone, Morinthone, 1, 3-dimethoxy-anthraquinone	(L. Z. Deng *et al*., 2019; B. Singh & Sharma, 2020)
Iridoids and Ligans	Lucidin 3-O-β-D-xylopyranosyl- (1-6)-β-D-glucopyranoside, nonioside L, nonioside M, N, nonioside, scopoletin	Singh and Sharma (2020) Mian-Ying *et al.*, 2008
Triterpenoids	Ursolic acid, β-sitosterol	Singh and Sharma (2020)
Polyphenols Coumarins	Scopoletin and Esculetin	Hsu and Yen (2007)

2.3.4.1 Colour Change in fermented Noni fruit extract

Chunhieng *et al.* (2005) reported that fresh noni fruit extract contains 0.29 mg of chlorophyll per gram, which is significantly more than the amount found in olive oil. Chlorophyll is highly sensitive to factors such as heat, light, oxygen, acidity, and enzymes, which can lead to its degradation and colour change (Özkan and Bilek, 2015). The main causes of the conversion of chlorophyll's colour from green to olive brown are acidic conditions and Mg-de-chelatase, an enzyme found in algae and plants (Marquez and Sinnecker, 2008). These changes occur due to the loss of the central magnesium atom in the chlorophyll's structure, which is replaced by hydrogen ions, leading to the transformation of chlorophyll's structure from native chlorophyll (green) to pheophytin, which exhibits an olive-brown colour as represented by fermented noni fruit extract.



2.4 Health and nutritional benefits of Noni fruit

2.4.1 Role of antioxidants in maintenance of health

The crux of metabolism (aerobic life) is the transfer of electrons from one atom to another referred to as oxidation because oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP (Kumar and Goel, 2019). Electron flow may become uncoupled (transfer of unpaired single electrons) thus generating oxygen-centred free radicals known as reactive oxygen species (ROS). This includes superoxide ($O_2^{\bullet-}$), peroxyl (ROO•), alkoxyl (RO•), hydroxyl (HO•), and nitric oxide (NO•). Hydroxyl

and alkoxyl free radicals are very reactive and rapidly attack the molecules in nearby cells damaging them but repair processes referred to as anti-oxidation are triggered to repair them (Pietta, 2000).

Unlike the hydroxyl and alkoxyl, the superoxide anion, lipid hydro-peroxides, and nitric oxide are less reactive. In addition to these ROS radicals, there are other ROS non-radicals in living organisms such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl) (Ames *et al.*, 1993). Some ROS are positive and are related to their involvement in energy production, phagocytosis, regulation of cell growth and intercellular signalling, and synthesis of biologically important compounds (Halliwell *et al.*, 1997). However, ROS may be very damaging, since they can attack lipids in cell membranes, proteins in tissues or enzymes, carbohydrates, and DNA, to induce oxidations that cause membrane damage, protein modification (including enzymes), and DNA damage (Kumar and Goel, 2019).

Noni fruits are rich in flavonoids and a range of other antioxidants (Blonska *et al*., 2004; Yang *et al*., 2010; Samarasiri *et al.*, 2019, 2022; Samarasinghe *et al*., 2023). Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilation actions (Blonska *et al.*, 2004; Mian-Ying *et al.*, 2008). Thus noni fruit extracts have the potential to reduce free radical formation and scavenge free radicals (Chan-Blanco *et al*., 2007; Yilmazer *et al*., 2016; Cimrin *et al*., 2019; Yu *et al.*, 2020; Samarasinghe *et al.*, 2023).

According to a study by Mhatre and Marar (2016), a school of thought believes that taking antioxidants during chemotherapy can reduce its effectiveness, as chemotherapeutic drugs

actively produce oxygen-derived free radicals that are blocked by antioxidants. They reported that the fruit extract, extracted from *Morinda citrifolia L.* fruit could reduce the toxic side effects of methotrexate (MTX) chemotherapy without affecting its effectiveness. Methotrexate (amethopterin) is a chemotherapy agent and immune-system suppressant used to treat cancer, autoimmune diseases and ectopic pregnancies (Bluett *et al.*, 2021). The anticancer effect of MTX does not rely on the formation of free radicals, thus the use of antioxidants can assist in lessening its side effects without compromising its efficacy. Therefore, using *Morinda citrifolia L.* extract concurrently with chemotherapy may increase the effectiveness of the chemotherapy treatment while reducing its harmfulness.

The polyphenols, mainly the coumarin group, scopoletin and esculetin have also been described for their free radical-scavenging activities (Lin *et al*., 2008; Sunder *et al.*, 2016) and for their anti-inflammatory activities in various models (Sousa *et al.*, 2017). The significant quantity of Vitamin C in the noni fruit has been reported to be present as a non-phenolic antioxidant. Iridoids are plant metabolites based on a monoterpene structure with a cyclopenta[c]puranoid skeleton. They provide a biogenetic and chemotaxonomic link between terpenes and alkaloids. The cleavage of the cyclopentane ring of iridoids produces secoiridoids. Feed additives that provide a rich source of secoiridoids have been used to provide immunomodulation benefits for broilers (Abou Assi *et al.*, 2017; Phillips *et al.*, 2023).

Generally, vitamin C, moisture, soluble protein, total carbohydrates, total acidity and phenolic compounds increased with maturation of the noni fruit (Luján *et al.*, 2014). Vitamin E and Niacin minerals, mainly potassium, manganese and selenium contribute to the

nutritional composition of the noni fruit (Mohd Zin *et al.*, 2002, 2007; West *et al.*, 2011; Wigati *et al.*, 2017).

2.4.2 Immunomodulation properties of Noni fruit extract

The phytochemical substances of noni have significant immunomodulatory ability to alter cell-mediated and humoral immunity (Lohani *et al.*, 2019). Noni fruit extract modulates the immune system by activating the cannabinoid 2 (CB_2) receptors and suppressing interleukin 4 (IL-4) and increasing the production of interferon (IFN)-gamma cytokines (Burke and Young, 2019). The noni fruit extract exerts beneficial immunomodulation effects in conditions involving inadequate immune responses (Bhatia *et al*., 2015). Noni-induced immunostimulatory activity is achieved by increasing nitric oxide (NO) production and the expression of IL - 1β, IL - 6, IL - 12, tumour necrosis factor-alpha (TNF - α,), interferon-gamma, (IFN - γ). Similarly, Lohani *et al*. (2019) reported that the immunostimulatory properties of noni have been attributed to facilitatory actions on intracellular signalling pathways involving extracellular signal-regulated kinase 1/2 (ERK1/2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).

Phytochemicals (flavonoids, lignans, iridoids, organic acid and anthraquinones) and polysaccharides (homo- and hetero-polysaccharides, pectic polysaccharides, arabinan and Type 1 arabinogalactan) present in noni enhance the immune response and suppress inflammation and can prevent a wide range of disease states or symptoms (Lohani *et al.*, 2019). Noni has been shown to reduce inflammation and tissue damage in ulcerative colitis with minimal side effects (Huang *et al.*, 2015). Noni augmented the anti-inflammatory mediators and significantly suppressed the pro-inflammatory cytokines in mouse models

(West *et al*., 2018). Low molecular weight anti-inflammatory phytochemicals of Noni could cross the blood-brain barrier and prove effective against neurodegenerative diseases (Lohani *et al.*, 2019). Several *in vitro* and *in vivo* studies have shown that Noni fruits have antioxidant, anti-inflammatory, anti-dementia, liver-protective, anticancer, analgesic, and immunomodulatory effects (Mian-Ying *et al.*, 2002; Muralidharan and Srikanth, 2010; Inada *et al.*, 2017; Ayunda *et al.*, 2020).

Sunder *et al*., (2016) reported that noni exerts its immunomodulatory effect by influencing the various components of the immune system of broiler chickens and significantly increasing the total antibody titers and reduced (75 %) the mortality of broilers challenged with the infectious bursal disease virus (IBDV).

2.4.3 Lipid metabolism of Noni fruit extract

2.4.3.1 Stimulation of lipid homeostasis by Noni fruit extract

Lipogenesis and lipolysis are the two primary metabolic events that take place in adipose tissues. They are coordinated to maintain lipid homeostasis under physiological conditions. During lipogenesis, non-esterified fatty acids (NEFAs) accumulate in white adipose tissue (WAT) and are then esterified into triacylglycerol by lipoprotein lipase (LPL), resulting in the synthesis of esterified fatty acids (EFAs). On the other hand, lipolysis is the mobilisation or hydrolysis of triglycerides (Inada *et al.*, 2017). During food deprivation, the body's demand for energy substrates is the primary cause of lipolysis, which results in the release of fatty acids from white adipose tissue. This process leads to a metabolic shift in energy-consuming tissues, from utilising glucose to using fatty acids. Moreover, the liver increases gluconeogenesis and glucose secretion, providing glucose to glucose-dependent cells and

tissues, thus ensuring their proper functioning (Grabner *et al.*, 2021). This physiological process can be stimulated by noni which acts as an appetite depressant (Aroche *et al.*, 2018).

2.4.3.2 Anti-obesity properties of Noni fruit extract

Nishioka (2007) observed lower adipose tissue weight and plasma triglyceride levels, and improved glucose tolerance in mice consuming a high-fat diet (HFD) plus noni fruit extract. The study suggested that noni fruit extract might have a positive impact on glucose and lipid metabolism. These observed beneficial effects were without any toxic effects and resulted in a lower final body weight compared to the high-fat diet (HFD) group. Numerous studies on rats, mice, and hamsters have reported the anti-obesity effects of noni fruit extract. These studies have revealed that the consumption of noni fruit extract can lead to a decrease in adipose tissue weight gain and an improvement in various metabolic parameters such as total cholesterol, low-density lipoprotein-cholesterol, glucose, insulin tolerance, fasting glucose levels, and hepatic insulin resistance (Inada *et al.*, 2017). Noni fruit extract, which contains high levels of phenolic acids like gentisic acid, p-hydroxybenzoic acid, and chlorogenic acid, is believed to be responsible for the fruit extract's effectiveness. Earlier research indicated that Phenolic acids (gentisic acid, p-hydroxybenzoic acid, and the derivative chlorogenic acid) and flavonoids (epicatechin, catechin, rutin, quercetin, and kaempferol) and several transcriptional factors, such as proliferator-activated receptor (PPAR)-γ and Cytosine-cytosine-adenosine-adenosine-thymidine/enhancer-binding proteins (C/EBPs), are involved in the early stage of adipocyte differentiation (Rosen *et al.*, 2000; Ramji and Foka, 2002). The PPAR-γ, for instance, influences glucose homeostasis and insulin sensitivity (Berger and Moller, 2002). Flavonoids and phenol acids inhibit adipogenesis in 3T3-L1 adipocytes (Hsu and Yen, 2007). 3T3-L1 is a fibroblast that was isolated from mouse embryo and has

the characteristics of both white and brown adipocytes and displays features of multiple adipocyte lineages (Morrison and McGee, 2015). Additionally, another flavonoid that was isolated from the fruit and leaves of *Morinda citrifolia* is kaempferol (Sang *et al.*, 2001; Shoeb *et al.*, 2016; Algenstaedt *et al.*, 2018; Pandy *et al.*, 2020). Kaempferol is the major component of soy leaf extract (SLE), and a recent study evaluated the anti-obesity effects of SLE extracts in HFD-obese male C57BL/6 mice (Inada *et al.*, 2017).

2.4.3.3 Cholesterol homeostasis properties of Noni fruit extract

The Noni fruit contains many antioxidants, such as scopoletin, nitric oxide, vitamin C, and vitamin A (Widjastuti *et al.*, 2019). These antioxidants are instrumental in stimulating the excretion of cholesterol through the droppings of chicken by increasing the secretion of bile and Nitric Oxide (NO) (Salleh *et al.*, 2002). Studies have shown that Noni fruit contains active substances like xeronine, which can reduce fat and cholesterol levels and play a crucial role in the formation of proteins for hormones (Heinicke, 1985). Hormones such as insulin can increase the number of hepatic and extrahepatic LDL (low-density lipoprotein) receptors, leading to a decrease in the concentration of cholesterol in the blood and tissues. This reduction is beneficial for reducing abdominal fat and improving the overall health of animal products while reducing production costs.

2.4.4 Enhanced gut integrity properties of Noni fruit extract

Zonula occludens proteins (ZO) are scaffolding proteins providing the structural anchor of the strands to the actin cytoskeleton of the cytoplasmic (cell-cell) surface junction of epithelial cells. They play an important role in the organisation of epithelial tissue. The family of zonula occludens proteins are ZO-1, ZO-2, and ZO-3 (Bauer *et al.*, 2010). ZO-1

is a peripheral membrane phosphoprotein with a molecular mass of 220 kilo Daltons [kDa] (Stevenson *et al.*, 1986), expressed in all epithelial and endothelial cells as well as in cell types lacking tight junctions (Howarth *et al.*, 1992). The ZO-1 levels appear to be early predictive markers in patients suffering from sepsis (Zhao *et al.*, 2016).

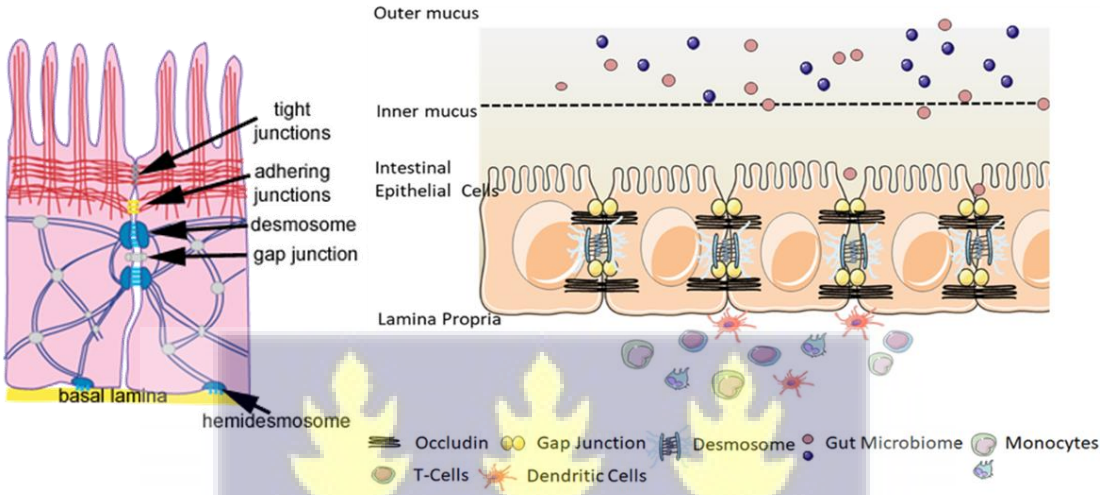


Plate 2.6. Structure of tight junction (Chelakkot *et al.*, 2018)

Tellez (2018) reported that noni increased the gene expression of ZO-1 and ZO-2 in broilers subjected to heat stress (HS) conditions. The role of ZO-1 and ZO-2 scaffolding proteins is to hold the tight junctions in certain locations to prevent permeability (Lu *et al.*, 2014).

The protein expression for both ZO-1 and ZO-2 decreased in those same broilers given a noni diet under heat stress (HS) conditions. It is important to note that ZO-2 protein expression decreased regardless of noni supplementation. When the amount of scaffolding proteins decreases, tight junctions (TJ; Plate 2.6) are no longer stabilised to certain crucial locations between cells and begin to fragmentise (Liao *et al.*, 2008) disrupting the intestinal

barrier and causing increased gut permeability and inducing leaky gut syndrome (Assimakopoulos, 2011).

2.4.5 Liver protection properties of Noni fruit extract

According to a study conducted by Mian-Ying *et al.* (2008), Noni fruit extract could be an effective way to protect the liver against damage caused by certain chemicals. The study involved six-week-old female Sprague-Dawley (SD) rats. It was found that Noni inhibited the inflammatory response and reduced the levels of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Elevated levels of these enzymes indicate damage to the liver cell membrane. Damaged liver cells release ALT and AST enzymes when exposed to toxins, making these enzyme levels good indicators of liver damage. Noni fruit extract prevented oxidative-pathological events caused by free radicals. Additionally, high doses of Noni did not lead to liver damage. This suggests that Noni fruit extract could potentially serve as a natural supplement to protect the liver from chemical-induced toxicity (hepatotoxicity).

2.5 Constituents of chicken blood

The blood of chicken is made up of plasma and cells containing nuclei and mitochondria, such as erythrocytes, leukocytes, and thrombocytes, which are similar to platelets in mammals (Scanes, 2022). Blood plays a critical role in the physiology of birds. It has several functions, including the transportation of respiratory gases such as oxygen and carbon dioxide, electrolytes such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, and PO_4^{3-}, nutrients like glucose, fatty acids, and amino acids, metabolites including lactate, waste compounds like urate and urea,

hormones, and heat (Scanes, 2022). Blood constituents such as antibodies, leukocytes offer protection to the body and aid in water balance, glucose, and electrolyte homeostasis (Scanes, 2022). In case of injury to blood vessels, blood also aids in clotting. However, blood can also carry pathogens and toxic substances. According to Benzo *et al.* (1986) and Scenes (2022), chicken blood has a viscosity of 27.6 x 10^{-4} Pas.

2.5.1 Blood haematological parameters of chicken

Haematological studies are crucial in assessing the condition of an animal's body. They are used to identify environmental, nutritional, and pathological factors that may impact an animal's health (Elagib and Ahmed, 2011; Graczyk, 2016; Sidharthan, 2023). The Full blood count (FBC) is one of the most frequently conducted blood tests (Sidharthan, 2023). The FBC measures several haematological parameters, such as the number of white and red blood cells, platelet count, packed cell volume (PCV), haemoglobin concentration, differential white blood count and other red blood cell indices (Plate 2.7). It is useful in the diagnosis of infections, anaemia, blood-related cancers, and inflammatory diseases (Sidharthan, 2023)

2.5.1.1 Physiological functions of red blood cell (RBC) in chicken

The main function of the red blood cells also known as erythrocytes is transportation and delivery of oxygen to peripheral tissues from the lungs (Moreno and Wiegand, 2014). This is facilitated by its biconcave circular disk-like shape, which increases its surface area and reduces its diffusion distance. Unlike mammalian erythrocytes, avian erythrocytes have nuclei and other intracellular organelles such as mitochondria (Sidharthan, 2023). The

normal physiological reference range of red blood cells for chicken is 2.50 - 3.90 x 10^6µL (Bounous and Stedman, 2000; Clinical Diagnostic Division, 1990).

High red blood cell (RBC) counts, or erythrocytosis, in chickens, can be caused by dehydration, leading to haemoconcentration and an apparent increase in RBC count due to a decrease in plasma volume (Chikumba *et al.*, 2013). Conditions that restrict oxygen availability, such as high altitude or chronic respiratory diseases, can trigger increased RBC production as the body compensates for reduced oxygen levels (Villafuerte *et al.*, 2022). Physical or environmental stress can also prompt erythropoiesis in chickens as part of the stress response *(*Abo-Al-Ela *et al.*, 2021). Certain infections can result in increased RBC production in chickens due to inflammation and the body's immune response (Silveira *et al.*, 2009). Furthermore, conditions involving elevated levels of erythropoietin in chickens, due to tumours or other endocrine pathologies, may stimulate erythropoiesis (Thomas, 2024).

On the other hand, low RBC count in chickens, known as anaemia, can be caused by various factors including iron deficiency. Iron is crucial for haemoglobin synthesis; Inadequate dietary iron intake can result in low red blood cell count (hypochromic) that results in microcytic anaemia. Parasitic infections, such as coccidiosis and haemo-protozoa, can cause anaemia through intestinal bleeding or malabsorption of dietary iron (AL-Saegh, 2018; Erhabor *et al.*, 2021). Diseases like Infectious Bursal Disease (IBD) and Avian Leukosis can disrupt normal haemopoiesis (Chaves, 2014). Conditions affecting bone marrow function, like aplastic anaemia or neoplasia, can significantly reduce RBC production. Exposure to certain chemicals, such as lead or other heavy metals, can lead to haemolytic anaemia in birds. Additionally, genetic predispositions affecting haematopoiesis and conditions

destroying RBCs, such as autoimmune disorders or certain infectious agents, can lead to decreased RBC survival.

The RBC differentials evaluate the oxygen-carrying capacity and production of red blood cells. They include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

The MCV is the measure of the average red blood cell size. The normal physiological range for chicken is 90.0 - 140.0 fL (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000).

The MCH measures the average amount of haemoglobin per red blood cell. The normal physiological range for chicken is 33.0 – 47.0 pg of blood (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000).

The MCHC is the haemoglobin concentration per red blood cell. The normal physiological range for the chicken is 26.0 – 35.0 g/dL (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000).

2.5.1.2 Physiological functions of white blood cell (WBC) in chicken

The white blood cell (WBC) count measures the number of white blood cells in the body. White blood cells are responsible for defending the body against diseases and infectious agents (Moore *et al.*, 2016). Werman and Brown (1986) reported that two populations of WBC are crucial for the body's immune response. Their development occurs in the bone marrow and lymphoid tissues. They are the granulocytes (heterophils, eosinophils, basophils, and monocytes) and the lymphocytes (β cells, T cells, and Neutral Killer cells). The

granulocytic cells are primarily involved in the phagocytic response to local inflammation and infection and are generated in the bone marrow (Ziaei *et al.*, 2021). The lymphocytic cells assist the immune system in identifying antigens (viruses, bacteria and cancer) and fighting them. Lymphocytes are generated in the bone marrow and are released into the blood and lymphatic system (Werman and Brown, 1986).

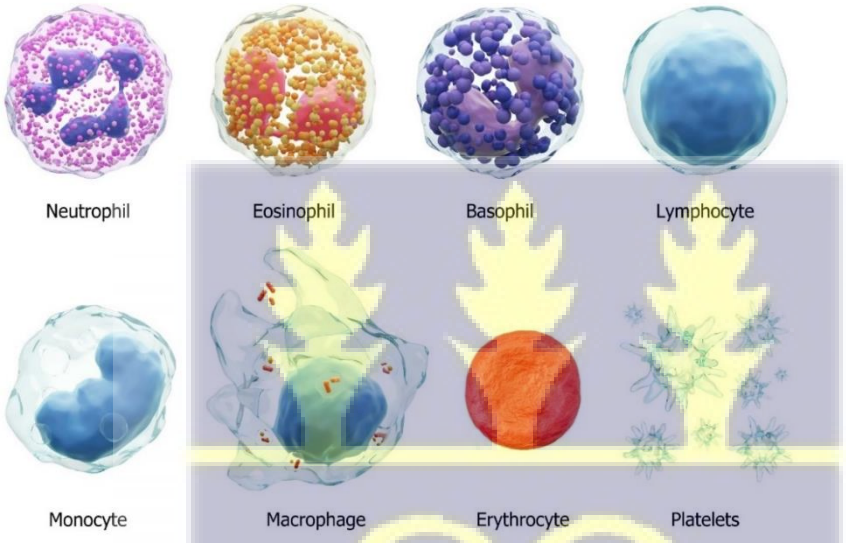


Plate 2.7. Blood Cells (Sidharthan, 2023)

The normal physiological range of WBC, Heterophil, Basophil, Eosinophils, Monocytes and Lymphocytes are 1.9 -9.5 x 10^9/L, 29.0-48.70 %, 0.0 - 6.4%, 0.0-11.51 %, 0.0-6.5 %, and 26.9 - 70.6 % respectively (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000).

2.5.1.3 Physiological functions of thrombocytes in chicken

Thrombocytes are small, disc-shaped cell fragments in the blood that play a crucial role in haemostasis (the process of stopping bleeding) and wound healing. They are derived from megakaryocytes in the bone marrow and are essential for blood clotting (Scanes, 2022). Thrombocytes adhere to damaged blood vessel walls and each other, forming a temporary "platelet plug" to stop bleeding. They release substances that help in tissue repair and regeneration and provide surfaces for the activation of various proteins involved in the coagulation cascade, contributing to the formation of a stable blood clot. The normal physiological thrombocyte counts range from 3.0 - 33.0 x 10^9/L of blood (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000).

Higher than normal values may be a result of various conditions such as inflammation, iron deficiency anaemia, or splenectomy (removal of the spleen). These may lead to a greater risk of abnormal blood clotting (thrombosis). Lower than normal values (Thrombocytopenia) can result from decreased production (bone marrow disorders), increased destruction (conditions like autoimmune diseases or certain infections), or an enlarged spleen (Benzo *et al.*, 1986). These may lead to an increase in the risk of bleeding and bruising (Scanes, 2022).

2.5.1.4 Physiological functions of haemoglobin (Hb) in chicken

Haemoglobin is a protein found within red blood cells (erythrocytes). It transports oxygen from the lungs (or air sacs in birds) to the tissues and facilitates the return of carbon dioxide from tissues to the lungs for exhalation. It is critical in maintaining the animal's metabolic needs and energy levels (Scanes, 2022). Normal haemoglobin levels in chickens typically

range from 7.0 to 13.0 g/dL (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000).

Increased haemoglobin levels may be due to dehydration, erythrocytosis (increased red blood cell production), or chronic conditions that require increased oxygen delivery, such as high-altitude adaptation. These may lead to increased blood viscosity, which may strain the heart and increase the risk of clotting (Villafuerte *et al.*, 2022). Reduced haemoglobin levels may result from anaemia due to blood loss, nutritional deficiencies (such as iron, vitamin B_{12}, or folic acid), infections, or bone marrow disorders. These may lead to reduced oxygen transport capacity, resulting in lethargy, weakness, and impaired growth or productivity in chickens (Benzo *et al.*, 1986; Scanes, 2022).

2.5.1.5 Physiological functions of packed cell volume (PCV) in chicken

Packed Cell Volume (PCV) or Haematocrit measures the proportion of blood volume that is occupied by red blood cells. It is an indicator of overall blood cell concentration and is used to assess hydration status and oxygen-carrying capacity (Villafuerte *et al.*, 2022). In chickens, normal PCV levels usually range from 22.0 % to 35 % (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000).

Higher than normal values for PCV (Polycythaemia) may be due to due to dehydration (concentration of red blood cells increases when plasma volume decreases), respiratory diseases that hinder oxygenation, or other conditions where the body compensates by producing more red blood cells (Scanes, 2022; Villafuerte *et al.*, 2022). These may lead to thickened blood and an increased risk of clots, leading to potential cardiovascular issues.

Lower than normal values may signify anaemia, blood loss, haemolysis (destruction of red blood cells), or insufficient production due to nutritional deficiencies or bone marrow problems (Scanes, 2022). A low PCV level can indicate inadequate red blood cell volume, leading to decreased oxygen transport and potential health issues in the chicken.

2.5.2 Effect of Noni on haematological parameters in chicken

Mhatre and Marar (2016) reported that administration of noni fruit extract could lower lipid peroxide levels in the blood, which may provide protection against a decline in leukocyte count, haemoglobin level, and mean osmotic fragility of erythrocytes. Mhatre and Marar (2016) assessed the haematological and biochemical profile of rats treated with methotrexate (MTX), to determine the potential of noni fruit extract treatment in reducing the harmful effects of MTX chemotherapy. The authors reported that MTX caused a decrease in RBCs, WBCs, and thrombocytes due to oxidative injury, leading to the premature death of RBCs and a subsequent decrease in haemoglobin levels in the control group. However, in the experimental group, pre-treatment with noni fruit extract had a beneficial effect by restoring the levels of RBCs, WBCs, and haemoglobin. This suggests that noni fruit extract has an ameliorative effect in preventing MTX-induced bone marrow suppression.

In another study, Adriani *et al.* (2017) found that incorporating noni-fruit flour into the diet of quail birds had a beneficial effect in reducing both oxidative stress and oxidative damage. This was evidenced by a reduction in malondialdehyde (MDA) levels and an increase in the number of erythrocytes and concentration of haemoglobin.

Widjastuti *et al.* (2019) reported that noni fruit extract may be used up to 5 ml/litre in drinking water without affecting Sentul chicken health. They observed that the haematological indicators (erythrocytes = 2.01 x 106 - 2.67 x 106/mm^3, leukocytes = 21.30 - 23.45 x 103/mm^3, haemoglobin 10.25 - 13.85 g/dL, and haematocrit = 27.53 - 32.41%) were not affected and remained within the normal physiological range for chicken.

2.5.3 Blood biochemical profile of chicken serum

The biochemical profile of chicken serum includes various parameters that help assess the overall health, organ function, and metabolic status (Harvey and Bruss, 1997) of poultry. Normal physiological reference ranges can vary depending on factors such as breed, age, sex, and the specifics of the laboratory methods used (Hagan *et al.*, 2022). The key components are Total Protein (TP), Albumin (ALB), Globulin (GLB), Albumin/Globulin ratio (ALB/GLB), Creatinine (CRE), Urea (U), Uric Acid (UA). Others are Total Bilirubin (TB), Direct Bilirubin (DB), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), AST/ALT ratio, Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), Total Cholesterol (TCHOL), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), Triglycerides (TG), Glucose (GLU), Calcium (Ca^{2+}), Sodium (Na^{+}), Chloride (Cl^{-}), and Potassium (K^{+}).

2.5.3.1 Physiological characteristics of serum liver enzymes in chicken

Aspartate Aminotransferase (AST) is an enzyme involved in amino acid metabolism and is found in liver and muscle tissues (Benzo *et al.*, 1986; Scanes, 2022). Its normal physiological range in chicken is 9.0 – 49.0 U/L (Clinical Diagnostic Division, 1990). Elevations can

indicate liver disease or muscle injury while low levels may indicate lower liver metabolic activity (Scanes, 2022).

Alanine Aminotransferase (ALT) is an enzyme that facilitates amino acid metabolism, often found in liver tissues (Scanes, 2022). The normal physiological range for Alanine Aminotransferase for chicken is 10.0 – 109.0 U/L (Clinical Diagnostic Division, 1990). Elevated levels beyond the normal physiological range suggest liver damage or dysfunction (e.g., hepatic necrosis or hepatitis). Lower than normal levels may indicate reduced liver function (Chikumba *et al.*, 2013)./The ratio of serum aspartate to alanine aminotransferase levels is used as a clue to the aetiology of underlying liver diseases (Chikumba *et al.*, 2013). The normal physiological range for Aspartate/Alanine Aminotransferase ratio (AST/ALT ratio) for chicken is 1.0 – 1.2 (Clinical Diagnostic Division, 1990). A higher than normal level could be a sign of hepatocellular damage associated with reactive oxygen species (ROS) production and a lower than normal value indicates less liver damage and fewer liver problems (Hong *et al.*, 2021; Azi *et al.*, 2023).

The ALP and GGT enzymes are useful markers in assessing chickens' liver health and metabolic functions (Scanes, 2022). Elevated levels of these enzymes often indicate liver or biliary dysfunction and may necessitate further diagnostic assessments. The normal physiological ranges of ALP and GGT are 1.0 – 114.0 U/L and 3.0 – 19.0 U/L respectively (Clinical Diagnostic Division, 1990).

2.5.3.2 Physiological characteristics of serum proteins in chicken

The serum proteins include total proteins, albumin, and globulin (Filipović *et al.*, 2007). They play roles in maintenance of homeostasis through the maintenance of colloid osmotic

pressure, such as in the transport of minerals and hormones, in building of enzymes, and in the immune system of the organism. The relation between the total proteins, albumin, and globulin fractions reflects the functional, metabolic and health status of birds (Tóthová *et al.*, 2016).

Total Protein indicates the overall protein levels in the serum, derived from albumin and globulin (Tóthová *et al.*, 2019). The normal physiological range of total protein for chicken is 54.0 – 75.0 g/L (Clinical Diagnostic Division, 1990; Harr, 2002). Higher than normal total protein levels exceeding 75.5 g/dL in the serum of laying hens suggest hyperproteinemia, which may indicate dehydration, inflammation, or organ disease (e.g., liver dysfunction). A lower than normal value often suggests malnutrition, liver disease, or possible immunodeficiency (Scanes, 2022).

Albumin maintains osmotic pressure and transports various substances in serum (Tóthová et al., 2016). Its normal physiological range in chicken is 23.0 – 31.0 g/L (Clinical Diagnostic Division, 1990). A high range indicates dehydration or an inflammatory response; a low range suggests liver dysfunction, kidney disease, or malnutrition.

Globulin comprises various proteins involved in immune response, binding proteins, and enzymes (Tóthová *et al.*, 2016; 2019) Its normal physiological range in chicken is 0.00 – 0.45g/L (Clinical Diagnostic Division, 1990; Harr, 2002). A higher than normal serum globulin level may indicate hyperglobulinemia, which may suggest issues like inflammation, infections, autoimmune disorders, or cancer (Mian-Ying and Su, 2001). A lower than normal range may indicate immunodeficiency, liver dysfunction, or excessive protein loss.

An Albumin/Globulin ratio is used to assess the relative levels of albumin and globulin in plasma proteins. The normal physiological range for chicken is 0.0 -10.0 (Clinical Diagnostic Division, 1990; Harr, 2002). A higher than normal Albumin/Globulin ratio may suggest unusually low globulin levels resulting from certain acute illnesses or specific types of cancer. A lower than normal Albumin/Globulin ratio, on the other hand, may indicate conditions such as chronic inflammation, liver disease, kidney disease, or immune disorders, where globulin levels are elevated or albumin levels are decreased (Tóthová *et al.*, 2016; Scanes, 2022).

2.5.3.3 Physiological characteristics of serum bilirubin in chicken

Bilirubin is categorised into two primary forms: total bilirubin (which includes both direct and indirect forms) and direct (conjugated) bilirubin (Creeden *et al.*, 2021)

They serve as an indicator of the overall bilirubin production and excretion in the body, reflecting the breakdown of haemoglobin from red blood cells. They help to evaluate liver function and the efficiency of the bilirubin excretion pathway as well as protect against oxidative stress and cellular damage (Zaefarian *et al.*, 2019; Creeden *et al.*, 2021). The normal physiological range for total bilirubin for chicken is 0.0 – 5.1 µmol/L (Clinical Diagnostic Division, 1990). Elevated total bilirubin levels may be indicative of liver diseases such as hepatitis or fatty liver syndrome. This suggests impaired processing and excretion of bilirubin, and increased breakdown of red blood cells (haemolysis), resulting in more bilirubin being produced than the liver can effectively handle. Alternatively, elevated levels could indicate a blockage in the bile duct, preventing the normal excretion of bilirubin. Normal levels, on the other hand, indicate efficient metabolism and excretion of bilirubin.

Extremely low levels of total bilirubin may suggest malnutrition or inadequate intake of nutrients necessary for blood cell production and liver function (Zaefarian *et al.*, 2019)

Direct Bilirubin also known as conjugated bilirubin, is the form that has been processed by the liver and is water-soluble (Zaefarian *et al.*, 2019) It is readily excreted through bile into the intestine, where it aids in fat digestion and absorption (Zaefarian *et al.*, 2019). Elevated levels of direct bilirubin can serve as markers of liver dysfunction or bile duct obstruction, highlighting issues that affect biliary excretion (Zaefarian *et al.*, 2019). Normal levels of direct bilirubin typically indicate normal liver function and efficient processing and excretion of bilirubin. The normal physiological range for chicken is 1.0 – 2.0 µmol/L (Clinical Diagnostic Division, 1990).

2.5.3.4 Physiological characteristics of serum lipid profile in chicken

The serum lipid profile is an important tool for monitoring the overall health of chickens. It helps detect metabolic disorders and provides insights into the birds' nutritional status. The lipid profile is used to inform optimal diet formulation, and correlate lipid levels with growth rates and feed efficiency (Scanes, 2022). Diagnosed health issues or stress conditions aid in early detection, evaluate the reproductive health of breeding hens for optimal egg production, assess the health status of breeding stock to guide selection and detect lipid metabolism changes due to environmental stressors and conditions, such as fatty liver syndrome through abnormal lipid levels (Scanes, 2022). The components of the serum lipid profile are triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein, and very low-density lipoprotein.

2.3.3.4.1 Physiological characteristics of triglycerides in chicken

Triglycerides serve as the primary form of fat storage, providing energy reserves during growth, reproduction, or stress (Benzo *et al.*, 1986; Scanes, 2022). They act as insulators to maintain body temperature and protect vital organs, contribute to cell membrane integrity, and influence cell function and transport.

Triglycerides serve as precursors for hormones and signalling lipids. They are involved in fat metabolism and energy production through the breakdown of fatty acids and glycerol (Benzo *et al*., 1986; Scanes, 2022). The normal physiological range for chicken is 0.20 – 2.80 mmol/L (Clinical Diagnostic Division, 1990). Higher than normal levels lead to obesity, metabolic disorders, liver dysfunction, stress, decreased fertility, and compromised immunity. However, lower than normal levels, are a result of malnutrition, chronic diseases, or hyperthyroidism. These may lead to inadequate energy reserves affecting growth and health (Benzo *et al.,* 1986; Scanes, 2022).

2.3.3.4.2 Cholesterol and Lipoproteins

Total Cholesterol is a key component of cell membranes, contributing to membrane fluidity and stability. It is a precursor for the synthesis of steroid hormones such as oestrogen, testosterone, and cortisol (Scanes, 2022). Cholesterol is essential for the production of bile acids, which aid in digestion and absorption of dietary fats. The normal physiological range for chicken is 3.34 – 7.7 mmol/L (Clinical Diagnostic Division, 1990; Bueno *et al*., 2017).

A higher than normal range may indicate metabolic disorders, fatty liver syndrome, or other stress-related conditions. It can also be a sign of poor diet, leading to increased fat deposition.

Lower than normal range may suggest malnutrition, chronic disease, or stress which can lead to hormonal imbalances and reduced immune function (Scanes, 2022).

High-density lipoprotein transports cholesterol from the tissues back to the liver for disposal or recycling hence referred to as "good" cholesterol because it helps to reduce the risk of cardiovascular disease (Scanes, 2022). The normal physiological range for chicken is 0.0 - 10.0 mg/dL (Clinical Diagnostic Division, 1990; Bueno *et al.*, 2017). Normal ranges of high-density lipoproteins are generally considered protective against cardiovascular diseases and may be indicative of a healthy diet, particularly one high in unsaturated fats, while a lower than normal range is associated with a higher risk of cardiovascular diseases and metabolic issues and may suggest an imbalance in dietary intake or a state of stress (Scanes, 2022). A lower than normal range may also indicate inadequate intake of dietary fats or an overall deficiency in caloric intake, which can affect growth and productivity. It may also mean that energy reserves are low, which can compromise the bird's ability to cope with stress, disease, or growth demands. It may also suggest liver dysfunction (Scanes, 2022).

Low-density lipoprotein delivers cholesterol from the liver to peripheral tissues for membrane synthesis, steroid production, and several cellular functions (Scanes, 2022). It is often referred to as "bad" cholesterol, and it can contribute to plaque formation in vessels if levels are excessively high (Scanes, 2022).. The normal physiological range is 0.0 – 10.0mmol/L (Clinical Diagnostic Division, 1990; Bueno *et al*., 2017). A higher than normal range indicates an increased risk of atherosclerosis and cardiovascular diseases. It is associated with dietary imbalances or obesity. Normal ranges are generally seen as

beneficial, indicating a healthier lipid metabolism in birds while low levels may impair tissue repair or hormone production (Scanes, 2022)..

Very low-density lipoprotein (VLDL) is primarily responsible for the transport of triglycerides synthesised in the liver to peripheral tissues, where they are used for energy or stored in adipose tissue (Scanes, 2022). They carry a proportion of cholesterol. As VLDL travels through the bloodstream, it interacts with enzymes that break down triglycerides, converting VLDL into LDL. The VLDL plays a major role in providing energy for growth and maintenance in poultry by carrying triglycerides to tissues and can reflect the metabolic state of the bird and may indicate how effectively they are utilising energy resources (Scanes, 2022). The normal physiological range of the VLDL for chicken is 0.0 - 10.0 mmol/L (Clinical Diagnostic Division, 1990). Implications of higher than normal range can suggest excess lipid accumulation in the liver, leading to fatty liver syndrome, which is common in intensively raised poultry (Benzo *et al.*, 1986; Scanes, 2022). This condition can impair liver function and has negative implications for overall health. It may also indicate underlying metabolic disorders or poor nutrition (e.g. excess energy-dense diets).

2.5.3.5 Physiological characteristics of serum urea and uric acid in chicken

Urea and uric acid are two important nitrogenous waste products that result from protein metabolism in poultry. They play significant roles in nitrogen excretion and overall metabolic processes (Scanes, 2022). Urea is a water-soluble compound formed in the liver from ammonia, primarily from the breakdown of proteins. Urea serves as a major method of nitrogen excretion in birds that have a mixed diet, especially in species that have more mammalian metabolic pathways (Assefa and Tesfaye, 2022). Urea requires water for

excretion, which is relevant to the overall fluid management in poultry, particularly in commercial operations where water availability is crucial. It reflects the dietary intake of protein; higher protein intake typically leads to increased urea synthesis (Assefa and Tesfaye, 2022). The normal physiological range for urea in chicken is 2.9 – 10.0 mg/dL (Clinical Diagnostic Division, 1990). According to Lin *et al.* (2017), elevated levels of urea may indicate impaired kidney function, suggesting that the kidneys are not effectively excreting urea. It may also result from increased dietary protein intake, which generates higher amounts of nitrogenous wastes or dehydration, as low water intake can concentrate urea in the bloodstream. Lower levels of urea than normal can signify inadequate protein intake or malnutrition, as there is insufficient protein breakdown to produce urea. Normal urea levels are indicative of effective renal function allowing for efficient nitrogen water excretion (Scanes, 2022).

Uric acid is a nitrogenous waste product formed from the breakdown of purines and is less soluble than urea. Birds excrete uric acid as a paste to conserve water. Uric acid is excreted with minimal water loss, making it an efficient waste product for birds in arid environments where water conservation is vital (Scanes, 2022). As the primary nitrogenous waste product in birds, uric acid contributes significantly to the elimination of excess nitrogen from the body. Uric acid has antioxidant properties and can play a role in protecting tissues from oxidative damage. The normal physiological range is 1.9 – 12.5 mg/dL (Clinical Diagnostic Division, 1990).

Similar to urea, elevated uric acid levels than normal can indicate dehydration, as birds conserve water and produce less dilute wastes (Assefa and Tesfaye, 2022). High uric acid

levels than normal may indicate renal problems, where the kidneys fail to eliminate uric acid effectively, leading to its accumulation while lower levels of uric acid than normal may suggest a diet low in protein or purines, which means reduced breakdown of nitrogenous compounds (Scanes, 2022).

2.5.3.6 Physiological characteristics of serum creatinine in chicken

Creatinine is a nitrogenous waste product formed from the normal metabolism of creatine, a compound important for energy production in muscle cells (Scanes, 2022). In poultry, creatinine serves several key functions and acts as an important indicator of kidney function and overall health (Benzo *et al.*, 1986; Scanes, 2022). Creatinine is produced from creatine phosphate, which is stored in muscle tissue and used for quick energy during muscle contractions. Since it is primarily linked to muscle metabolism, its levels can reflect muscular health and activity (Benzo *et al.*, 1986). The kidneys mostly excrete creatinine; consequently, its concentration in the blood can be a valuable marker for assessing renal function. Elevated levels than normal may suggest impaired kidney function or reduced clearance (Benzo *et al*., 1986; Scanes, 2022). It can also be an indicator of protein metabolism and overall nutritional status and therefore can provide insight into whether the poultry are receiving adequate protein in their diet (Benzo *et al.*, 1986). The normal physiological range for creatinine in chicken is 0.9 – 1.8 µmol/L (Clinical Diagnostic Division, 1990).

Normal creatinine levels can indicate effective kidney function. The kidneys efficiently filter and excrete creatinine. Lower than normal creatinine levels may occur in birds with low muscle mass or in cases of muscle-wasting diseases, where there is less creatine available for conversion to creatinine (Benzo *et al*., 1986; Scanes, 2022). Additionally, reduced dietary

protein or malnutrition can lead to lower production of creatinine, reflecting inadequate muscle development or energy reserves.

2.5.3.7 Physiological characteristics of serum minerals in chicken

Calcium (Ca) is crucial for the formation and maintenance of strong bones and skeletal structures in poultry, especially during growth and development (Onyango *et al.*, 2003). Ca plays a vital role in eggshell formation. Hens require adequate Ca to produce eggs with strong, intact shells (Zhang and Coon, 1997; Dumoulin, 2018). Ca is essential for the coagulation of blood, contributing to the haemostatic process following injury. It is involved in muscle contraction and relaxation, affecting overall mobility and physical activity in poultry (Benzo *et al.*, 1986). Ca is involved in neurotransmitter release and proper nerve function, facilitating communication between muscles and the nervous system. The normal physiological range for Ca in chicken is 2.2 - 3.0 mmol/L. (Clinical Diagnostic Division, 1990).

Elevated Ca levels than normal may indicate hypercalcemia, which may result from dietary imbalances or disorders such as nutritional secondary hyperparathyroidism, and kidney damage due to the deposition of calcium salts in tissues and organs, negatively affecting eggshell quality and may lead to production issues (Benzo *et al.*, 1986).

Lower levels of Ca than normal can result in poor bone development, increased susceptibility to fractures, and weak eggshells. Insufficient calcium can lead to decreased egg production or eggs with thin shells, influencing overall poultry farming efficiency (Benzo *et al.*, 1986).

Potassium K play a critical role in maintaining fluid balance and osmotic pressure within cells and tissues. It is essential for proper nerve impulse transmission, contributing to muscle movement and coordination. K helps regulate muscle contractions, influencing overall locomotion and physical performance. It aids in maintaining the acid-base equilibrium in the body, which is vital for various metabolic processes (Echols, 2006). Adequate K levels are important for cardiovascular function, helping to regulate heart rhythms (Echols, 2006), The normal physiological range for chicken is 3.5 – 5.2 mmol/L (Clinical Diagnostic Division, 1990). Elevated levels of K than normal (hyperkalaemia) can disrupt normal heart function and lead to arrhythmias (irregular rhythm of heartbeat). High K levels can also suggest kidney problems, where the kidneys cannot adequately excrete K from the body. For the more excessive K can lead to metabolic disorders and muscle dysfunction (Echols, 2006; Scanes, 2022).

Lower than normal K levels can cause hypokalaemia, leading to weak muscles, reduced growth, and poor overall health. Insufficient K in the body can affect nerve transmission and muscle contractions, impairing coordination and mobility. Low K levels can contribute to metabolic alkalosis, leading to disturbances in acid-base balance (Echols, 2006; Scanes, 2022).

Sodium (Na) is vital for fluid balance and nerve transmission (Scanes, 2022). The normal physiological range for chicken is 139.0 – 155.0 mmol/L (Clinical Diagnostic Division, 1990). Higher than normal values may indicate dehydration or renal failure while lower levels than normal can reflect excessive fluid loss or kidney problems (Echols, 2006; Scanes, 2022).

Chloride (Cl) assist in maintaining fluid balance and acid-base status (Scanes, 2022). The normal physiological range for chloride in chicken is 108.0 – 124.0 mmol/L (Clinical Diagnostic Division, 1990). A higher than normal value may indicate dehydration or renal problems while lower than normal values may suggest various disturbances, including metabolic alkalosis (Scanes, 2022).

2.5.3.8 Physiological characteristics of serum glucose in chicken

Glucose serves as a primary energy source for cellular metabolism (Kuzmina *et al.*, 2021). The normal physiological range for chicken is 4.2 – 6.6 mmol/L (Clinical Diagnostic Division, 1990). A higher value than the normal physiological range could indicate stress or other metabolic disorders while a lower value may suggest starvation, liver disease, or other metabolic problems (Grabner *et al*., 2021; Kuzmina *et al.*, 2021).

2.5.4 Effect of Noni on blood biochemical profiles in birds

Serum biochemical indices help to explain nutrient metabolism and the body's physiological status (Toghyani *et al.*, 2012). Sunder *et al.* (2011a) observed that noni fruit extract decreased the level of total protein, serum creatinine, and albumin in poultry but levels were within the normal physiological range. Also, Sunder *et al.* (2016) reported of lower concentrations of protein, albumin and creatinine in poultry fed noni fruit extract compared to their counterparts on the control treatment without noni fruit extract. However, the concentration of the above serum biochemical constituents was within the normal range of values for poultry.

Feeding noni fruit extract to birds was observed to improve immune status (Sunder *et al.*, 2015a). Noni fruit extract contains phytochemicals and polysaccharides which enhance its immune stimulating properties enabling it to exhibit immunostimulatory activity on T and ß lymphocytes (Kamiya *et al*., 2004; Nayak and Mengi, 2010; Lohani *et al.*, 2019). Sunder *et al.*, (2011a) reported that feeding noni fruit extract to Nicobari fowl reduced blood cholesterol level. This may be due to the phytochemicals (eg. 3, 3-bisdementhylpinoresinol, amercanol A, americanin A, americanoic acid A, morindolin, and isoprincepin) that inhibit the enzyme HMG-COA Reductase (3-hydroxy-3-methyl-glutaryl-COA reductase) and beta-sitosterol, a plant sterol both with potential for anti-cholesterol activity (Mian-Ying *et al*., 2002; Kamiya *et al*., 2004; Palu *et al*., 2012).

2.6 Pre-Lay sexual development

2.6.1 Body weight gain (BWG)

Body weight is a key factor in managing layers to influence the future performance of birds. Consequently, their body weight should be controlled throughout the whole life of layer flocks. Management of nutrition and lighting programs in particular assist to control body weight and allow the birds express their full genetic potential (Dumoulin, 2018). The active substances in Noni fruit, including polysaccharide, scopoletin, ascorbic acid, β-carotene, L-arginine, proxeronine, and proxeroninase, are essential for the metabolic activity that supports the addition of weight, ultimately resulting in a high percentage of the carcass (Ali *et al.*, 2016; Widjastuti *et al.*, 2019).

Sunder *et al.* (2011a) tested the growth enhancing ability of the noni fruit in the local Nicobari fowl; an indigenous poultry bird of Andaman and Nicobar Islands, India. The body

weight gain, feed conversion ratio and feed efficiency of the noni-fed group had a performance index superior (93.6 ± 16.15) to the control groups. Similarly, the highest dressing percentage was obtained in the noni-fed group (69.05 %) than in the control (68.38 %). The highest body weight gain occurred from 3 to 4 months of age which is the growing phase of the bird during which the bird attained more body weight gain than compared to the other phases of the growth (Sunder *et al.*, 2011a; Ahlawat *et al.*, 2004).

Dumoulin (2018) reported that the growth of layer birds is not consistent throughout their development. The most rapid growth occurs between weeks 6 and 11, followed by a gradual deceleration until around 16 weeks. During this time, growth experiences a slight increase due to the interplay of pre-lay calcium increase, medullary bone deposition, and uterus maturation. Afterwards, growth slows down once more until the point of lay (Figure 2.1).

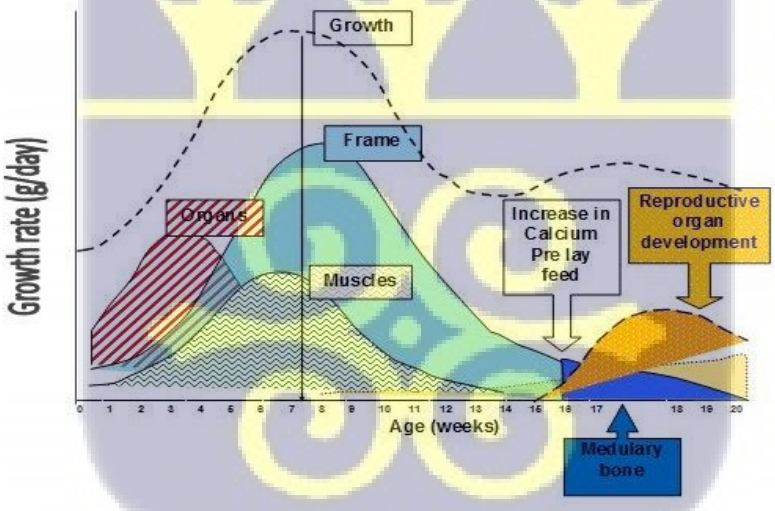


Figure 2.1. Growth rate (g/day) to the point of lay (Dumoulin, 2018)

The deposition of calcium in the medullary bones is important for strong eggshell quality. Throughout the rearing period, it is imperative to aim for optimal body weight of the birds between 4 - 5 weeks old. This stage is when their organs and muscles are developing at their peak, making it a critical time for growth. Establishing consistent uniformity among the birds at the end of the rearing period is essential. This guarantees that each bird has received proper management decisions, including their feeding regimen (Dumoulin, 2018).

2.6.2 Uterus development and egg production

The rate of development of the uterus in young female chickens (pullets), commences between the 16th and 20th week of their maturation process. This is a vital phase in the reproductive cycle of chickens, wherein the uterus undergoes significant modifications to prepare for oviposition. It reaches its maximum level at week 18 and then gradually decreases until the birds start laying eggs. Figure 2.1 illustrates this process. During this period, the average daily weight gain is likely to increase from weeks 16 to 18 and then decline again (Dumoulin, 2018). Oestrogen has been suggested to be an essential factor involved in the development and maintenance of the female reproductive system and ovum formation (Plate 2.9).

Vitellogenesis is one of the key factors necessary for egg production facilitated by oestrogen (Tramunt *et al.*, 2021), but the level of oestrogen decreases gradually with age resulting in a decline in laying performance in post-peak laying hens. Thus, raising the level of oestrogen using phytoestrogens may be a method to increase the laying performance in post-peak laying hens. Although *Morinda citrifolia* has been found to show very weak oestrogenic activity *in vivo* (Chearskul *et al.*, 2004), studies by some researchers have indicated that noni

supplementation increased egg production (Sunder *et al.*, 2013; Sunder *et al.*, 2016; Asmara *et al.*, 2019; Widjastuti *et al.*, 2023). This may be due to the adjuvant characteristic of noni fruit extract.

2.6.3 Bone regeneration

Hussain *et al.*(2016) reported that noni fruit extract enhanced the proliferation rate of the bone marrow mesenchymal stem cells (BMSC). It upregulated the osteogenic differentiation marker genes: osteocalcin and runt-related transcription factor-2 and the enzyme alkaline phosphate (ALP), which increased bone density thus revealing neo-angiogenesis for bone formation in male Wistar rats. They concluded that noni increases the proliferation and osteoblast differentiation of BMSC and plays an important role in the regeneration of the bone. This suggests that noni may be one of the anti-osteoporotic phytogenics free from side effects. Thus, noni fruit extract fed to layers could enhance medullar bone density and subsequent improvement in eggshell quality.

2.6.4 Thickening of long bones

As bones grow longer, they also widen; growth in diameter can continue even after longitudinal growth ceases. This is called appositional growth (Norton *et al.*, 1996). Osteoclasts are cells that work to break down bone and resorb old bone that lines the medullary cavity. At the same time, osteoblasts via intramembranous ossification produce new bone tissue beneath the periosteum.

The erosion of old bone along the medullary cavity and the deposition of new bone beneath the periosteum not only increase the diameter of the diaphysis but also increase the diameter

of the medullary cavity. This process is called modelling (Plate 2.8). The amount of mineral contents and adequacy in poultry diets reflects the strength of the bone (Rath *et al.*, 2000). The degree of bone density is also affected by the rate of bone mineralisation (Reichmann and Connor, 1977).

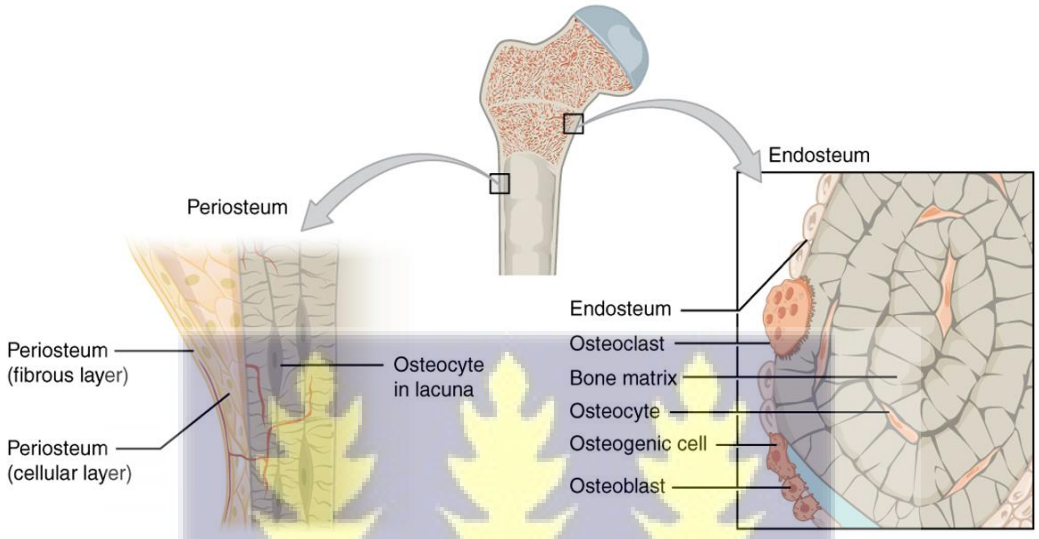


Plate 2.8. Periosteum Anatomy (Firdous and Singh, 2023)

Brittle legs are associated with reduced feed intake and calcium availability, which can decrease the number of eggs laid and weight gain (Orban *et al.*, 1999). At 16 weeks of age, birds begin to develop medullary bone (Fleming *et al.*, 1998). To support the heightened calcium deposition in these bones, it is crucial to provide them with a calcium-rich pre-lay diet. As depicted in Figure 2.1, this phase is accompanied by a slight increase in the expected average daily weight gain rate, which subsequently tapers off at week 20 (Dumoulin, 2018).

2.7 Production performance

2.7.1 Feed conversion ratio (per kg egg mass)

The feed conversion ratio per kilogram egg mass is the amount of feed required in kilograms to produce one kilogram of egg mass (TNAU, 2012). Singh (2012) and Aroche *et al.* (2018) observed that Noni could act as an appetite suppressant. Pu *et al.* (2004) used noni to control the rate of feed consumption in the stomach by stimulating cholecystokinin (CCK) secretion to activate CCK1 receptors. The CCK is a brain-gut peptide that functions both as a neuroreceptor and as a gut hormone. The CCK stimulates the secretion of pancreatic enzymes and causes gall bladder contraction and release of bile into the small intestines hence, inhibiting gastric emptying and suppressing feed intake. The bile acids inhibit gastric emptying by reducing small intestinal transit time but stimulate colonic peristalsis thereby increasing colonic transit time as the CCK1 receptor is found primarily in peripheral tissues throughout the gut (Asmara *et al.,* 2019). Asmara *et al.* (2019) observed that in general egg production in female Grey Sentul Chickens aged 22 to 30 weeks was higher in groups treated with Noni fruit extract. They had a lower FCR of 2.96 compared to the control group of 4.71.

This is because the treated groups had lower feed consumption, yet their egg weight and hen day production (40.18 g, 71.42 %) were higher compared to the control group (36.36 g, 57.14 %).

2.7.2 Egg mass and feed conversion ratio

Better comparisons of egg production of bird flocks or strains are made with egg mass instead of egg numbers (TNAU, 2012). The Feed consumed per kilogram egg mass considers feed intake, egg weight, and production. This was calculated as the ratio between feed consumed and egg mass. A value of 2.2 or less is advantageous to the farm.

2.7.3 Hen Day Egg Production (HDEP %)

Hen day egg production (HDEP %) is usually expressed as a percentage and is used to measure the production capacity of birds in a house. A farm average of 85% or higher per year is desirable. This was calculated by first computing the number of hen days in the period by summing up the number of hens alive each day and used to divide the number of eggs laid during the same period and multiplied by a hundred (equation 5.1).

HDEP % = (Total number of eggs produced during the period) / (Total number of hen-days in the same period) X 100……5.1 (TNAU, 2012)

2.7.4. Net Feed Efficiency Index (NFEI)

According to Narahari *et al.* (1983), net feed efficiency index values significantly correlate with egg production, feed conversion ratio and egg mass values. Flocks showing net feed efficiency values of 40 or above may be considered better layers.

The net feed efficiency index evaluates layers' performance efficiency and it is the sum of the mean egg mass and body weight gain (or loss) by the layer(s) during the study period divided by their mean feed intake for the same period and multiplied by 100.

2.8 Internal egg quality

The interior egg quality is based on air cell size, albumen quality, yolk quality, and the presence of blood or meat spots (Table 2.2; Jacob *et al.*, 2000).

Table 2.2. USDA standards for the interior quality of chicken eggs by candling

Interior Quality Factor	AA Quality	A Quality	B Quality	C Quality – (Inedible)
Air cell	3.2mm or less in depth	4.8mm or less in depth	More than 4.8mm	Doesn't apply
White (albumen)	Clear- Firm	Clear-may be reasonably firm	Clear- may be weak and watery	Doesn't apply
Yolk	Outline slightly defined	Outline may be fairly well-defined	Outline clearly visible	Doesn't apply
Spots (blood or meat)	None	None	Blood or meat spots aggregating not more than 3.2mm in diameter	Blood or meat spots aggregating more than 3.2mm in diameter

(Jacob *et al.*, 2000)

2.8.1 Haugh Unit as a measure of egg freshness

The Haugh unit score has been generally accepted as a measure of albumen quality in egg quality studies (Haugh, 1937). The Haugh unit [HU] is related to albumen height [H] and egg weight [W] (Haugh, 1937) and is calculated by the following formula.

$HU = 100 \times \log (H - 1.7W^{0.37} + 7.57)$ …… 2.2 (Haugh, 1937)

Albumin quality or Haugh unit (HU) are considered a standard measure to judge the freshness of an egg in the poultry industry. It is influenced by genetic factors (Johnson and Merritt, 1955) and environmental factors such as temperature, time and humidity of storage (Lee *et al.*, 2016). The Haugh Unit is the precise measurement of opened egg quality, the

higher the HU value, the better the albumen quality of the egg (Silversides and Scott, 2001). Several factors, such as the storage time, temperature, age of the hen, the strain of the bird, nutrition, disease, supplements, artificial exposure to ammonia, induced moult and medication affect the HU (Zaboli *et al.*, 2017). According to Al-Obaidi *et al*. (2011) cited by Kumari *et al.* (2020) and Nabel (2016), the quality grade AA of eggs must have at least 72 Haugh Units. Eggs of quality grade A must have between 60 and 71 Haugh Units. Quality grade B eggs require Haugh Units between 30 and 59. Conversely, eggs with quality grade C have Haugh Units lower than 30 and are not suitable for consumption as shell eggs.

2.8.2 Effect of storage time on Yolk index

The yolk of a freshly laid egg is round and firm (Chukwuka *et al.*, 2011). Albumen and yolk index reflect the freshness of the egg, and the index decreases along with storage duration (Eke *et al.*, 2013). In addition, hen age and laying duration influence the albumen and yolk index. The indices decrease with the progression of the laying period (Zita *et al.*, 2012). Poor storage conditions can result in a deterioration of the vitelline membrane, which can cause the yolk to become more susceptible to breaking due to the osmotic flow of the albumen through the membrane (Osei-Amponsah *et al.*, 2014). These findings are based on research conducted by Zita *et al*. (2012) and Nadia *et al*. (2012), as cited by Eke *et al.* (2013). This can lead to swelling of the yolk, causing the membrane to weaken and stretch, potentially impacting the egg's weight (Watkins, 2007 cited by Kumari *et al.*, 2020; Qi *et al.*, 2020). This pressure eventually causes the yolk to change from a spheroid shape to a round flattened flabby shape mass with increased water content and decreased yolk solids concentration (Stadelman and Cotterill, 2013). The range of the yolk index for freshness is as follows:

Extra Fresh > 0.38, Fresh 0.38 > X > 0.28, Regular 0.28 > X < 0.15, and Poor 0.15 > X (Nabel, 2016). Studies conducted by Sunder *et al.* (2011a; 2013) revealed that birds fed with higher dosages of Noni fruit extract exhibited higher yolk index values. These studies demonstrated the positive impact of Noni on the egg quality of Japanese quails and Nicobari fowls, respectively.

2.8.3 Effect of storage time on Yolk colour

The permeability of the vitelline membrane can increase under unfavourable storage conditions, such as extended storage time and high temperature. This can result in a mixture of albumen and yolk content, which leads to yolk mottling. Numerous pale spots and blotches of varying colour, size, and shape characterise this condition. The severity of mottling can increase if albumen proteins enter the yolk, causing it to appear even paler (Chukwuka *et al.*, 2011). Yolk colour is a crucial quality attribute of eggs influencing consumer preferences. Consumers generally favour eggs with more yellowish yolks, ranging from golden yellow to orange, corresponding to an 8 to 14 score on the Roche Yolk Colour Fan (Vuilleumier, 1969). Moreover, yolk colour enhances visual appeal and indicates the presence of lutein and zeaxanthin compounds, which offer health benefits (Wardiny and Sinar, 2013).

2.8.4. Causes of blood and meat spot in chicken eggs

According to van de Braak (2023), the inside of an egg should appear clean and fresh, and the egg white, the chalazae, and the yolk should be visualized clearly. Chemically and nutritionally, eggs that contain blood or meat spots are fit to eat (Jacob *et al.*, 2000). The spot can optionally be removed and the egg used, however, United State Department of

Agriculture (USDA) regulations classify eggs with blood or meat spots aggregating more than 3.2mm in diameter as inedible (Table 2.2).

Blood spots result from haemorrhage of a small blood vessel in the ovary or oviduct (Plate 2.9). If the blood spot is on the yolk, the haemorrhage was probably in the ovary at the time of ovulation or in the infundibulum part of the oviduct before albumen was laid down. If a spot of blood is found within the albumen, this may suggest that bleeding took place in the wall of the magnum portion of the oviduct. Meat spots, on the other hand, can arise from a variety of factors, including degenerated blood spots, fragments of ovary or oviduct tissue, or remnants of cuticle that were swept up to the magnum and subsequently became incorporated into the albumen. (Jacob *et al.,* 2000).

Although blood and meat spots occur between white and brown egg layers, white egg layers have a lower probability of producing eggs containing blood and meat spots (van de Braak, 2023). Blood and meat spots in eggs can be caused by various factors. One of the main reasons for their occurrence is underlying diseases such as infectious bronchitis, which may lead to a sudden increase in the appearance of blood and meat spots in eggs (van de Braak, 2023).

Additionally, research has shown that high ambient temperature may also contribute to a higher incidence of blood spots (Jacob *et al.,* 2000). On the other hand, Amoah *et al*. (2022) found no significant correlation between blood and meat spots and egg weight, temperature variation, or noise source (generator). Interestingly, Leghorn hens seem to produce fewer blood spots at 32°C compared to their brown counterparts who produce more even at 21°C (Jacob *et al.*, 2000).

The USDA standards for the quality of individual shell eggs (Table 2.2) were developed based on both interior and exterior quality factors. Currently, commercial eggs are graded simultaneously for both exterior (size) and interior quality (albumen, yolk and presence or absence of blood or meat spots) according to van de Braak (2023). Some egg grading machines grade the eggs according to the presence or absence of blood spots or meat spots thereby resulting in a loss of the other qualities in an AA or A egg.

The factor with the lowest grade determines the overall grade of the egg. In the United States, egg grades include AA quality, A quality, B quality, and Dirty. Only AA and A quality eggs are sold at supermarkets. Egg grading equipment takes out most grade B and dirty eggs containing blood or meat spots. Thereby, grading down the extra value of grade AA and A eggs with blood or meat spots (van de Braak, 2023).

Eggs are further graded based on their HU values: AA: 72 or more, A: 71 – 60, B: 59 – 31, C: 30 or less. AA and A values are considered eggs of high quality while those with B and C values are eggs of low quality and safe for consumption (Okiki and Ahmed, 2017). Eggs with 70 HU value are considered acceptable market standards in the United States of America (USDA, 2000).

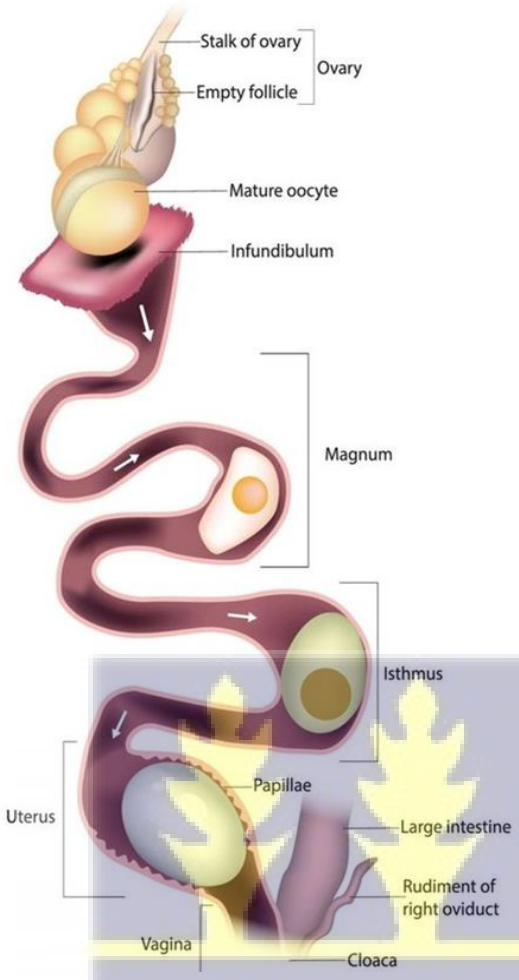


Plate 2.9. Reproductive system of the layer

(van de Braak, 2023)

Eggs are categorised into one of three consumer grades:

- USDA Grade AA – The freshest and highest quality eggs receive a Grade AA.
- USDA Grade A – Very high-quality receive a Grade A.
- USDA Grade B – Grade B eggs are usually used for breaking stock (liquid eggs) and baking, depending on the number of defects.

2.8.5 Machine grading

In Ghana, grading is done based on size and weight. The internal quality is not considered. Egg grading machines grade the eggs according to their weight (Adei and Asante, 2012; Anene *et al*., 2020).

2.8.6 Grading by internal egg quality

An egg has two types of albumen, thin albumen and thick albumen, based on their morphology. The thick albumen of a fresh egg evenly adheres to the yolk and raises it so that a vertical micrometre can be used to calculate the Haugh unit by measuring the height of the thick albumen. China has four egg grades: AA, A, B and C while the USA has three grades: AA, A and B. Stale eggs have no thick albumen because the thick albumen is gradually hydrolysed during storage and thins out with time and cannot be measured. The Haugh Unit (HU) is a measure of the protein quality and freshness of an egg based on the height of its thick albumen (Qi *et al.*, 2020). When the HU is above 72mm, the egg was given an AA quality rating for very fresh eggs. The quality rating given when the HU is between 60 and 72mm is A for fresh eggs, while a B quality rating is given when the HU is between 31 and 59mm for regular eggs. Eggs with a HU below 31mm were rated as C quality for poor quality eggs (Qi *et al.*, 2020).

The fresh yolk has a raised surface, while the stale yolk is flat because fat and protein in the yolk gradually hydrolyse during storage, which causes a decrease in yolk viscosity. The salt concentration of yolk is higher than the albumen, which forms osmotic pressure between them. The longer an egg is stored, the longer the penetration of water from the albumen into the yolk lasts, eventually leading to a flattened and mottled yolk. Therefore, the yolk index (YI) can express egg freshness. Thus, the staler the eggs are, the flatter and wider the yolk

and the smaller the YI. The YI of extra fresh eggs with an AA grade ranges from 0.38 to 0.5 with a mean value of 0.44. Fresh eggs with an A grade range from 0.28 to 0.37, regular eggs with a B grade range between 0.25 and 0.28 and poor eggs with a grade of C, fall below 0.25. When YI is less than 0.25, the vitelline membrane breaks and loose yolk appears (Eke *et al*., 2013; Nabel, 2016).

2.8.7 Effect of storage time on air cell of chicken eggs

The internal quality of eggs starts to decline as soon as the hens lay them (Gavril and Usturoi, 2012). The major difference between freshly laid eggs and stored eggs is albumen pH and albumen quality 'Firm albumen height' (Nadia *et al*., 2012 cited by Eke *et al.*, 2013). Inadequate handling conditions increase the air cell volume, the thick albumen portion's liquefaction, and the vitelline membrane's weakening that separates the yolk and albumen (Berardinelli *et al.*, 2008). Sufficiently high storage temperature conditions lead to the drying of the cuticle and the shell membrane in the egg, resulting in an increase in the pore area and permeability of the egg (Berardinelli *et al*., 2008; Krisnaningsih *et al.*, 2023).

During storage, moisture from the egg is lost through evaporation at a rate that is influenced by the temperature of the storage environment (Eke *et al.*, 2013). Carbon dioxide is also lost through the shell pores while oxygen gets into the egg and creates an air bubble inside in place of moisture and carbon dioxide, causing the egg to float when placed in water due to loss of weight (Hasan and Aylin, 2009) and this forms the basis of the floatation method of egg freshness. The movement of carbon dioxide and moisture through the shell of the egg increases the pH of the albumen and the yolk but decreases the percentage moisture of egg albumen and albumen weight. A significant increase of naturally occurring psychrophilic

bacteria, coliform, staphylococci, yeast and moulds occurs on eggshell surfaces and in the egg content during egg storage (Kumari *et al.*, 2020). A properly handled and refrigerated intact egg will retain its nutritional value and wholesomeness for a considerable long time (longer than 5 weeks). Eggs produced in farms could have good quality (more than 75 Haugh units) but because of poor handling and storage conditions in farms and markets, it could lead to losses in quality (Kumari *et al.*, 2020).

2.9 Extraction of Noni fruit extract

2.9.1 Noni fruit extraction methods

Commercial noni fruit extract is traditionally made by fermentation of noni fruits in sealed containers for 2 months at ambient temperature (Newton, 2002; Nelson, 2006). Direct squeezing of ripened noni fruits produces fresh noni fruit extract. Some noni fruit extracts are made by boiling noni fruits for hours. Many Pacific islanders use the fermentation method. The fruits are fermented in sealed jars and either placed outdoors, exposed to full sunlight and temperature variation, or indoors at an ambient temperature of 24°C (Nelson, 2006). Natural mutualistic microorganisms present in the fruit produce the intrinsic enzymes that initiate the fermentation of noni fruit extract (Yang *et al.*, 2007).

2.9.2 Odour change during extraction process

Cheng *et al.* (2021) and Zhao *et al.* (2022) identified and reported that hexanoic acid, octanoic acid and their methyl esters such as methyl octanoate, ethyl octanoate, methyl benzoate, ethyl hexanoate, methyl salicylate and ethyl lactate were the key odorants for the smell and flavour substances in ripe fresh noni fruits and freshly crushed noni fruit extract.

Ethyl lactate had the highest sensory threshold of 154.6 mg/l. These organic acids and esters contribute to the pungent cheesy smell and taste of fresh ripe noni.

The substance that had the greatest influence on the flavour of fermented noni fruit extract was the alcohol linalool. During fermentation, the ester (methyl salicylate) and alcohol (linalool) with sensory thresholds of 0.06 mg/l and 0.006 mg/l respectively. Though present in fresh noni fruit, they had little effect on the flavour, but they became the main flavour substances of the fermented noni fruit extract. Hence, the main flavour substances of fresh noni fruit extract are changed by intensive fermentation, resulting in the development of an increasing rose and fruity smell of fermented noni fruit extract which is more appealing.

2.9.3 pH variation during fermentation of Noni fruit extract

Samarasiri *et al.* (2022) reported that the pH value of noni fruit extract decreased with fermentation time. At the start of the process, it was about 3.88 and finally, it reached 3.31. The pH of the noni fruit extract decreased dramatically during the first half of the first month, and after that, the pH slightly reduced with a few fluctuating points. For two months, the highest pH was about 3.98, while the least was around 3.23. A previous study showed that the pH of the fermenting fruit extract reduced with the fermentation time from 5 to 3 (Samarasiri *et al.*, 2019). The study by Konsue *et al.* (2018) showed that the fermentation led to a decrease in pH from 3.72 to less than or equal to 3.5. This decrease in pH was mainly caused by the formation of lactic acid during the fermentation process. For the whole drip fermentation period, noni fruit extract remained in the acidic phase.

2.9.4 Maintaining the integrity of Noni fruit extract

According to Yang *et al.* (2010) the antioxidant properties of noni fruit extract are estimated by its antioxidant capacity (AC) and total phenolic content (TPC) and the most effective way to retain the quality of antioxidant characteristics of noni fruit extract is a combination of controlling both light and temperature.

Yang *et al.* (2007) showed that refrigeration and freeze storage of fresh noni fruit extract significantly retained 90 % of its radical scavenging activity (RSA) while fermentation of the fresh noni fruit extract for three months caused more than 90 % loss of RSA. Sina *et al.* (2021) also recommended freezing and refrigeration of fresh noni fruit extract. According to Yang *et al.* (2010), protection of noni products from illumination significantly decreased the degradation of antioxidant capacity, total phenolic, and ascorbic acid for 2 – 4 weeks in noni fruit extract. In addition to illumination, controlling temperature at 4 °C further stabilised the antioxidant and total phenolic capacity during storage. Thus, the most effective way to retain the quality of antioxidant characteristics of fresh noni fruit extract is a combination of controlling both light and temperature.

Samarasiri *et al.* (2022) compared three forms of noni fruit extract namely traditional fermented fruit extract, freshly extracted fruit extract and fermented fresh extracted fruit extract for their antioxidant capacity and total phenolic content over two months. They observed that both indoor traditional fermented and fresh fruit extract extraction provided satisfactory levels of antioxidant capacity and total phenolic content in the first two weeks of extraction whereas fermentation of fresh noni fruit extract resulted in a severe loss of antioxidants and phenolic compounds inherited by fresh noni fruit extract. However, Guo *et*

al. (2020) and Zhang *et al.* (2021) reported that the biological effect of noni fruit extract was improved through traditional fermentation extraction (referred to as drip fermentation in this study).

2.9.5 Antibacterial sensitivity of fermented and non-fermented Noni fruit etract

Sina *et al.* (2021) observed that fresh noni fruit extract showed greater antibacterial activity against eight strains of bacteria than fermented noni fruit extract. The reference strains were Gram-positive strains (*Staphylococcus aureus, S. epidermidis, Streptococcus oralis* and *Enterococcus faecalis*) and gram-negative strains (*Pseudomonas aeruginosa, Proteus mirabilis, P. vulgaris* and *Escherichia coli*). The inhibition diameter of fresh noni fruit extract was either greater than or equal to that of fermented noni fruit extract. The greatest antibacterial activities were obtained with fresh noni fruit extract on *Pseudomonas aeruginosa* (14.5 ± 2.12 mm) followed by *Escherichia coli* (12.75 ± 1.06 mm). The lowest activities were obtained with fermented noni fruit exxtract on *Enterococcus faecalis* (10 mm) and *Staphylococcus aureus* (9.5 ± 2.12 mm). They inferred that fermentation for long periods could also reduce not only the antioxidant activity but also the antibacterial activity of the noni fruit extract. Cimrin *et al.* (2019) suggested that tannins and flavonoids present in the fresh fruit extract might allow the extract to overcome the bacterial cell wall barrier hence the greater antibacterial activity observed against Gram-negative bacteria. Natheer (2012) observed that noni fruit extract has more antibacterial activity on Gram-negative than on Gram-positive bacteria since Gram-negative strains are much more sensitive than Gram-positive strains. They inferred that the accumulation of peptidoglycan layers by Gram-positive strains could be responsible for this bacterial resistance observed in the Gram-positive strains

CHAPTER 3

3.0 GENERAL MATERIALS AND METHODS

3.1 Study area

The study was carried out at different locations. The birds were raised at the University of Ghana Livestock and Poultry Research Centre (LIPREC) of the University of Ghana, Legon. LIPREC is located near a small settlement called Ashale-Botchwe on the Accra Plains in the Coastal Savannah Zone of Ghana. It lies on latitude 05° 40′ N and longitude 00° 16′ W. The Research Centre receives an annual rainfall of 785 mm, ranging from 128 mm to 1,709 mm, and follows a bi-modal distribution. The long rainy season is typically from March to July, peaking in June, while the short rainy season occurs from August to November, peaking in October. The mean monthly temperatures range from 24.8 °C in August to 28.3 °C in February, with an average of 26.9 °C. At 1500 hours, the relative humidity ranges from 58% to 83.7 % and is slightly lower at 0900 hours. The topography consists of gently rolling hills with low elevation and is covered by natural grassland with medium tussock growth, along with scattered fire-resistant trees and shrubs (Osei-Amponsah *et al.*, 2014).

The extraction of the noni fruit extract and the proximate analysis of the feed were carried out at the Nutrition laboratory of LIPREC. The total antioxidant capacity of the noni fruit extract was determined at the Graduate Students Laboratory of the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) of the University of Ghana, Legon, while the analysis for the haematological and blood biochemical parameters was done at the laboratories of the Veterinary Services Directorate, La-Accra, and the Small Animal Teaching Hospital (SATH) of the School of Veterinary Medicine University of Ghana, Legon.

3.2 Sample collection

3.2.1 Harvesting of Noni fruits

Mature unblemished pale-yellow hard *Morinda citrifolia* (Noni) fruits were manually harvested from Adentan, Ashale-Botchwe and Haatso in the Greater Accra Region of Ghana. The noni fruits were randomly collected from different parts of the tree (Plate 3.1) to ensure a representative sample of the entire tree's production. This was done to exclude vertical variability due to nutrient distribution, temperature and sunlight thus providing a more accurate picture of fruit quality and characteristics (Stuart *et al.*, 1988). The noni fruits were placed in a pre-cooled ice chest at 25°C and transported to the Nutrition Laboratory of LIPREC for fruit extract extraction.

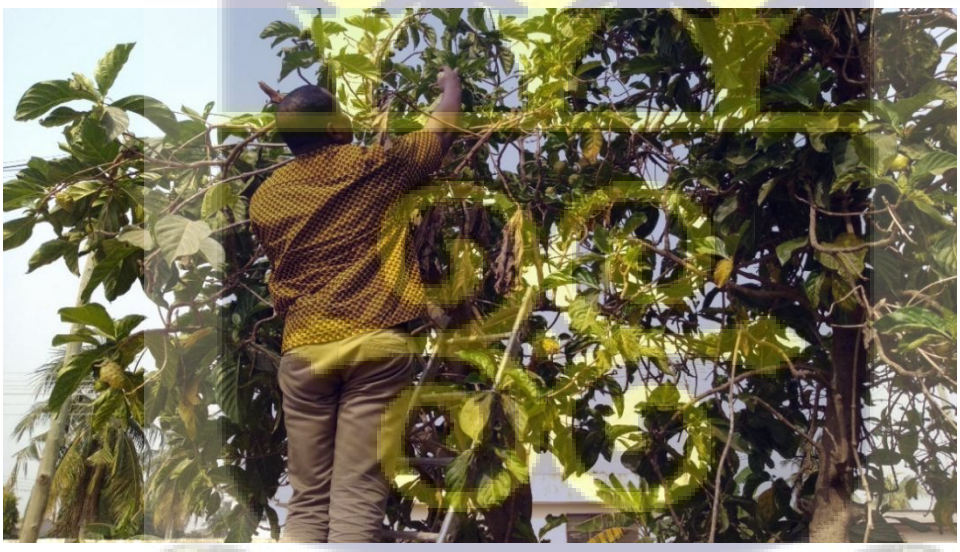


Plate 3.1. Harvesting of noni fruits from all levels of the tree

3.2.2 Preparation of Noni fruit extract

The noni fruits were sorted, washed and sanitised with 50g/l salt water; rinsed with distilled water, and air-dried for 5 minutes at 26ºC. The fruits were fermented at their pale-yellow-white stage of maturity. About 6 to 8 unblemished pale-yellow-white hard noni fruits weighing 1,000g were placed into twelve airtight opaque polypropylene (PPL) food-grade containers each to determine the total antioxidant capacity (TAC) of the noni fruit extract. They were fermented over 12 weeks in a dark cool room of the Nutrition Laboratory of LIPREC and allowed to drip. The containers were sanitised with the same concentration of the salt solution, rinsed with distilled water, and air-dried immediately before use.

The total antioxidant capacity (TAC) of the fermented noni fruit extract was determined to be. 3.9 mg/ml at a maximum fermentation period of eight weeks of drip fermentation at the Graduate Students Laboratory of WACCBIP (see sections 4.4.2 and 4.4.3). Subsequent extracts were stored in 5 and 2 litre polypropylene food-grade gallons in the cold room of LIPREC at -18ºC. These were retrieved and used as and when the need arose.

3.3 Experimental animals and design

Four experiments were conducted involving 600 layer-type birds in two groups of 300 birds. The two groups of 300 layer-type birds were fed a regular layer ration. The first Group of 300 birds were allocated to the first three experiments (experiments 1, 2 and 3) and the second group of 300 birds were used for the fourth experiment (experiment 4).

The 300 commercial layer pullets, with a mean body weight of 1.3 ± 0.233 kg, were randomly assigned to three experimental treatments, with 20 pullets per treatment, each treatment of 20 birds were replicated five times in a completely randomised design. The pullets were

housed in groups of 20 birds per replicate in a slated floor house. After selection, subsequent weighing was done in groups of 10 using a plastic bird transfer basket and a suspended scale. The birds were reared with standard industry husbandry procedures throughout the experimental period of 6 weeks. These included provision of feed and water, routine vaccination, diet management, daily monitoring, recording of ambient temperature and relative humidity, routine cleaning adhering to standard biosafety measures and good production practices. The birds were used for experiments 1, 2 and 3 in this study.

The treatments were:

- Treatment 1 (control group) received plain water with 0 mg/ml of noni fruit extract
- Treatment 2 received 20 mg/ml (equivalent to 5 ml) of noni fruit extract per litre of water
- Treatment 3 received 40 mg/ml (equivalent to 10 ml) of noni fruit extract per litre of water

Administration of the noni fruit extract was through drinking water for 6 weeks beginning from week 16. The daily feed and water allocated to the birds were recorded. The guidelines for brown-layer farming (Growel, 2017) were followed. At week 22, ten birds per treatment (2 from each of the five replicates) were randomly selected and euthanised by rapid decapitation according to the protocol outlined by AVMA (2020).

The following organs were harvested for assessment: abdominal fat, reproductive tract (uterus) and the right tibia bone (representing medullary bone). Blood samples were taken from 10 birds per treatment (2 birds per replicate) randomly selected at four stages of

growth/production namely weeks 16 (pre-lay), 22 (50 % lay), 30 (peak lay) and 48 (late-lay). The proper handling of the bird with two hands and using venipuncture to extract the blood were followed. A control group served as the baseline for the study.

The second group of 300 commercial pullets, with an average body weight of 1.3 ± 0.233 kg, was randomly assigned to three experimental treatments, with 20 birds in each treatment and five replicates. The pullets were housed in groups of 20 birds per replicate in a completely randomised design. These birds were used for experiment 4 in chapter 7 of this study. The birds were fed a standard commercial layer diet (Table 3.1). After acclimatising for two weeks, the pullets were put on the experimental treatment at the end of week 15. Pullets in treatment 1 (control) received 0 mg/ml of noni extract from 16 weeks of age. Treatment 2 comprised of 16-week-old pullets receiving 40 mg/ml of noni extracts in their drinking water. In treatment 3, the pullets were started on 0 mg/ml of noni fruit extract at 16 weeks of age. At 20 weeks of age, the layers were provided with 40 mg/ml of noni fruit extracts in their drinking water. The experiments were terminated when the layers reached 48 weeks of age. The guidelines for brown-layer farming (Growel, 2017) were followed. All the birds were kept under uniform management conditions throughout the experimental period of 32 weeks.

3.3.1 Water management

Water was made available *ad-lib* to both control and treatment groups by ensuring that the water in the water fountains were not completely depleted by the next morning. Each morning the residual water was decanted into a measuring cylinder and recorded and fresh

water provided. The weekly water intake (litres) was computed by summing up the daily water intake per bird for the week.

The daily intake of water (litres) was computed using equation 3.1 as follows:

$$\text{DWI per bird} = \frac{\text{Inital water - Residual water}}{\text{Number of birds}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

Where:

DWI = Daily water intake per bird.

3.3.2 Feeding management

The two groups of 300 commercial birds were fed an experimental diet that was isocaloric and isonitrogenous. The feed used was the regular ration used at LIPREC (Table 3.1). The feed was administered to the birds as follows: layer chick starter from day 1 to week 8, grower mash from week 9 to week 15, pre-layer mash from week 16 to week 20, and layer mash from week 21 to the end of the experiment, as per the recommended standardised nutrient requirements for layers. Daily ambient temperature and relative humidity records were recorded.

Table 3.1. Composition of experimental diet

FEED COMPOSITION				
Ingredient (%)	**0-8 wks**	**9-15 wks**	**16-20 wks**	**>21 wks**

Maize	54.50	58.20	55.00	53.50
Soybean meal	28.50	12.00	20.00	17.70
Wheat bran	14.70	27.45	14.10	22.00
Oyster shell	1.00	1.00	9.40	5.00
Di-calcium phosphate	0.20	0.20	0.25	0.50
Salt	0.50	0.50	0.50	0.50
Methionine	0.15	0.15	0.15	0.15
Lysine	0.15	0.15	0.15	0.15
Layer Premix	0.30	0.25	0.25	0.40
Toxin binder	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**
	Proximate Analysis			
% Crude protein	19.43	18.04	15.81	14.19
Crude fibre	5.42	7.84	7.04	6.94
Energy (kcal ME kg^{-1})	2,702	2,617	2,543	2,461
Fat	0.46	3.86	1.71	2.19
Ash	8.20	10.08	11.16	10.31

3.3.3 Vaccination programme for layers used

The vaccination schedule for layers is shown in Table 3.2

Table 3.2. Vaccination programme for layers used

AGE			VACCINE
Week	**Day**		
1	7	*	Gumboro Intermediate
2	14		HB1(1st Newcastle Vaccine)
3	21		Gumboro Intermediate Plus
4	28		LaSota (2nd Newcastle Vaccine)
5	35	**	Gumboro Intermediate/Plus
8	56		1st Fowl Pox
10	70		LaSota
12	84		2nd Fowl Pox
16	112	***	3rd Newcastle Vaccine

* Skip if parent was given Gumboro Maternalin Vaccine

** Use (Intermediate Plus) if farm is Gumboro endemic.

*** Repeat Lasota / I-2 Vaccine / Inactivated Newcastle Vaccine every 3 months after 16weeks

(Veterinary Services Directorate, 2021)

Vaccination of the birds was carried out as informed by National Poultry Vaccination Schedule (2021) and as being practiced at LIPREC. The Veterinary Clinic at LIPREC approved all the vaccines and medication used.

3.4 Egg break-out evaluation

Internal egg quality indicators were measured after breaking the eggs. Fifteen trays of eggs were evaluated for their internal quality over 4 weeks with eggs collected on the same day. Two eggs per replicate per treatment comprising of 150 eggs (5 trays of eggs) were used. The evaluation was conducted at weeks 22, 30 and 48 for both studies. The temperature and humidity of the storage period was recorded.

The eggs were cracked with a sharp knife close to the equator region and opened out from one side with a hinge-like motion onto the flat tray of a digital egg tester (DET-6000®, Nabel, Kyoto, Japan). The egg tester graded and evaluated each egg for its quality. First, the egg weight was measured before the breaking-out evaluation by the tester. The tester measured the yolk colour score based on Roche Yolk Colour Fan™ (Vuilleumier, 1969), albumen height, and Haugh unit and the grade of the egg based on the presence or absence of blood or meat spots.

The Haugh unit (HU) is related to albumen height (H) and egg weight (W) (Haugh, 1937) and the tester automatically calculated it by the following formula in equation 3.2.

$$HU = 100 \times \log (H - 1.7W^{0.37} + 7.6) \quad \text{..... 3.2 (Haugh, 1937)}$$

Where:

H = observed height of the ablumen in millimetres
W = weight of an egg in grams

Yolk index (YI) also related to the yolk height (YH) and the diameter of the yolk (YD1+YD2), was calculated using a Microsoft Excel work sheet by the following formula.

$$YI = \frac{2(YH)}{(YD1+YD2)} \qquad \text{……..3.3} \qquad \text{(Qi } et\ al.\text{, 2020)}$$

Where:

YI is the yolk index. YH is the height of the yolk (mm), which is measured by holding the stem of the vernier calliper vertically and piercing the yolk at right angles; YD_1 and YD_2 are the diameters of the yolk (mm) measured at right angles to each other using a vernier calliper to give a more accurate measurement. The shell thickness was measured with a vernier calliper (Anene *et al*., 2020).

3.4.1 Test for Freshness using Floatation method

The floatation test is one of the most popular methods for assessing whether an egg is edible (Taylor, 2023). An egg was submerged in a glass beaker filled with water at room temperature. A very fresh egg sunk to the bottom, turned on its side and settled there. A week to two weeks old eggs sink but floats at an angle or stands on end with the broad end of the egg elevated. An egg beyond 3 weeks old floated. This method is based on air cell enlargement with age of eggs (Hasan and Aylin, 2009).

3.5 Blood sampling

To ensure the birds were comfortable, their wings and feet were gently held with two hands during blood collection. The blood was drawn from the wing vein located on the inside of the wing above the elbow joint using a puncture method. This vein is easily identifiable as the largest vessel in the area, which was cleaned of small feathers and down before the procedure. To prevent blood clotting, the vein was first cleaned with a cotton swab soaked

in ethyl alcohol and then treated with an anticoagulant liquid (Trilon B – 40 % aqueous solution of Ethylenediaminetetraacetic acid [Na_4EDTA]). After the blood was drawn, the vein was clamped with a cotton swab for 1-2 minutes to prevent bleeding before releasing the bird.

A total of 10mls of blood were sampled from the brachial wing vein of two birds per replicate into heparin tubes using a syringe by the procedure outlined by Kelly and Alworth (2013). 5mls of the blood sample were placed in heparin tubes containing the anticoagulant tri-potassium-ethelyne-diamine-tetra acetic acid (K_3EDTA). The samples were stored immediately in an ice chest containing ice packs and transported to the laboratories of the Veterinary Services Directorate for haematological parameters and thrombocytes (Trb).

The other 5ml samples were placed in vacutainer tubes containing the clot activator (Gel) and kept at room temperature and not on ice to prevent an increase in viscosity of the serum (Ashworth *et al.*, 2021). The samples were immediately transported to the laboratory and then processed for serum and analysis of biochemical parameters.

3.5.1 Estimation of MCV, MCH and MCHC

The mean corpuscular volume (MCV) is estimated as:

$$\text{MCV (fl)} = \frac{\text{PCV}(\%)}{\text{RBC}\left(10^6 \mu\text{l}\right)} \times 10 \quad \text{…… …3.4 (Hagan } et\ al.\text{, 2022)}$$

The mean corpuscular haemoglobin (MCH) is estimated as follows:

$$\text{MCH (pg)} = \frac{\text{Hb(g/dl)}}{\text{RBC}(10^6\mu\text{l})} \times 10 \ldots\ldots\ldots\ldots 3.5 \text{ (Hagan et al., 2022)}$$

The mean corpuscular haemoglobin concentration (MCHC) is estimated as follows:

$$\text{MCHC (\%)} = \frac{\text{Hb(g/dl)}}{\text{PCV(\%)}} \times 10 \ldots\ldots\ldots 3.6 \text{ (Hagan } et\ al.\text{, 2022)}$$

3.6 Evaluation of internal organs

The 10 birds per treatment (2 birds per replicate) were randomly selected per treatment and euthanised by rapid decapitation according to the protocol outlined by the guidelines of the euthanasia of animals, sub-section S3.4.2.2 (AVMA, 2020). The following organs were harvested for assessment: right tibia bone (representing the medullary bone), abdominal fat and reproductive tract (uterus).

3.7 Statistical analysis

The statistical model for estimating the parameter variables in the study is shown in equation 3.3 as follows:

$$Y_{ij} = \mu + T_i + e_{ij}. \ldots\ldots\ldots\ldots\ldots\ldots 3.7$$

Where:

Y_{ij} = The response variable

μ = Overall mean

T_i = Effect of the i^{th} treatment i = 1, 2, 3

e_{ij} = Random residual error term, assumed ≈ NID (0, $\sigma^2 e$)

Where interactions between noni fruit extract and physiological stage on parameters were measured, the statistical model used is shown in equation 3.4 as follows:

$$Y_{ijkl} = \mu + T_i + P_j + TP_{(ij)} + e_{ijkl}. \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.8$$

Where:

Y_{ijkl} = The response variable

μ = Overall mean

T_i = Effect of the i^{th} treatment i = 1, 2, 3

P_j = Effect of the j^{th} physiological stage j = 1, 2, 3…

$TP_{(ij)}$ = Effect of the i^{th} treatment and j^{th} physiological stage (ij) = 1, 2, 3…

e_{ijkl} = Random residual error term, assumed ≈ NID (0, $\sigma^2 e$)

The statistical model was implemented using the generalised linear model (GLM) in GenStat 12.1 for Windows (VSN International, 2009). Means that were significantly different ($p < 0.05$) were separated using the Students' Newman-Keuls test in the same software.

‘

CHAPTER 4

4.0 EXPERIMENT 1

EFFECT OF NONI (*Morinda citrifolia*) FRUIT EXTRACT ON FEED AND WATER INTAKE, WEIGHT GAIN AND SELECTED INTERNAL ORGANS OF LAYER PULLETS IN EARLY-LAY

4.1 Summary

The antioxidant capacity of the noni fruit extract, and the effect of noni fruit extract on the feed and water intake, weight gain, egg weight, egg mass and selected internal organs of commercial pullets fed two levels of noni fruit extract in drinking water was assessed in this study. Three hundred pullets with an average body weight of 1.3 ± 0.233 kg were randomly allotted to three treatments in a completely randomised design with five replicates per treatment. Birds in Treatment 1 (control group) received plain water with no noni fruit extract; Treatment 2 received 20 mg/ml of noni fruit extract; Treatment 3 received 40 mg/ml of noni fruit extract all administered through drinking water from 16 to 22 weeks of age. The daily feed and water intake in the birds were recorded. At 22 weeks of age, 10 birds per treatment (2 birds per replicate) were randomly selected per treatment and euthanised by rapid cervical dislocation. The internal organs collected for examination included the medullary bone (from the right tibia), abdominal fat, and reproductive tract (uterus).

Providing noni fruit extract in drinking water increased ($p < 0.05$) the final body weight, daily weight gain, egg weight, and egg mass of commercial pullets. Feed intake decreased ($p < 0.05$) with increase in concentration of noni fruit extract, however water intake was similar ($p > 0.05$) among treatments. Increased Noni fruit extract concentration reduced ($p <$

0.05) abdominal fat, but improved ($p < 0.05$) the weight of the uterus and medullary bone growth and mineralisation characteristics. The 40 mg/ml had a better effect than the 20 mg/ml. Results from this study demonstrate the positive effects of noni fruit extract on pre-lay sexual development, egg characteristics and internal organs affecting egg production in commercial pullets during the early-lay period.

4.2 Introduction

Optimisation of the growth and productivity of pullets is crucial for sustainable and efficient egg production (Dumoulin, 2018; Du *et al.,* 2022). Recently, there has been a surge of interest in natural feed additives that can enhance pullet performance without any adverse effects on animal welfare or the environment (Agyarko, 2013; Gyan, 2015; Sarfo *et al.*, 2019; El-Sabrout *et al.*, 2022). Noni (*Morinda citrifolia)* fruit extract, has long been used in traditional medicine for its perceived health properties, including antioxidant, anti-inflammatory, and immunomodulatory properties (Ayunda *et al.*, 2020). Despite the poultry industry's efforts to find innovative and natural approaches to improve bird growth and productivity, the potential applications of noni in poultry nutrition have not received the required attention (Diarra *et al.*, 2019).

The growth performance of birds is a critical factor in poultry production, influencing meat yield, feed efficiency, egg production and quality and overall profitability (Dumoulin, 2018). With the increasing demand for natural and organic products, identifying alternative strategies to promote bird growth without relying on antibiotics or hormones is essential. Noni fruit extract, rich in bioactive compounds, may offer a promising solution. Its antioxidant and anti-inflammatory properties may enhance nutrient absorption, immune

function, and overall health, potentially leading to improved growth performance in birds (Sunder *et al.*, 2016).

Presently there is a knowledge gap regarding the influence of noni fruit extract on pullet development, weight gain, and pre-laying traits. More specifically, the extent to which varying concentrations of noni fruit extract in drinking water may affect pullet performance, and the dosage required to achieve desirable results, remains uncertain.

4.3 Objectives

The experiment was conducted with the following objectives:

1. To determine the effect of duration of fermentation on yield and antioxidant capacity of Noni fruit extract.
2. To assess the effect of noni fruit extract on feed and water intake, weight gain, egg weight, egg mass and selected internal organs of pullets administered with two concentrations (20 mg/ml and 40 mg/ml) of noni fruit extract in drinking water.

4.4 Material and methods

4.4.1 Source of noni fruits

The Noni fruits were harvested and carefully selected for maturity, ensuring the absence of vertical variation and blemishes. The fruits were harvested by hand from plants grown locally in various communities in the Greater Accra Region of Ghana.

4.4.2 Yield of noni fruit extract

Preparation of noni fruit extract has been indicated at section 3.2.2 of Chapter 3 (General materials and methods). The yield was measured from three food-grade plastic containers

fermented for 2, 4, 8 and 12 weeks to determine the maximum fermentation period that results in a maximum yield. The fluid leach from the water holding capacity of the fruits was measured as Drip Yield in ml/kg. The fermented soft fluid-laden fruits were squeezed through a double-layer nylon mosquito-grade net to obtain the squeezed extract in ml/kg (Plate 4.1) and added to the drip yield to give the total yield (Nelson and Elevitch, 2006).

Plate 4.1: Squeezing fermented soft fluid-laden fruits

The fruit extract was poured into falcon tubes and arranged and set in a centrifuge (biobase; Plate 4.2 a and b). it was then centrifuged at 3,000 rpm at 4°C to remove the pellets (fruit fibre) to obtain a clear fluid (Plate 4.3 a and b).

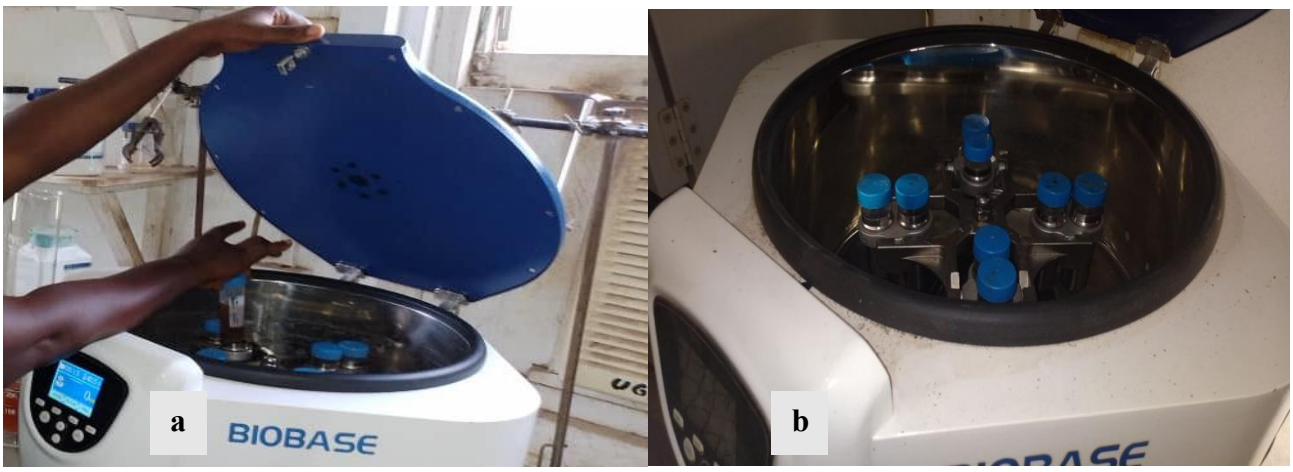


Plate 4.2: (a) Arranging and (b) setting falcon tubes containing noni fruit extract in centrifuge

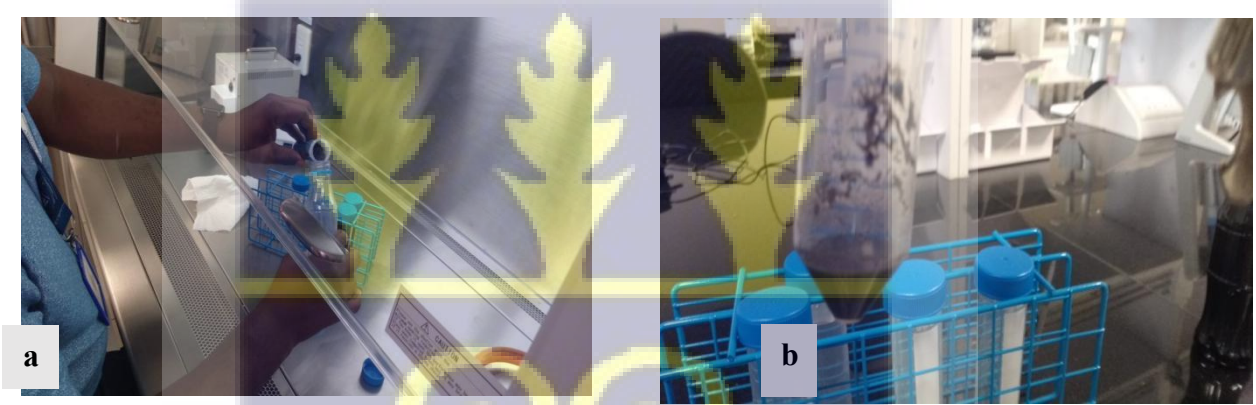


Plate 4.3: (a) Decanting centrifuged noni fruit extract (b) Pellets remaining in a falcon tube after decanting noni fruit extract

After 2 weeks of fermentation, 200 ml of noni fruit extract was taken from each of the three containers, mixed and stored at -18ºC pending chemical analysis. The noni fruit extract was analysed for total antioxidant capacity. This was repeated with the remaining containers for the specified timelines of 4, 8 and 12 weeks respectively.

4.4.3 Measurement of anti-oxidant capacity of noni fruit extract

The antioxidant capacity of noni fruit extract was determined by scavenging of 2.2-diphenyl-1-picrylhydrazyl (DPPH) free radicals as described by Brand-Williams *et al.* (1995). Four standard concentrations of ascorbic acid (0.2, 0.4, 0.6 and 0.8 mg/ml) were prepared and placed together with 100 µl aliquot of the noni fruit extract in triplicate into a 96-well plate. To all wells, freshly prepared 100 µl of 0.5 mM of DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) and 100µl of Ethanol were added to obtain a purple-coloured solution. It was then placed in a dark room for 30 minutes to allow the DPPH to be scavenged. After 30 minutes, the absorbance of the DPPH solution was measured at 517nm with a spectrophotometer (Varian Cary 100 Bio U-visible Spectrometer, Spectra Lab., Ontario Canada).

The percentage inhibition [DPPH remaining after 30 min] was calculated from equation 4.1:

$$\%\ Inhibition = \frac{\text{AbsCon} - \text{AbsScav}}{\text{AbsCon}} \text{ x } 100 \quad \ldots\ldots\ldots 4.1 \qquad \text{(Brand-Williams et al., 1995)}$$

Where:

- AbsCon is the initial absorbance of DPPH free radicals
- AbsScav is the absorbance of DPPH free radicals after 30 min

$$\%\ Inhibition = 1 - \left[\frac{\text{AbsScav}}{\text{AbsCon}}\right] \text{ x } 100 \quad \ldots\ldots\ldots 4.2 \qquad \text{(Brand-Williams et al., 1995)}$$

The results from equation 4.2 were used to plot a calibration curve with an R^2 of 0.9886. Where R^2 represents the proportion of the variance of the dependent variable that is explained by the variance of the independent variable, which at 98.9 % indicates the 'goodness of fit'.

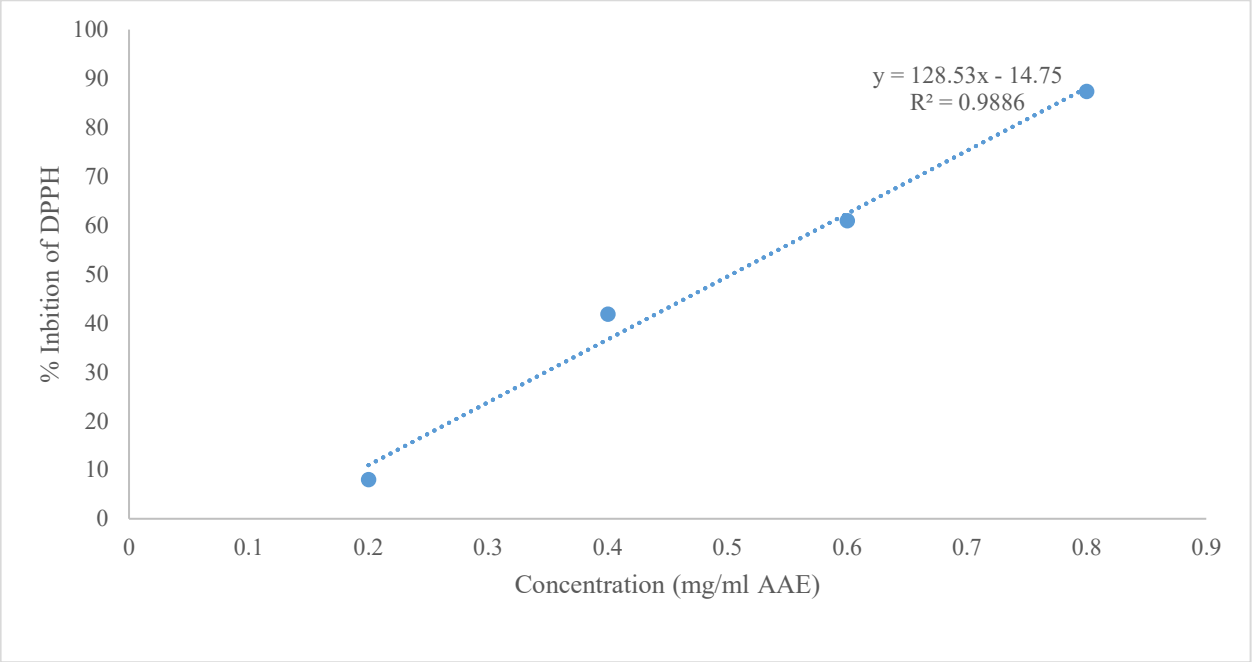


Figure 4.1. Calibration curve of DPPH by the standard concentration of ascorbic acid

DPPH = 2, 2-diphenyl-1-picryl-hydrazyl-hydrate; AAE = ascorbic acid equivalence

The concentrations of noni fruit extract were determined from the calibration curve using equation 4.3:

$$Y_{(517)} = 128.53x - 14.5 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 4.3$$

Where:

Y = % inhibition of noni fruit extract calculated from equation 4.2

x = antioxidant capacity (concentration) of noni fruit extract (mg/ml)

128.53 = the slope

14.75 = intercept of Y when x = 0

The amount of noni fruit extract necessary to reduce the initial DPPH concentration by 50 % was determined and expressed as Ascorbic Acid Equivalence also referred to as:

Vitamin C Equivalent Anti-Oxidant Capacity [VCEAC] in mg vitamin C per ml fruit extract (Kim *et al.*, 2002).

Therefore, following from equation 4.3 the concentration of noni fruit extract X was calculated using equation 4.4.

$$X = \frac{Y + 14.75}{128.53} \quad \ldots\ldots\ldots 4.4$$

Where:

X = antioxidant capacity (concentration) of noni fruit extract (mg/ml)

Y = % inhibition of noni fruit extract calculated from equation 4.2

128.53 = the gradient of the line

14.75 = intercept of Y when X = 0

4.4.4 Experimental diets

The experimental diets administered to the birds are shown in Table 4.1.

Table 4.1: Composition of experimental diets

Ingredient (%)	**9-15 wks**	**16-20 wks**	**>21 wks**
Maize	58.20	55.00	53.50
Soybean meal	12.00	20.00	17.70
Wheat bran	27.35	14.10	22.00
Oyster shell	1.00	9.40	5.00
Di-calcium phosphate	0.20	0.25	0.50
Salt	0.50	0.50	0.50
Methionine	0.15	0.15	0.15
Lysine	0.15	0.15	0.15
Layer Premix	0.25	0.25	0.40
Toxin binder	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**
	Proximate Analysis		
Crude protein (%)	18.04	15.81	14.19
Crude fibre (%)	7.84	7.04	6.94
Energy (kcal ME kg^{-1})	2,617	2,543	2,461
Fat (%)	3.86	1.71	2.19
Ash (%)	10.08	11.16	10.31

4.4.5 Experimental design and management of experimental birds

The 300 commercial layer pullets with a mean body weight of 1.3 ± 0.233 kg were randomly assigned to three experimental treatments with 20 pullets per treatment in a completely randomised design that was replicated five times. The pullets were housed in groups of 20 birds per treatment and replicated 5 times in a slated floor house. The birds had been raised on a standard chick starter diet up to 10 weeks of age and subsequently placed on grower mash (Table 4.1). At week 16, the birds were placed on the experimental treatments. Birds in Treatment 1 (control group) received plain water that is 0 mg/ml noni fruit extract; Treatment 2 received 20 mg/ml of noni fruit extract and Treatment 3 received 40 mg/ml of noni fruit extract all administered through drinking water for 6 weeks.

The daily feed and water allocated to the birds in each replicate of the experiment was recorded. The guidelines for brown-layer farming (Growel, 2017) were followed. The trial ended at 22 weeks and 10 birds per treatment (2 birds per replicate) were randomly selected and euthanised by rapid decapitation according to the protocol outlined by AVMA (2020). The following organs were harvested for assessment: the right tibia bone (to represent the medullary bone), abdominal fat and reproductive tract (uterus).

4.4.6 Data collection and statistical analysis

The data collected in this experiment were daily feed intake, daily water intake. Days to first egg was calculated as the average days the first egg was dropped in all replicates of a treatment. The egg weight at week 22, abdominal fat, uterus, ash representing the mineral content of the right tibia bone, right tibia bone length and diaphysis diameter were also determined. The general analysis of variance procedure of Genstat (VSN International, 2009)

was used to determine significant differences among treatment means. Where significant ($p < 0.05$) differences were indicated, the Student Newman-Keuls procedure was used to separate means.

The statistical model used was as presented below:

$$Y_{ij} = \mu + T_i + e_{ij}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 4.5$$

Where:

Y_{ij} = The response variable

μ = Overall mean

T_i = Effect of the i^{th} treatment i = 1, 2, 3

e_{ij} = Random residual error term, assumed $\approx$ NID (0, $\sigma^2 e$)

4.5 Results

4.5.1 Total yield (TY) of noni fruit extract

The total yield of noni fruit extracts obtained at weeks 2, 4, 8, and 12 are illustrated in Figure 4.2. The yield obtained for the various weeks ranged from 397 to 580 ml/kg respectively over the 12 weeks. Extracts obtained from week 8 and week 12 (with symbols a) were not significantly different ($p > 0.05$) from each other, but both were significantly ($p < 0.05$) higher than those of weeks 2 and 4 (with symbols b). After week 8 there was no further change in the yield of noni fruit extract. The ratio of the yield to weight of fruits was approximately 50 % (Figure 4.2).

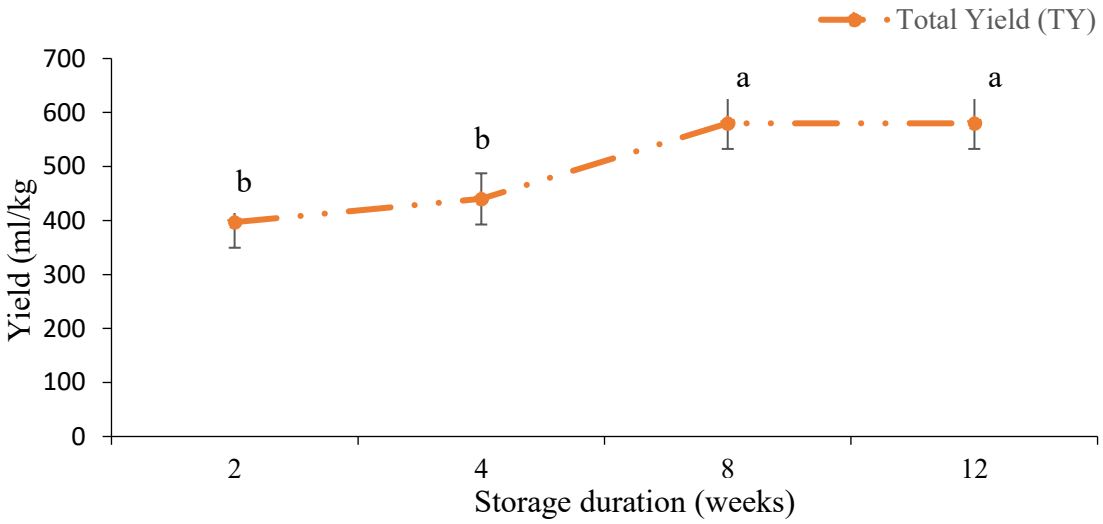


Figure 4.2: Yield of noni fruit extract over 12 weeks of fruit storage

4.5.2 Anti-oxidant capacity (concentration) of noni fruit extract

The antioxidant capacities of noni fruit extracts are illustrated in Figure 4.3. The anti-oxidant capacity (concentration) of noni fruit extract varied from 3.2 to 3.9 mg/ml over 12 weeks. Extracts obtained from week 8 and week 12 (with symbol a) were significantly different ($p < 0.05$) from those of weeks 2 and week 4 (with symbol b). Among them, the extract from week 8 had the highest value of approximately 4.0 mg/ml and was chosen for the experiment.

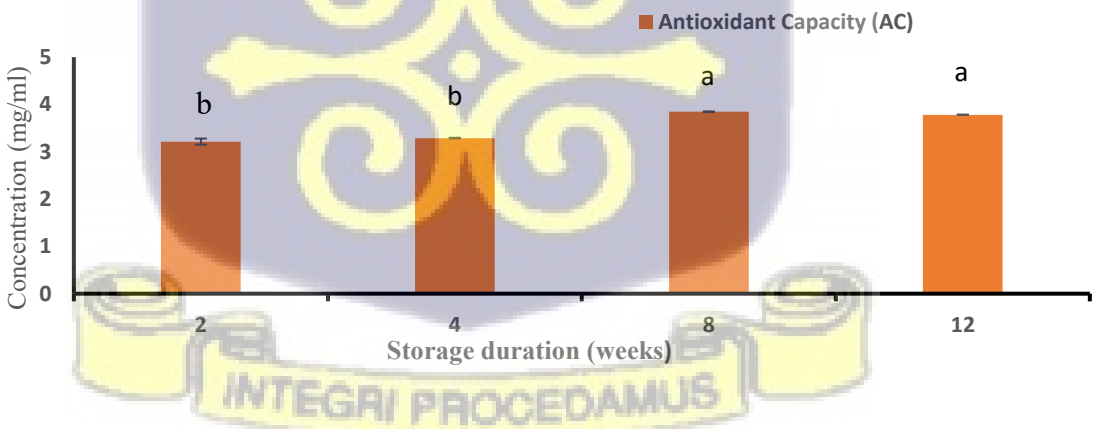


Figure 4.3: Variation in antioxidant capacity of noni fruit extract over 12 weeks

4.5.3 Early-lay performance of pullets

The influence of the concentration of noni fruit extract on the early-lay performance indices is shown in Table 4.2. The daily weight gain was significantly ($p < 0.05$) affected by the concentration of noni fruit extract. It was higher for the birds that received 40 mg/ml noni fruit extract (T_3) than those that received 20 mg/ml noni fruit extract (T_2) or 0 mg/ml the control group (T_1). Also, birds that received 20 mg/ml (T_2) of noni fruit extract had higher ($p < 0.05$) daily weight gain than those on 0 g/ml of noni fruit extract (control, T_1). The effect of the noni fruit extract on final body weight at early lay, egg mass and egg weight followed a similar trend as that for the daily weight gain and were higher ($p < 0.05$) for the birds that received 40 mg/ml noni fruit extract followed by those that received 20 mg/ml noni fruit extract and 0 mg/ml noni fruit extract respectively.

Daily feed intake decreased with an increase in the concentration of noni fruit extract with birds on the control (0 mg/ml) having higher ($p < 0.05$) feed intake than those on the 20 mg/ml and 40 mg/ml treatment. Water intake was however similar ($p > 0.05$) across treatments. The age at first egg was significantly ($p < 0.05$) affected by the concentration of noni fruit extract with the number of days to first egg being longer for the birds that received 20 mg/ml noni fruit extract than those that received 40 mg/ml noni fruit extract or 0 mg/ml noni fruit extract respectively.

Table 4. 2 Early lay performance indices of commercial layer pullets

Parameters	Concentration of Noni Fruit Extract			SEM	p-value
	T_1 (0 mg/ml)	T_2 (20 mg/ml)	T_3 (40 mg/ml)		
Initial body weight (g)	1259.44	1259.82	1259.72	0.233	0.243
Final body weight (g)	1756.22^c	1817.61^b	1826.57^a	0.397	0.001
Daily weight gain (g/d)	6.59^c	8.85^b	9.19^a	0.072	0.001
Daily feed intake(g/d)	102.04^a	101.04^b	99.46^c	0.0002	0.001
Daily water intake (l/d)	0.176	0.178	0.178	0.000	0.999
Age at first egg (days)	125.9^c	129.3^a	128.7^b	0.169	0.001
Egg weight (g)	45.92^c	48.33^b	52.39^a	0.250	0.001
Egg mass	23.02^c	25.75^b	29.19^a	0.057	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$).
SEM = Standard error of means

The effect of the concentration of noni fruit extract on the internal organs of the birds at early lay is shown in Table 4.3. The abdominal fat decreased significantly ($p < 0.05$) with an increase in concentration of noni fruit extract (Table 4.3; Plate 4.2).

Table 4.3 Internal organ parameters at early lay

Parameters	Concentration of Noni Fruit Extract			SEM	p-value
	T_1 (0 mg/ml)	T_2 (20 mg/ml)	T_3 (40 mg/ml)		
Abdominal fat (g)	31.38^a	21.55^b	19.63^c	0.230	0.001
Weight of uterus (g)	37.10^c	52.68^b	53.15^a	0.124	0.001
Ash (g)	2.99^c	3.34^b	3.48^a	0.055	0.001
Right tibia bone length (mm)	111.61^c	119.21^b	122.26^a	0.099	0.001
RTDD (mm)	5.92^c	6.56^b	6.73^a	0.010	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$);
SEM = Standard error of means; RTDD = Right tibia bone diaphysis diameter.

Weight of the uterus, right tibia bone length, right tibia bone diaphysis diameter and ash increased ($p < 0.05$) with increase in concentration of noni fruit extract (Table 4.3; Plate 4.3).

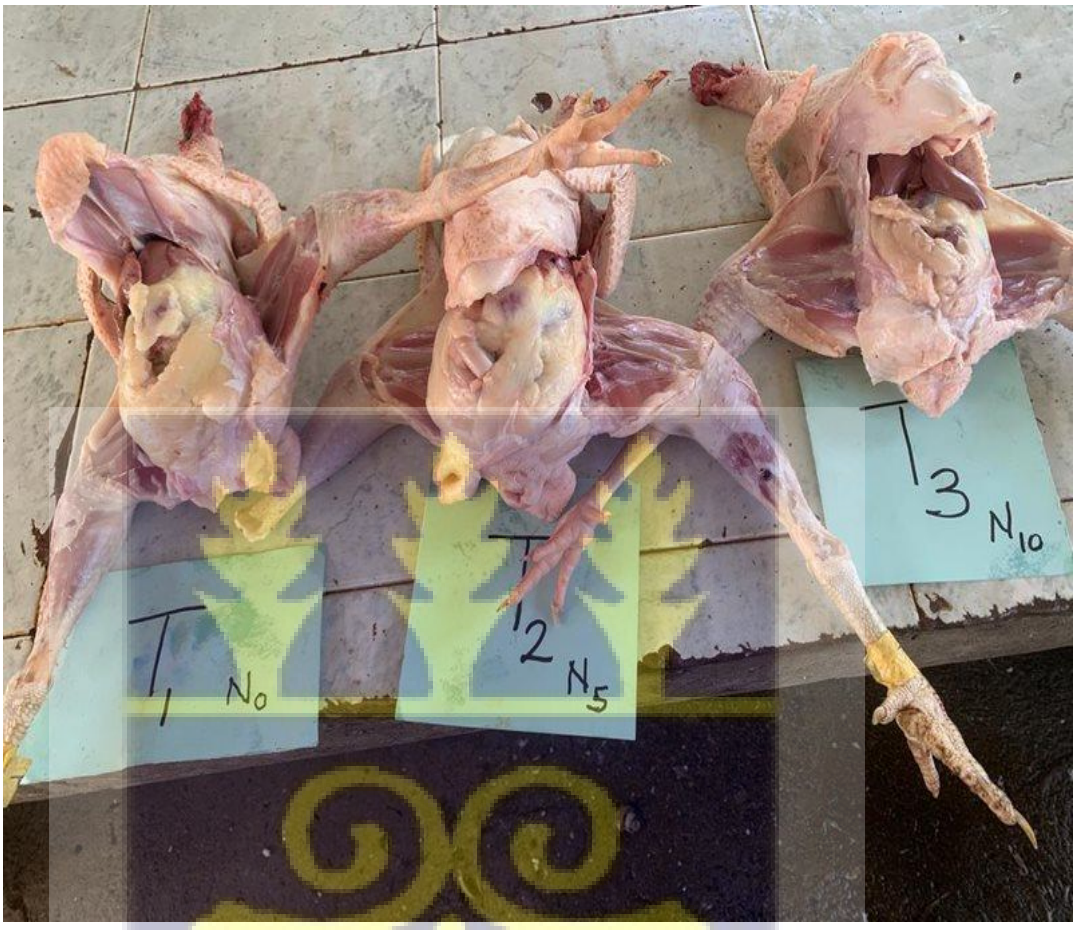


Plate 4.2 Comparison of the effect of noni fruit extract on fat-laden abdomen

0 mg/ml noni fruit extract (control) = T_{1No}; 20 mg/ml noni fruit extract = T_{2N5}; 40 mg/ml noni fruit extract = T_{3N10}

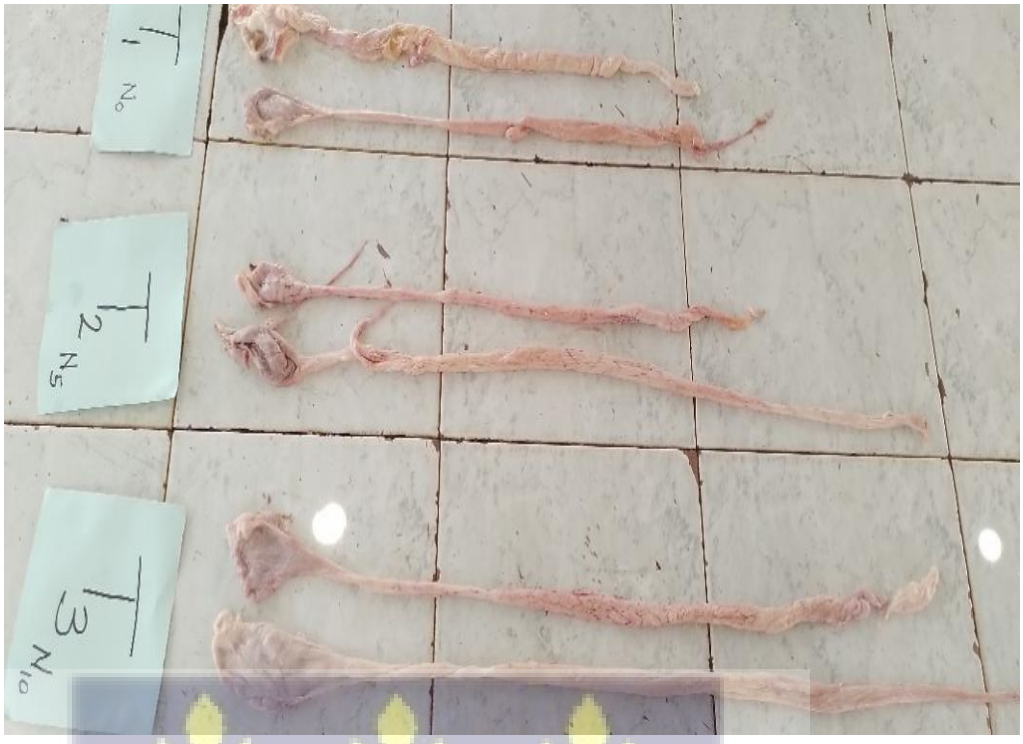


Plate 4.3 Effect of noni fruit extract on uterus development

0 mg/ml (control) noni fruit extract = T_{1No}; 20 mg/ml noni fruit extract = T_{2N5}; 40 mg/ml noni fruit extract = T_{3N10})

4.6 Discussion

4.6.1 Yield of noni fruit extract

The higher yield of noni fruit extract at week 8 (580 ml/kg) and week 12 (580 ml/kg) compared to that of week 2 (397 ml/kg), and week 4 (440 ml/kg) may be due to the lower extent of fruit tissue disintegration of the fruits at weeks 2 and 4 at the onset of fermentation. Samarasiri *et al.* (2022) reported a different pattern in their study. They kept the fruits indoors for ripening for three days beyond the hard-yellow stage to attain the soft grey succulent fruit stage prior to initiating the drip fermentation process. They reported a dramatic release of fruit extract in first 2 weeks of fermentation and thereafter a gradual

increase reaching a peak at week 4 followed by a gradual release of extract till the end of fermentation at week 8.

Noni fruit extract yield is influenced by the stage of ripeness, and processing techniques, and the method used for extraction such as pressing, blending or fermentation (Nelson and Elevitch, 2006). Temperature and duration also play a role in the yield as higher temperatures break down the cellular structure of the fruit faster, potentially enhancing fruit extract release thereby influencing the final yield. Nelson and Elevitch (2006) reported the recovery of fruit extract by the drip method to be approximately 40 – 50 % of the original fruit weight. They also proposed that the residual pulp may be pressed to express the remaining fruit fluids.

4.6.2 Anti-oxidant capacity (concentration) of noni fruit extract

The higher antioxidant capacity of the noni fruit extract at 8 and 12 weeks of drip fermentation compared to that of 2 and 4 weeks may be attributed to the activity of the increased population of microorganisms, particularly Acetobacter and Gluconobacter bacteria that increase the level of antioxidant compounds such as ascorbic acid and flavonoids. Drip fermentation modified the flavonoid composition to enhance bioactive compounds like quercetin and kaempferol (Jakfar *et al*., 2023; Zhang *et al*., 2021). Almeida *et al*. (2019) reported that the accumulation of bioactive compounds in the noni fruit depends on the ripening stage. Thomson (2011) found that the total content of phenolic compounds, the antioxidant potential and the ascorbic acid content in the noni fruit increased in the transition from the green stage to the hard white-yellow stage.

4.6.3 Early-lay performance of pullets

4.6.3.1 Feed intake

Feed intake decreased with increasing levels of noni fruit extract in drinking water. This may be due to the appetite suppressant properties of the noni fruit extract.

Studies by Singh (2012) and Aroche *et al.* (2018) suggest that noni fruit extract may have appetite-suppressing properties. Additionally, Pu *et al.* (2004) found that noni can regulate food consumption through the action of the gut by increasing the secretion of cholecystokinin (CCK) and activating CCK1 receptors. The CCK stimulates the secretion of pancreatic enzymes, leading to gall bladder contraction and bile release into the small intestine thus slowing down small intestine transit time. This reduces the emptying of the stomach and feed intake.

4.6.3.2 Water intake

Water intake was similar across dietary treatments, the water intake by the birds administered noni fruit extract appeared not to have any deterrent effect on acceptability of the water which may be due to the appealing rose colour and fruity flavour developed by the noni fruit extract during fermentation (Cheng *et al.*, 2021).

4.6.3.3 Body weight gain

The noni fruit is rich in essential nutrients including amino acids, vitamins, minerals, coenzymes, carbohydrates, and alkaloids that aid in the metabolism of other nutrients and promote cell and tissue growth (Sunder *et al.*, 2016) including protein accretion during muscle development. This may account for the higher daily weight gain in the birds

administered with the noni fruit extract compared to their counterparts, which received no noni fruit extract (control). According to Dumoulin (2018), medullary bone deposition and uterus maturation are essential components of early-lay development and contribute to body weight gain. The final body weight obtained in the present study was similar to observation by Sunder *et al.* (2011a) who recorded an average final body weight of 1,864 ± 89.22 g for the noni-fed birds compared to the control group (1748.5 ± 83.22g) in the Nicobari fowl.

4.6.3.4 Age at first egg

The results for the days-to-first egg in the present study were contrary to the observation of Churchil *et al.* (2019) who reported a significant reduction in the days-to-first egg in layer type Japanese quails fed noni fruit extract as compared to the control. The days-to-first-egg is an important economic factor that determines the age at which chickens attain sexual maturity, as noted by Guni *et al.* (2021). According to Olawumi (2011), body weight is one factor that affects the age at which hens lay their first egg, the age at which they reach peak egg production and their overall performance. Birds with higher body weight have been reported to lay their eggs earlier (Olawumi, 2011). Contrary to the findings concerning body weight, the birds in the present study that had higher body weight and lower abdominal fat that received noni fruit extract rather had a delay of 3 days at age at first egg. This suggested a delay in attaining sexual maturity which may be explained by the report of Bornstein *et al.* (1984) who observed that a high degree of fatness due to an accelerated rate of fat accumulation appeared to be associated with an early start of ovulation, which shortened the age at first egg. The delay of the days to first egg in the current study was more than compensated for due to the heavier and larger size of eggs laid by the noni-treated groups

(20 mg/ml and 40 mg/ml) compared to the control (0 mg/ml). This may be due to the continued rate of development of the uterus of the birds on noni fruit extract beyond week 18 (Dumoulin, 2018; Yin *et al.,* 2020). The presence of proxeronine in the noni fruit extract may have stimulated further development of the uterus and delayed sexual maturity.

4.6.3.5 Egg mass and egg weight

The egg weight and egg mass improved with increasing level of noni fruit extract in drinking water of the birds. The increased final body weight associated with increasing concentration of noni fruit extract administered to the birds may have conferred an advantage in terms of the laying performance of the pullets over their counterparts that received no noni (control). The body weight of a layer has been reported to be a crucial factor that influences the laying performance (Dumoulin, 2018; Olawumi, 2011). However, Churchil *et al.* (2019) did not find any significant increase ($p < 0.05$) in egg weight and egg mass probably due to the lower concentration of noni fruit extract administered to their birds as compared to what was used in this current study.

4.6.4 Selected internal organs affecting egg production

4.6.4.1 Abdominal fat

The reduction in abdominal fat with increasing concentration of noni fruit extract compared with birds on the control may be due to the anti-obesity effects Research has shown that noni fruit extract has anti-obesity effects that reduce adipose tissue weights and plasma triglyceride levels while improving glucose tolerance without any toxicity in C57BL/6 mice (Nishioka, 2007). In addition, Jambocus *et al.* (2017) suggested that noni leaf extracts exhibit

anti-obesity properties through the inhibition of pancreatic and lipoprotein activity, which positively affects lipid profiles and reduces LDL levels and visceral fat deposition in male Sprague-Dawley rats. The reduction in abdominal fat observed as the concentration of the noni fruit extract in the drinking water increased agreed with the trend reported by Widjastuti *et al.* (2019) for Sentul Debu Chicken.

4.6.4.2 Uterus development

Typically, maturity of the bird's reproductive organ begins at around 16 weeks of age. The uterus gradually increases in size until it reaches its maximum level by week 18. Following this point, there is a gradual decrease in the rate of increase in the size of the uterus until the birds begin to lay eggs (sexual maturity; illustrated in Chapter 2, subsection 2.4.1, Figure 2.1). Noni fruit extract contains compounds such as scopoletin, xeronine, flavonoids, and vitamin C, which may affect cellular function in birds and also impact their reproductive cycles by influencing hormonal regulation of oestrogen and progesterone essential for uterus maturation for egg formation and egg laying (Abdel-Wareth and Lohakare, 2014; Saki *et al.*, 2014 Ali *et al.*, 2016; Pandiselvi *et al.*, 2019). This may account for the uterus weight increase observed in the birds administered with the noni fruit extract compared to the control.

Various studies conducted that have suggested that the supplementation of noni can increase egg production performance include Sunder *et al.* (2013), Sunder *et al.* (2016), Asmara *et al.* (2019) and Widjastuti *et al.* (2023).

4.6.4.3 Bone mineralisation

The increase in bone mineralisation depicted by increase in ash content in the bird's right tibia bone with increase in concentration of noni fruit extract may due to the fact that it stimulates osteoblastogenesis leading to bone mineralisation with large deposition of calcium. Hussain *et al.* (2016) has reported that noni fruit extract stimulates osteoblastogenesis by increasing the proliferation rate of bone marrow mesenchymal stem cells (BMSC), and upregulating the genes associated with osteogenic differentiation such as osteocalcin and runt-related transcription factor-2, as well as the enzyme alkaline phosphate (ALP) which tends to increase bone density.

4.6.7.4 Tibia bone length and diaphysis diameter

The increase in the tibia bone length and diaphysis diameter as concentration of noni fruit extract increased may be due to the growth-promoting characteristics of noni fruit extract, which is phytogenic in nature. In a study conducted by Javid *et al.* (2022), the impact of pre- and probiotics on the morphometric characteristics (length, weight, thickness of lateral and medial walls, tibiotarsal index of bone, bone ash percentage) of tibia bones of broilers was examined. The study concluded that using pre- or probiotics as growth promoters can improve the quality and density characteristics of bones in broiler chickens compared to the use of antibiotics for growth control. Norton *et al*. (1996) has also reported that bones grow not only longer but also wider in diaphysis diameter as osteoclasts break down old bone in the medullary cavity, while the osteoblasts generate new bone tissues beneath the periosteum through intramembranous ossification.

4.7 Conclusion

1. The maximum yield and antioxidant capacity (concentration) obtained for the noni fruit extract using drip fermentation were 580 ml/kg fruit and 3.9 mg/ml at 8 weeks.
2. Feed intake decreased with increasing concentration of noni fruit extract administered in the drinking water/ while water intake remained similar. Final body weight, daily weight gain, egg weight and egg mass were improved. The abdominal fat reduced, but weight of the uterus, tibia bone growth and mineralisation characteristics improved with increasing concentration of the noni fruit extract. The 40 mg/ml concentration of noni fruit extract generally had a better effect on most of the parameters examined than the 20 mg/ml.

CHAPTER 5

5.0 EXPERIMENT 2

EFFECT OF NONI FRUIT EXTRACT AND PHYSIOLOGICAL STAGE ON PRODUCTION PERFORMANCE AND EGG QUALITY CHARACTERISTICS OF LAYERS

5.1 Summary

This study was conducted at the Livestock and Poultry Research Centre, University of Ghana, Legon to determine the effect of noni fruit extract on egg production performance (egg mass, Hen day egg production, feed conversion ratio and net feed efficiency index) and egg quality characteristics (such as the Haugh Unit, Yolk Index, Yolk Colour etc) in commercial layers. Also, the effect of physiological stage of birds (early-lay, peak-lay and late-lay) on egg production and egg quality characteristics was assessed. The 270 commercial layers used for this study were placed in three treatment groups of 90 birds per treatment with five replicates of 18 birds per replicate in a completely randomised design (CRD). Treatment 1 (T_1) received plain water (0 mg/ml of noni fruit extract), Treatment 2 (T_2; received 20 mg/ml of noni fruit extract), and Treatment 3 (T_3; received 40 mg/ml of noni fruit extract) all administered through drinking water. These treatments were administered from the age of 16 weeks. Eggs were collected at week 22, week 30, and week 48 to represent early-lay, peak-lay, and late-lay physiological stages. Two eggs per replicate per treatment (30 eggs) were used to evaluate the effect of levels of noni fruit extract (0 mg/mi, 20 mg/ml and 40 mg/ml) and the physiological stage on the internal quality (albumin height, Haugh unit, yolk index and yolk colour) as well as, the egg weight and estimated shell thickness.

To determine the effect of storage period on egg quality, 10 eggs per treatment were used for each of the 5 evaluation weeks of storage (weeks 0, 1, 2, 3 and 4), totalling 150 eggs. The eggs were collected at 9 a.m., labelled on the shell, and promptly transported to the Nutrition Laboratory of the Animal Science Department at the University of Ghana, Legon. The temperature and humidity of the storage area were recorded daily using a thermometer and humidity gauge. The first day after eggs were collected (Week 0), 10 eggs per treatment (2 eggs per replicate) were weighed using a digital egg tester. The analysis of variance procedure of Gen Stat (VSN International, 2009) was used to analyse the egg quality parameters measured. The data was tested at 5 % level of significance. Where significant differences were indicated, the Student Newman-Keuls multiple comparisons procedure was used to separate the means. The results showed that adding noni fruit extract to drinking water improved ($p < 0.05$) all egg production performance indices and egg quality indices determined. As the dosage of noni fruit extract increased, egg production and quality also improved. Over time, egg quality decreased, but noni fruit extract administration aided in slowing down this process and preserved the yolk colour. Generally, egg production performance (egg mass, HDEP % and FCR) and egg quality indices (albumen height and egg weight) improved ($p <0.05$) with increasing physiological stage from early-lay through peak-lay and late-lay periods. The results suggest that noni fruit extract could be used as a natural supplement beyond the rate of 40 mg/ml of drinking water to enhance egg production, quality, and extend shelf life of eggs kept under ambient temperature as no negative performance were recorded.

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5.2 Introduction

Concerns about antibiotic resistance due to sub-therapeutic use in animals as growth promoters and performance enhancers have sparked controversy in poultry production, due to the rise in antibiotic resistance in humans (Selaledi *et al.*, 2020). It has been reported (Abdel-Wareth and Lohakare, 2020) that phytogenics could replace synthetic products to enhance the effective use of feed nutrients, which may subsequently result in faster body weight gain, higher production rates, and improved feed efficiency. Interest in using phytogenics in animal production and studies to identify natural growth promoters and performance trait enhancers in poultry production is therefore increasing (Sunder *et al.*, 2011a; Abd El-Hack *et al.*, 2019; Asmara *et al*., 2019; Abdel-Wareth and Lohakare, 2020; Phillips *et al.*, 2023). Dietary supplementation with herbs, essential oils, and active components stimulates the productive performance, egg quality, digestibility of nutrients, and some blood biochemical parameters of laying hens (Abdel-Wareth and Lohakare, 2014; Abdel-Wareth, 2016; Akbari *et al.*, 2016; Olgun, 2016; Ding et al., 2017). Sunder *et al.* (2015b) and Churchil *et al.* (2019) have suggested that *Morinda citrifolia*, commonly known as noni, has egg productivity-stimulating properties.

Unfortunately, most poultry farmers in Ghana lack access to well-equipped storage facilities to prolong the shelf life of eggs. Consequently, they mainly store their eggs in open-ventilated rooms, resulting in increased egg losses (Mensah *et al.*, 2022).

The commercial layers are extensively used in Ghana but they often experience a decline in egg production and quality during the transition from early laying to peak laying, resulting in significant economic losses for farmers (Hagan and Apori, 2013). Despite various efforts

made to address this issue, an effective solution remains elusive. Hence, the question arises as to what effect would Noni fruit extract have on production and quality indices in laying hens and whether it can be used as a natural feed supplement to improve egg production and quality during this critical phase. Also, what effect will the physiological stage of birds (early-lay, peak-lay and late-lay) have on egg production performance, egg quality characteristics and storage time of eggs.

5.3 Objectives

The objectives of this study were:

- To evaluate the potential benefits of noni fruit extract as a natural feed supplement on egg production and egg quality indices in commercial layers.
- To investigate the effect of physiological stage (early-lay, peak-lay and late-lay) on egg laying performance and egg quality characteristics in commercial layers
- To assess the effect of noni fruit extract on the extension of shelf-life in eggs of commercial layers.

5.4 Materials and methods

5.4.1 Study area and experimental diets

The study area has already been indicated in section 3.1. (Chapter 3). The experimental diets administered to the birds was in the form of layer mash as shown in Table 3.1. (Chapter 3). The initial feed offered to the birds was 2.0 kg per 20 birds per treatment. The daily feed intake was obtained by deducting the residual feed weight each morning from the initial

weight. This was further divided by the number of birds per pen to obtain the daily feed intake per bird and summed weekly to obtain the weekly feed intake per bird.

5.4.2 Management of experimental birds

The 270 commercial layers with an average body weight of 1.8 ± 0.397 kg were assigned to three treatments. Treatment 1(control received 0 mg/ml of noni fruit extract), Treatment 2 received 20mg/ml of noni fruit extract) and Treatment 3 (received 40 mg/ml noni fruit extract) administered through drinking water. These treatments were administered from the age of 16 weeks as stated in Chapter 3 Section 3.3.1. The birds had been raised to the point-of-lay on a standard grower and pre-lay mash and were placed on layer mash (Table 3.1, Chapter 3). The daily feed and water allocated to the birds were recorded. The trial ended at 48 weeks when the hen house egg production started to decline.

5.4.3 Experimental design and analysis

The 270 commercial layers were randomly assigned to three treatments of 18 birds per treatment with five replicates per treatment in a completely randomised design. The general analysis of variance procedure of GenStat (VSN International, 2009) was used. The data was tested at 5 % level of significance, the Student Newman-Keuls multiple comparisons procedure was used to separate the means.

The statistical model used was as presented below:

$$Y_{ijkl} = \mu + T_i + P_j + TP_{(ij)} + e_{iikl}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 5.1$$

Where:

Y_{ijkl} = The response variable

μ = Overall mean

T_i = Effect of the i^{th} treatment i = 1, 2, 3

P_j = Effect of the j^{th} physiological stage j = 1, 2, 3

$TP_{(ij)}$ = Effect of the interaction between the i^{th} treatment and j^{th} physiological stage (ij) = 1, 2, 3

e_{ijkl} = Random residual error term, assumed ≈ NID (0, $\sigma^2 e$)

5.4.4 Egg Collection and determination of egg weight, egg mass and egg quality

The eggs were collected at week 22, week 30 and week 48 representing early-lay (E-Lay), peak-lay (P-Lay) and Late-lay (L-Lay). The number of eggs used was 2 eggs per replicate per treatment (30 eggs) for each of the 5 evaluation weeks of storage (150 eggs) per the 3 physiological stages of lay totalling 450 eggs. The eggs were collected at 7 am daily and then each egg was labelled on the shell and immediately transported to the Nutrition Laboratory of the Department of Animal Science at the University of Ghana, Legon for egg internal quality analysis. The temperature and humidity of the storage area were recorded using a thermometer and humidity gauge. The next day (Week 0), 10 eggs per treatment (2 eggs per replicate) were weighed using a digital egg tester (DET-6000® with an accuracy of 0.01g, Nabel, Kyoto, Japan). Each egg was carefully cracked near the equator with a sharp knife and then gently opened on one side in a hinge-like motion onto the flat tray of the digital tester to prevent the yolk membrane from breaking (Nabel, 2016; Jang, 2022). The digital

egg tester then evaluated each egg for the albumen height, Haugh Unit and yolk colour (see Chapter 3 section 3.4).

The yolk height and diameter were measured using a vernier calliper for determination of the yolk index (Qi *et al*., 2020). This procedure was repeated at week 30 (peak-lay) and week 48 (late lay) to determine the effect of noni fruit extract treatment and the physiological stages (early, peak and late lay) on the internal quality of the eggs. To determine the effect of storage period on egg quality, ten eggs per treatment were used for each of the five evaluation weeks of storage, totalling 150 eggs. The procedure for determining the internal egg quality was repeated weekly over four weeks.

Data obtained from the egg tester to determine egg quality traits were albumen height (AH), Haugh Unit (HU), and yolk colour (YC). The estimated shell thickness (EST) was measured with a vernier calliper from three points at the top, equator and base of the broken-out shell and the average was recorded as the eggshell thickness of each egg (Anene *et al.*, 2020). The storage ambient temperature (T) and relative humidity (RH) were recorded.

5.4.4.1 Derived data from eggs collected

Derived data computed were percent hen day egg production (HDEP %), egg mass (EM), feed conversion ratio per kg egg mass (FCR), net feed efficiency index (NFEI), and Yolk Index (YI). Weight loss (WL) of eggs during storage was also computed.

5.4.4.1.1 Hen day egg production (HDEP %)

This was obtained by dividing the total number of eggs produced during the period by the total number of hen days (sum of number of hens alive each day) during the same period and multiplied by hundred (equation 5.2).

$$\text{HDEP \%} = \frac{\text{Total number of eggs produced during the period}}{\text{Total number of hen-days in the same period}} \text{ X } 100 \quad \ldots 5.2 \text{ (TNAU, 2012)}$$

5.4.4.1.2 Egg mass

To calculate egg mass, representative samples of the eggs were weighed to obtain the average weight of eggs and then multiplied by the percent hen day egg production (equation 5.3).

$$\text{Egg mass} = \text{(Percent HDEP) x (Average egg weight in grams)} \ldots 5.3 \text{ (TNAU, 2012)}$$

5.4.4.1.3 Feed Conversion Ratio (FCR)

This was calculated as the ratio between feed consumed and egg mass.

$$\text{FCR} = \frac{\text{Weight of feed consumed}}{\text{eggs mass}} \ldots\ldots\ldots 5.4 \text{ (TNAU, 2012)}$$

5.4.4.1.4 Net feed efficiency index (NFEI)

NFEI is the sum of the mean egg mass and body weight gain (or loss) by the layer (s) during the study period multiplied by 100 and divided by their mean feed intake for the same period.

The NFEI thus takes into account egg mass, average body weight gain and feed consumed in the period as shown by equation 5.5.

$$NFEI = \frac{(Egg\ mass + BWG)}{Feed\ consumed} \times 100 \ldots\ldots 5.5 \quad \text{(Narahari, 1983)}$$

Where:

EM = Mean egg mass in grams during a particular period

BWG = Mean body weight gain or loss in grams during a particular period

FC = Mean Feed consumption/hen in g during a particular period

A NFEI value of 45 and above is considered desirable.

5.4.4.1.5 Weight Loss in Eggs under Storage

The weekly weight loss in eggs under storage was calculated by deducting the mean final egg weight at the end of each week of storage from the mean egg weight of the preceding week using a Microsoft excel worksheet.

5.5 Results

5.5.1. Egg production performance indices

The influence of the noni fruit extract on egg production performance indices is presented in Table 5.1. Treatment had a significant ($p < 0.05$) effect on the daily feed intake, body weight gain, egg mass, percent hen day egg production, feed conversion ratio and net feed efficiency index. Daily feed intake significantly ($p < 0.05$) decreased with increasing dosage of the noni fruit extract. The values for the 40 mg/ml noni fruit extract (T_3) were lowest compared to the 20 mg/ml noni fruit extract (T_2) and the control (0 mg/ml, T_1). On the other hand, weight gain, egg mass, hen day egg production, feed conversion ratio and net feed efficiency index

increased ($p < 0.05$) with increasing concentration of noni fruit extract. The values for the 40 mg/ml noni fruit extract (T_3) were highest compared to the 20 mg/ml noni fruit juice (T_2) and the control (0 mg/ml; T_1).

The effect of the physiological stage of the birds on egg performance indices was significant ($p < 0.05$). Egg mass increased with the physiological stage. Feed conversion ratio improved with increasing physiological stage (from early-lay to late-lay) being better at late-lay than early-lay and peak-lay (Table 5.1). Percent hen day egg production increased from early lay to peak lay and declined at late lay. Net feed efficiency on the other hand decreased ($p < 0.05$) with increase in physiological stage. The value obtained at early-lay (82.9 %) was higher ($p < 0.05$) than that at peak-lay (63.27 %) and late-lay (51.19 %).

The interaction effects between noni fruit extract and physiological stage on production performance indices daily feed intake, body weight gain, egg mass, HDEP %, FCR and NFEI were significant at $p < 0.5$ (see Appendix 1).

Table 5.1 Effect of Noni fruit extract and physiological stage of birds on egg performance indices

Treatment (Noni fruit extract)					
Parameters	**T_1 (0 mg/ml)**	**T_2 (20 mg/ml)**	**T_3 (40 mg/ml)**	**SEM**	**p-value**
Daily Feed Intake (g/d)	107.3^{a}	106.7^{b}	104.8^{c}	0.023	0.001
B.Wt. gain (g/d)	21.49^{c}	29.21^{b}	30.75^{a}	0.291	0.001
Egg Mass	35.85^{c}	42.28^{b}	48.91^{a}	0.031	0.001
HDEP %	67.49^{c}	75.02^{b}	78.65^{a}	0.052	0.001
FCR	3.22^{c}	2.74^{b}	2.38^{a}	0.004	0.001
NFEI	53.77^{c}	67.87^{b}	75.77^{a}	0.056	0.001
Physiological Stage					
Parameters	**Early-Lay (Week 22)**	**Peak-Lay (Week 30)**	**Late-Lay (Week 48)**	**SEM**	**p-value**
Daily Feed Intake (g/d)	100.9^{c}	108.4^{b}	109.0^{a}	0.023	0.001
B.Wt. gain (g/d)	57.52^{a}	20.00^{b}	3.93^{c}	0.029	0.001
Egg Mass	25.97^{c}	48.91^{b}	51.48^{a}	0.031	0.001
HDEP %	53.05^{c}	86.08^{a}	82.02^{b}	0.052	0.001
FCR	3.93^{c}	2.25^{b}	2.17^{a}	0.004	0.001
NFEI	82.90^{a}	63.27^{b}	51.19^{c}	0.056	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$).
SEM = Standard error of means: HDEP % = Percent Hen Day Egg Production;
FCR =Feed conversion ratio, NFEI = Net feed efficiency Index. BWt.gain = Body Weight Gain

5.5.2. Egg quality performance indices

All the egg quality traits were influenced ($p < 0.05$) by the administration of the noni fruit extract as shown in Table 5.2. The mean values of albumen height, Haugh unit, estimated shell thickness, yolk index, yolk colour and egg weight were higher ($p < 0.05$) in the birds that were offered 20 mg/ml of noni fruit extract (T_2) and 40 mg/ml noni fruit extract (T_3) compared to the control (0 mg/ml; T_1). The mean values of the Haugh unit, yolk index estimated shell thickness and yolk colour were similar ($p > 0.05$) in the treatment groups T_2 and T_3.

The albumen height and egg weight significantly ($p < 0.05$) increased with physiological stage with late-lay having the highest values (Table 5.2). Estimated eggshell thickness were higher ($p < 0.05$) at early-lay compared to peak-lay and late-lay. The Haugh unit and yolk index decreased ($p<0.05$) with physiological stage with late-lay having the lowest value. The change in yolk colour was not significantly ($p > 0.05$) affected by physiological stage.

The interaction effects between noni fruit extract and physiological stage on egg weight was significant ($p < 0.05$). Interactions for all other parameters such as albumen height, Haugh unit, yolk index, yolk colour and estimated eggshell thicknesses were not significant ($p > 0.05$) (see Appendix 2 and 3).

Table 5.2 Effect of Noni fruit extract, and physiological stage of birds on egg quality indices

Parameters	Treatment (Noni Fruit Extract)			SEM	p-value
	T_1 (0 mg/ml)	T_2 (20 mg/ml)	T_3 (40 mg/ml)		
Albumen height (mm)	4.28^{c}	5.12^{b}	5.31^{a}	0.061	0.001
Haugh Unit	65.55^{b}	71.44^{a}	71.35^{a}	0.551	0.001
EST (mm)	0.42^{b}	0.47^{a}	0.48^{a}	0.001	0.001
Yolk Index (mm)	3.36^{b}	0.40^{a}	0.40^{a}	0.005	0.001
Yolk Colour	4.13^{b}	4.38^{ab}	4.53^{a}	0.159	0.047
Egg Weight (g)	52.23^{c}	55.74^{b}	59.91^{a}	0.572	0.001
	Physiological Stage				
Parameters	**Early-Lay (Week 22)**	**Peak-Lay (Week 30)**	**Late-Lay (Week 48)**	**SEM**	**p-value**
Albumen height (mm)	4.82^{c}	4.86^{b}	5.03^{a}	0.061	0.002
Haugh Unit	73.78^{a}	68.73^{b}	65.82^{c}	0.551	0.001
EST (mm)	0.463^{a}	0.459^{b}	0.458^{b}	0.001	0.001
Yolk Index (mm)	0.42^{a}	0.38^{b}	0.37^{c}	0.005	0.001
Yolk Colour	4.27	4.37	4.40	0.159	0.685
Egg Weight (g)	48.58^{c}	56.69^{b}	62.60^{a}	0.572	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$).
SEM = Standard error of means; EST = Estimated Eggshell Thicknesses

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5.5.3 Effect of noni fruit extract, and storage time on egg freshness

5.5.3.1 Egg weight loss

During the storage period, significant ($p < 0.05$) egg weight loss was observed in the control group T_1 (0 mg/ml) as shown in Figure 5.1. The control group (T_1) had the highest weight loss value of 1.95g by week 4, while group T_3 (40 mg/ml noni fruit extract) had the lowest weight loss value of 1.34g by week 4. There were no significant differences ($p > 0.05$) in weight loss between T_2 (20 mg/ml) and T_3 (40 mg/ml) each week.

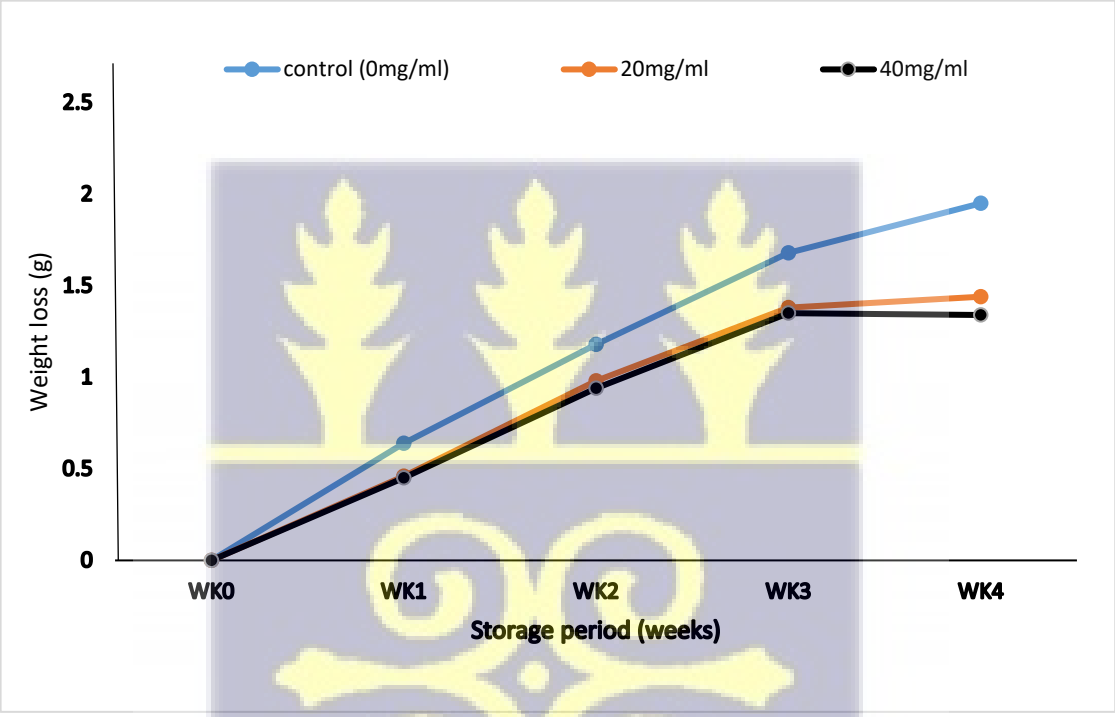
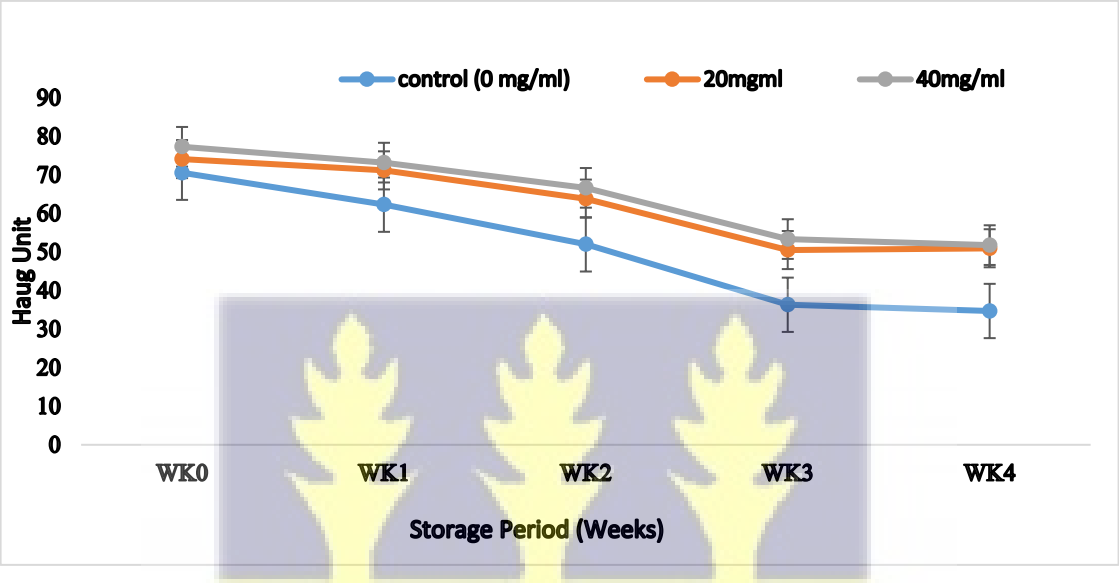


Figure 5.1 Weight loss in eggs during 4 weeks of storage

5.5.3.2 Haugh unit (HU)

The Haugh unit was significantly ($p < 0.05$) influenced by the storage period as shown in Figure 5.2. The Haugh Unit of the control group T_1 (0 mg/ml) declined ($p < 0.05$) rapidly compared to groups T_2 (20mg/ml) and T_3 (40mg/ml). The mean values of the Haugh unit of the treatment groups T_2 and T_3 were similar ($p > 0.05$).



5.5.3.3 Yolk index

The influence of the noni fruit extract on the yolk index over a 4-weeks storage period is shown in Figure 5.3. The yolk index of the control group T_1 (0mg/ml) declined ($p < 0.05$) between week 3 and week 4 from 0.31 (grade B) to 0.16 (grade C). Those of the treatment groups T_2 (20mg/ml) and T_3 (40mg/ml) declined over the 4 weeks but did not fall below 0.33 for regular eggs grade B (Figure 5.3).

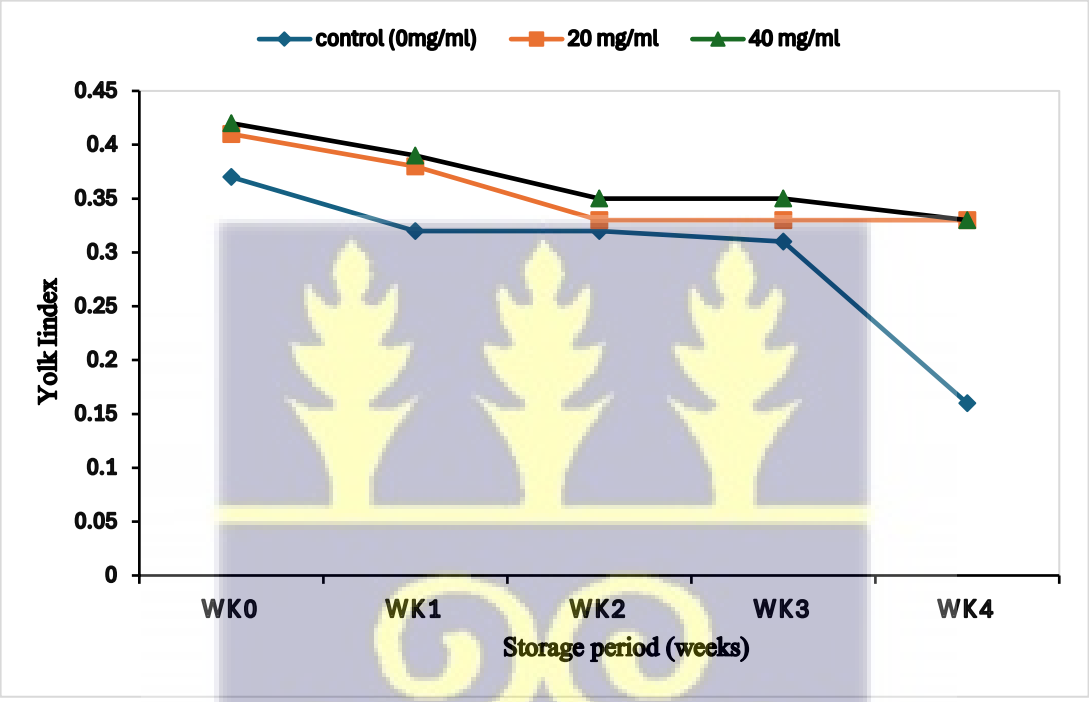


Figure 5.3 Change in yolk index of eggs during 4 weeks of storage

5.5.3.4 Yolk colour

The yolk colour was significantly ($p < 0.05$) influenced by the noni fruit extract over the 4-week storage period (Figure 5.4). The yolk colour of the control group T_1 (0 (mg/ml) changed from 4.0 on the Roche Colour Scale to 2.0 (Vuilleumier, 1969). The yolk colour of the noni

fruit extract treatment groups T_2 (20 mg/ml) and T_3 (40 mg/ml declined from 4.0 and 4.1 respectively to 3.0.

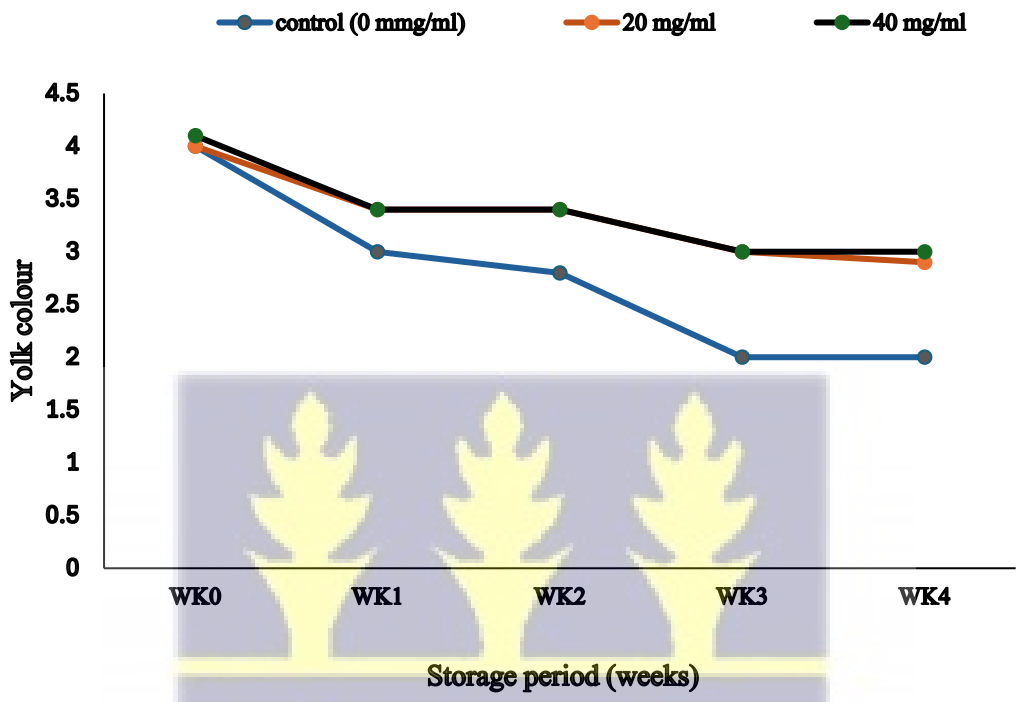


Figure 5.4 Change in yolk colour of eggs during four weeks of storage

5.6 Discussion

5.6.1 Effect of Noni fruit extract, and physiological stage on egg production performance indices

In this study, the significant influence of noni fruit extract (a phytogen) on egg mass may be due to the beneficial nutraceutical components of noni fruit extract. That may have improved gut integrity, increase in ileal digestibility, and absorption of calcium increasing egg mass (Sunder *et al.*, 2011, 2016; Growel, 2017; Dumoulin, 2018; Asmara *et al.*, 2019). Olgun and

Yildiz (2014) reported that the dietary addition of essential oil mixtures (EOM; 400 or 600 mg/kg) containing a mixture of essential oils from thyme, black cumin, fennel, anise and rosemary increased eggshell weight and hence egg mass by reducing the excretion of minerals such as calcium (Ca^{2+}), phosphorous (P^{3-}), magnesium (Mg^{2+}), manganese (Mn) and (Zn^{2+}) in breeder quails.

Amad *et al.* (2011) had reported that the addition of phytogenic feed additives such as thyme and star anise oils to broiler diets caused an increase in the apparent ileal digestibility of Ca and P, respectively. They concluded that the improvement in the digestibility of nutrients could be due to the stimulatory effect of the phytogenic feed, which increased endogenous digestive enzymes and the absorptive surface area in the intestine. The improved feed conversion ratio with increasing levels of noni fruit extract administration over the control in the present study (Table 5.1) may be due to decreased feed intake with increased body weight gain as the levels of noni fruit extract in the drinking water of the birds increased. Components of noni fruit extract including flavonoids, alkaloids, minerals and vitamins may have, contributed to improved weight gains observed in the birds administered with noni fruit extract. This coupled with higher egg mass and decreased feed intake with increasing level of noni fruit extract may have contributed to the high net feed efficiency index in birds on the noni treatment compared to those on the control. In agreement with the results obtained in this present study, Churchil *et al.* (2019) reported in a study of layer type Japanese quails that noni-fruit extract supplementation resulted in better feed conversion ratio for both growth and egg production compared to non-supplemented birds (control). Similar improvements in feed conversion ratio have been observed in Japanese quails (Sunder *et al.*,

2016), and Nicobari Indigenous chicken (Sunder *et al.*, 2011a) when noni fruit extract was administered through drinking water.

The increased egg mass with physiological stage in this study may be due to maturation of the reproductive system and an increased capacity to produce larger eggs over time (Yavuz 2014; Growel 2017; Dumoulin 2018). Factors associated with reproductive ageing and increased susceptibility to diseases (Growel 2017) may account for the increased egg production from earl-lay to peak-lay and its decline at late-lay.

Typically, feed conversion ratio worsens (increases) with physiological stage as older hens require more feed to produce the same amount of egg mass compared to younger birds (Growel 2017), but in this current study the feed conversion ratio rather improved ($p < 0.05$) with increase in physiological stage (early-lay, peak-lay and late-lay) respectively (Table 5.1). This may be due to the increased daily feed intake with physiological stage and a consequent increase egg mass (Table 5.1). Also, net feed efficiency index tends to decline with age because as physiological stage progresses the hens' overall efficiency of converting feed into egg mass and body weight gain decreases (Narahari *et al*. 1983; TNAU, 2012). Hence, the observed decline in net feed efficiency index in this study.

5.6.2 Effect of noni fruit extract, and physiological stage on internal egg quality characteristics

The higher albumen height and Haugh Unit observed in this study for the noni treatments (T_2, 20 mg/ml; T_3, 40 mg/ml) compared to the control T_1 (0 mg/ml) may be due to the stimulation of the oviduct's albumen-producing glands and enhanced protein synthesis and nutrient utilization by the bioactive compounds (flavonoids and phenolics) in noni fruit

extract (Almeida *et al*., 2019). The increase in estimated eggshell thickness of eggs from the noni administered birds over the control T_1 (0 mg/ml) may be accounted for by the rich mineral and vitamin contents of the noni fruit extract. The results observed in this study agree with that of Más-Toro *et al.* (2015), who supplemented 0.0 %, 0.5 %, 1.0 % and 1.5 % powdered leaves of noni to birds and observed an increase in eggshell thickness compared to those not provided noni (control).

The yolk index of eggs in birds that were given noni fruit extract improved compared to the eggs of birds that did not receive the extract (control). This improvement may be attributed to the nutritional value and health benefits of the noni fruit extract, as well as the strengthening of the perivitelline and vitelline membranes of the egg yolk (Más-Toro *et al.*, 2015). The increased yolk colour of eggs observed in the birds administered with noni fruit extract compared to the control without any noni may be due to the chlorophyll contents of the noni fruit extract imparting colour to the yolk of the eggs. In a study by Wardiny and Sinar (2013), noni leaf extract was added to the drinking water of birds. The inclusion of noni leaf extract in the drinking water resulted in a higher yolk colour intensity compared to the control. The increase in egg weight with increasing level in noni fruit extract (T_2, 20 mg/ml; T_3, 40 mg/ml) in the water of birds compared to those on the control (T_1, 0mg/ml) may be due to the noni fruit extract's increased influence on absorption and transport of nutrients to the uterus for egg formation (Fleming *et al.*, 1998). Sunder *et al*., (2011a, 2016) reported increased egg weight in noni fed group of birds compared to a no noni fed group (control).

Under normal circumstances, albumen height decreases with physiological stage due to changes in protein and water content of the albumen (Williams, 1992; Zita *et al*., 2012). The rather increased albumen height with increased physiological stage in this study (Table 5.2) may be due to the improved nutritional status of the birds (Perić *et al.*, 2017) which improved ovarian follicle maturation and increased protein synthesis and colloid osmotic tension between the albumen and yolk, thus causing the albumen to be more viscous (Zita *et al.,* 2009; 2012).

The Haugh Unit decreased with the physiological stage of laying in this study and agrees with the report of Williams (1992) and Perić *et al.* (2017) who indicated that the physiological stage/age of hens adversely affect the quality of albumen in freshly laid eggs from healthy flocks. The decrease in Haugh unit with increasing physiological stage may be as a result of the larger yolk sizes of bigger eggs laid by older birds which causes the yolk to lose their ovoid shape and flatten out hence causing the albumen height to reduce resulting in a lower Haugh unit (Stadelman and Cotterill, 2013).

The decrease in estimated eggshell thickness with physiological stage (Table 5.2) may be due to less efficient calcium metabolism in older hens leading to thinner shells. Camargo *et al.* (2022) observed that eggshell quality declined with the advance of physiological stage, which coincided with an increase in egg size. Therefore, the progression of physiological stage results in a lesser ability to mobilise bone calcium to form the eggshell.

The decrease in yolk index with increasing physiological stage (Table 5.2) may be due to the less firm larger yolks in eggs laid by older birds which caused them to flatten hence reducing the yolk index which is a ratio between the yolk height to yolk diameter (TNAU, 2012). This

agrees with the study by Perić *et al.* (2017) who reported that the yolk index of freshly laid eggs reduced with the physiological stage of the birds. According to Armitage (2023), yolk colour of laying hens is affected by their diet, while those of younger birds are lighter than their older counterparts as xanthophyll metabolism efficiency is affected by age. However, in this current study, yolk colour was similar in all the physiological stages examined.

The increase in egg weight with physiological stage in this study may be due to the maturation of the bird's reproductive system and their increased capacity to produce larger eggs over time.

5.6.3 Effect of Noni fruit extract and storage period on internal egg quality

Harnsoongnoen and Jaroensuk (2021) reported that weight and freshness of eggs stored at room temperature decreased with storage time. The weight loss in the eggs of the noni fruit extract treated groups T_3 (40 mg/ml) and T_2 (20 mg/ml) compared to those of the control group T_1 (0 mg/ml) over the 4-week period (Figure 5.1). This may be due to the influence of noni fruit extract on calcium mobilisation in favour of eggshell formation (Fleming *et al.*, 1998; Dumoulin, 2018) making the eggshells thicker, heavier and less porous. The less porous nature of the resulting eggshells tend to restrict moisture loss.

All the treatment groups T_1 (0 mg/ml), T_2 (20 mg/ml) and T_3 (40 mg/ml) showed a decreasing trend in their mean Haugh Unit values and yolk index (indicators of egg freshness) with increasing storage time (Figures 5.2 and 5.3). Also, Osei-Amponsah *et al.* (2014) reported a negative effect of storage time on egg quality irrespective of the ecotype of chicken. They recommended that chicken eggs should be kept at temperatures cooler than ambient temperatures to minimise deterioration of their quality.

The slower decline in Haugh unit and yolk index in the noni treatment groups T_2 (20 mg/ml) and T_3 (40 mg/ml) compared to the control T_1 (0 mg/ml) may be due to the improved colloid osmotic tension between the albumen and the yolk resulting in a more viscous albumen (Williams 1992; Zita *et al*., 2009; 2012) as a result of the noni fruit extract administration. The egg freshness reduced from very fresh (AA) 73.78 HU in the noni fruit extract-treated groups to regular (B) 51.78. The HU was comparable to eggs stored under refrigeration indicating an extension of its shelf life due to the treatment effect of the noni fruit extract. For the control group, the egg freshness reduced from fresh (AA) 74.09 HU to regular (B) 34.70 HU. This is consistent with the reports of Gandi *et al.* (2009), Eke *et al*. (2013) and Osei-Amponsah *et al.* (2014) who reported that egg quality decreased with an increase in storage time and temperatures. The primary cause of the decrease in Haugh units during storage is the loss of water and carbon dioxide from the egg whites. Additionally, the reduction in the value of the yolk index results from the weakening and stretching of the vitelline membrane and an increase in water content in the egg yolk, which flattens the egg yolk and decreases the empirical value of the yolk index (Scott and Silversidest, 2000) as observed in this study.

The lesser decline in yolk colour over the 4-week storge period in the noni fruit extract treatment groups T_2 (20 mg/ml) and T_3 (40 mg/ml) compared to the control T_1 (0 mg/ml) may be may be as a result of the reduced degeneration of the pre-vitelline membrane and reduced movement of water and proteins from the albumen to the yolk brought about by the noni fruit extract administration. The degeneration of the pre-vitelline membrane due to water from egg white entering the yolk and diluting the yellow pigment has been reported by (Lee *et al.,* 2016). Additionally, with longer storage periods, some proteins from the egg

white may leach into the yolk, leading to mottling and a decrease (lightening) in yolk colour as has been reported by some authors (Chukwuka *et al.*, 2011; Lee *et al.*, 2016).

5.7 Conclusion

1. Introducing noni fruit extract in drinking water of layers improved egg mass, hen day egg production, feed conversion ratio and net feed efficiency index. Also, the noni-treated groups had improved albumen height, Haugh unit, yolk index, yolk colour and estimated eggshell thickness
2. Egg production performance (egg mass, HDEP % and FCR) and egg quality characteristics (albumen height and egg weight) improved with increasing physiological stage from early-lay through peak-lay and late-lay periods.
3. Storage time increased egg weight loss and reduced the Haugh Unit, yolk index and yolk colour. The noni fruit extract slowed down the rate of degeneration of the egg freshness and preserved the yolk colour.
4. Noni fruit extract can be included at the rate of 40 mg/ml in drinking water as a natural feed supplement to improve egg production performance, egg quality characteristics and shelf life in commercial layers.

CHAPTER 6

6.0 EXPERIMENT 3

EFFECT OF NONI FRUIT EXTRACT, AND PHYSIOLOGICAL STAGE ON BLOOD HAEMATOLOGICAL AND BIOCHEMICAL PROFILES OF LAYERS

6.1 Summary

Commercial layers bred for high egg production experience physiological and oxidative stress that may affect their overall health, liver and kidney functions. As birds age, their kidney function declines, leading to a decrease in egg production and increased mortality. Noni fruit extract has been shown to have antioxidant and anti-inflammatory properties, which may aid in the mitigation of age-related liver and kidney dysfunction in commercial layers and improve the general health and productivity of poultry. This study determined the effect of noni fruit extract, and physiological stage on haematological and blood biochemical profiles of commercial layers. The three hundred pullets (~ 1.3 ± 0.233 kg) were randomly assigned to three experimental treatments. Birds in Treatment 1 (T_1) control group received plain water with no noni fruit extract, those in Treatment 2 (T_2) received 20 mg/ml of noni fruit extract while those in Treatment 3 (T_3) received 40 mg/ml of noni fruit extract all administered through drinking water throughout the experimental period. The pullets were housed in groups of 20 birds per replicate in a 15-cage house in a Completely Randomised Design (CRD). Ten birds per treatment (2 birds per replicate) were randomly selected for blood sampling at four physiological stages of growth namely weeks 16, 22, 30 and 48 representing pre-lay, early-lay, peak-lay and late-lay respectively. The results of the

experiment showed that administration of noni fruit extract up to 40 mg/ml significantly ($p < 0.05$) affected levels of haematological and biochemical indices in the laying hens. These indices were also significantly ($p < 0.05$) influenced by physiological stage of the birds. The levels of most of the blood parameters determined fell within the normal physiological range for poultry demonstrating no adverse effects of noni fruit extract on the health and physiology of the hens.

These findings imply that noni fruit extract holds promise as a natural feed additive for enhancing the health of laying hens, influenced by environmental, nutritional, and pathological factors.

6.2 Introduction

As a natural supplement, phytogenics are used in poultry feeds to improve poultry health and productivity (Sunder *et al.*, 2011a, 2013, 2016; Asmara *et al.*, 2019; Diarra *et al.*, 2019). Commercial layers are bred for high egg production, which can lead to physiological stress and oxidative stress, affecting their liver and kidney function, and overall health (Fulton, 2018).

Seo and Lee (2022), reported that a haematological profile, also referred to as full blood count (FBC), includes a series of tests that evaluate different components of blood, providing a comprehensive understanding of blood cell production, immune function, oxygen-carrying capacity, and coagulation. These parameters are crucial for diagnosing and monitoring various blood-related disorders and diseases (Erhabor *et al.*, 2021). Blood biochemical profiles on the other hand are useful in the assessment of organ function, metabolic status

and overall health Harvey and Thoroughly assessing the impact of feed ingredients on the haematological and blood biochemical indices of chickens is essential as such evaluation can yield valuable insights into the suitability of feed ingredients for poultry consumption, and facilitate early detection of diseases and disorders (Toghyani *et al.*, 2012; Sidharthan, 2023). The liver is an organ, that plays a critical role in digestion (bile production), metabolism, immunity, nutrient storage, and detoxification (Ozougwu, 2017). Studies have demonstrated that consuming noni fruit extract can enhance hepatic antioxidant capacity, and lipid homeostasis, and protect the liver from environmental and chemical stressors (Wang *et al.*, 2008; Lin *et al.*, 2017). Almeida *et al*. (2019) reported that noni fruit extract contains various potential active ingredients, such as polysaccharides, fatty acid esters, glycosides, iridoids, anthraquinones, flavonoids, phytosterols, carotenoids, vitamin A, and potassium. These compounds have been suggested to act as downstream mediators for reactive oxygen species, potentially aiding in alleviating liver fibrosis and inflammation by impacting Hepatic Stellate Cell (HSC) activation and collagen synthesis (Lin *et al*., 2017).

As birds age, their kidney function declines, leading to decreased egg production and increased mortality (Fulton, 2018) thus negatively affecting their productivity. Noni fruit extract has been shown to have antioxidant and anti-inflammatory properties, which may assist in mitigating age-related kidney dysfunction in laying birds (Lin *et al.,* 2017). A haematological and serum biochemical profile is essential for assessing flock health, detecting potential issues at an early stage through monitoring nutritional effectiveness, identifying individual birds with health problems, and informing breeding programs and genetic selection decisions (Benzo *et al.*, 1986; Hagan *et al.*, 2022; Scanes, 2022). Scanes (2022) highlighted the significance of analysing the blood biochemical profile of laying

chickens to assess various health aspects and reported that it provides valuable insights into kidney function, liver function, metabolic activity, calcium and phosphorus homeostasis, enzyme activity, and immune response. Through monitoring of haematological and serum biochemical parameters, farmers and Veterinarians can effectively optimise the health and productivity of egg-producing birds, thereby ensuring a sustainable and efficient egg-production process. Presently data on the influence of noni fruit extract on the health and physiology of commercial layers in Ghana is scanty.

6.3 Objective

This study sought to investigate the effect of varying concentrations of noni fruit extract, and physiological stage on liver and kidney function, lipid profile and overall health in commercial layers.

6.4 Material and methods

6.4.1 Experimental diets

The experimental diets administered to the birds was in the form of layer mash as shown in Table 3.1. in Chapter 3.

6.4.2 Management of experimental birds

The three hundred commercial pullets with a mean body weight of 1.3 kg were randomly assigned to three experimental treatments with 20 pullets per treatment replicated five times in a completely randomised design. The pullets were housed in groups of 20 birds per replicate in a slated floor house. The birds were fed standard chick starter diet and subsequently placed on grower mash (Chapter 3; Table 3.1). At the end of week 15, the birds

were placed on the experimental treatments. Birds in Treatment 1 (T_1) control group received plain water (0 mg/ml noni fruit extract). Treatment 2 (T_2) received 20 mg/ml of noni fruit extract, while those in Treatment 3 (T_3) received 40 mg/ml of noni fruit extract all administered through drinking water throughout the experimental period. The daily feed and water allocated to the birds were recorded. The guidelines for brown-layer farming (Growel, 2017) were followed. The trial ended at 48 weeks. Ten birds per treatment (2 birds per replicate) were randomly selected for blood sampling at four physiological stages of growth namely weeks 16, 22, 30 and 48 representing pre-lay, early-lay, peak-lay and late-lay respectively.

6.4.3 Blood sampling and analysis

The birds were subjected to a 12-hour fast by withdrawing the feeding troughs before blood samples were collected at 07.00hrs. To ensure the birds were comfortable, their wings and limbs were gently held with two hands during blood collection. The blood was drawn from the wing vein located on the inside of the wing above the elbow joint using the venipuncture method (Plate 6.1). To prevent blood clotting, the vein was first cleaned with a cotton swab soaked in ethyl alcohol and then treated with an anticoagulant liquid (Trilon B -40 % aqueous solution of tetrasodium-methylene-diamintetra acetate [Na_4EDTA]). After the blood was drawn, the vein was clamped with a cotton swab for 1-2 minutes to prevent bleeding before releasing the bird.

Approximately 10 ml of blood were sampled from each bird by venipuncture of the brachial wing vein using a sterile syringe and needle. For the haematological parameters, 5mls out of the 10mls of the sampled blood was collected into vacutainer tubes containing the

anticoagulant tri-potassium-ethelyne-diamine-tetra acetic acid (K_3EDTA) and immediately gently flipped from side to side for a few seconds to prevent clotting.

Plate 6.1 Sampling blood from the brachial wing vein

The samples were promptly placed in a refrigerated container with ice packs for preservation before being sent to the Veterinary Services Directorate, Ministry of Food and Agriculture Laboratory for the haematological analysis. The laboratory utilised an Automatic Haematology Analyser (Auto Haematology Analyser, HEMA-D6031, Bioevopeak Ltd, China) to assess various haematological parameters. The parameters determined were White blood cells (WBC), Heterophils (HET), Lymphocytes (LYMP), Monocytes (MON), Eosinophils (EOS), Basophils (BASO), Red blood cells (RBC), Haemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), and Thrombocytes (Trb).

The other 5ml samples were placed in the second vacutainer tube containing a gel clot activator and the samples were then transported to the laboratory of the Small Animal

Teaching Hospital – School of Veterinary Medicine University of Ghana, for analysis of the serum biochemical profiles. At the laboratory, the samples were centrifuged at 1,500rpm at 4ºC, to separate serum and stored for analysis. The blood biochemical parameters were analysed using the URIT-8021a Vet® Chemistry Analyser (MEDSINGLONG Co. Ltd, China). The biochemical profile measurements were, Total Protein (TP), Albumin (ALB), Globulin (GLB), Albumin/Globulin ratio (ALB/GLB), Creatinine (CRE), Urea, Uric Acid (UA). Others were Total Bilirubin (TB), Direct Bilirubin (DB), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), AST/ALT ratio, Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), Total Cholesterol (TCHOL), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), Triglycerides (TG), Glucose (GLU), Calcium (Ca^{2+}), Sodium (Na^{+}), Chloride (Cl^{-}) and Potassium (K^{+}).

6.4.4 Statistical analysis

The analysis of variance procedure of GenStat (VSN International, 2009) was used to analyse the data and the Student Newman-Keuls procedure was used to separate means at the 5 % level of significance.

The statistical model used was as presented below:

$$Y_{ijkl} = \mu + T_i + P_j + TP_{(ij)} + e_{iikl}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 6.1$$

Where:

Y_{ijkl} = The response variable

μ = Overall mean

T_i = Effect of the i^{th} treatment i = 1, 2, 3

P_j = Effect of the j^{th} physiological stage j = 1, 2, 3

$TP_{(ij)}$ = Effect of the interaction between the i^{th} treatment and j^{th} physiological stage (ij) = 1, 2, 3

e_{ijkl} = Random residual error term, assumed ≈ NID (0, $\sigma^2 e$)

6.5 Results

6.5.1. Effect of Noni fruit extract on haematology of birds

The influence of noni fruit extract on the haematological parameters is shown in Table 6.1. Treatment had a significant ($p < 0.05$) effect on the red blood cell (RBC) counts, haemoglobin concentration (Hb), and packed cell volume (PCV) with birds on T_2 and T_3 having higher ($p < 0.05$) values than those on the control (T_1). The values obtained for birds on T_2 and T_3 were however similar ($p > 0.05$).

The values for MCV and MCH for the birds on the control (T_1) were higher ($p < 0.05$) than those on the noni fruit extract treatments (T_2) and (T_3). The values were however similar ($p > 0.05$) for T_2 and T_3. The MCHC values were similar ($p > 0.05$) among treatments. The Trb levels, WBC counts and its deferential values for basophils, eosinophils, monocytes and lymphocytes were higher ($p < 0.05$) in birds on the control (T_1) than those on the noni fruit extract treatments (T_2 and T_3). Among the noni fruit extract treatment groups, birds on T_2 had higher ($p < 0.05$) Trb, WBC counts, basophils and eosinophils levels than their counterparts on T_3, while the levels of monocytes and lymphocytes were similar ($p > 0.05$) for the treatment groups T_2 and T_3.

Table 6.1: Blood haematological parameters in commercial birds administered Noni fruit extract

Parameters	Reference range	Treatment (Noni fruit extract)			SEM	p-value
		T_1 (0 mg/ml)	T_2 (20 mg/ml)	T_3 (40 mg/ml)		
RBC (10^6µL)	2.5 -3.9 [1,2]	3.30[b]	3.62[a]	3.63[a]	0.056	0.001
Hb (g/dL)	7.0 – 13.0[1,2]	10.26[b]	11.73[a]	11.75[a]	0.230	0.001
PCV (%)	22.0-35.0[1,2]	32.10[b]	36.54[a]	36.61[a]	0.717	0.001
MCV (fL)	90.0 – 140.0[1,2]	114.00[a]	112.9[b]	112.80[b]	0.212	0.001
MCH (pg)	33.0 - 47.0[1,2]	39.80[a]	32.94[b]	32.08[b]	0.111	0.001
MCHC (g/dL)	26.0 – 35.0[1,2]	31.98	32.07	32.08	0.066	0.262
Trb (10^9/L)	3.0-33.0[1,2]	31.55[a]	28.15[b]	25.97[c]	0.475	0.001
WBC (10^9/L)	1.9 - 9.5[1]	8.43[a]	7.88[b]	7.65[c]	0.064	0.001
Heterophils (%)	29.0 - 48.7[1]	37.00[a]	32.58[b]	32.57[b]	0.490	0.001
Basophils (%)	0.0 -6.4[1]	0.65[a]	0.43[b]	0.28[c]	0.031	0.001
Eosinophils (%)	0.0 -11.5[1,2]	6.59[a]	5.29[b]	4.29[c]	0.176	0.001
Monocytes (%)	0.0 -6.5[1,2]	2.02[a]	1.12[b]	1.13[b]	0.105	0.001
Lymphocytes (%)	26.9 - 70.6[1,2]	52.73[a]	47.16[b]	46.77[b]	0.562	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$). SEM = Standard error of means; RBC = Red blood cells; Hb = Haemoglobin, PCV = Packed cell volume; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; ..
MCHC = Mean corpuscular haemoglobin concentration; Trb = Thrombocytes; WBC = White blood cells; [1]Clinical Diagnostic Division (1990); [2]Bounous and Stedman (2000)

6.5.2. Effect of physiological stage on blood haematology of birds

Physiological stage significantly ($p < 0.05$) affected all the haematological parameters measured (Table 6.2).

The RBC, Hb, PCV, MCHC and Trb levels increased with physiological stage. The RBC levels at 22, 30 and 48 weeks were higher ($p < 0.05$) than in week 16. Hb levels were higher ($p < 0.05$) at weeks 30 and 48 than at weeks 16 and 22 and the PCV levels at week 48 was higher ($p < 0.05$) than at weeks 16 and 22. The MCV value at week 48 was higher ($p < 0.05$) than those of the other physiological stages (weeks 16, 22 and 30). The MCHC values at weeks 30 and 48 were higher ($p < 0.05$) than those at weeks 16 and 22. The MCH values rather decreased with the physiological stage, with that of week 48 being lower ($p < 0.05$) than that for all the other physiological stages (weeks 16, 22, and 30). The WBC counts, heterophils, basophils and lymphocyte levels were higher at week 16 than at all other physiological stages (weeks 22, 30, and 48). The levels of monocytes and eosinophils decreased with the physiological stage, with levels at week 16 being higher ($p < 0.05$) than those at all other physiological stages (weeks 22, 30, and 48). Eosinophil levels were lowest at week 30, while monocyte levels were lowest at week 48.

Table 6.2: Effect of physiological stages (pre-lay, early-lay, peak-lay, late-lay) in layer-type birds

Parameter	Ref. Range	Physiological Stage				SEM	p-value
		Week 16 (pre-lay)	Week 22 (early-lay)	Week 30 (peak-lay)	Week 48 (late-lay)		
RBC (10^6µL)	2.5 -3.9[1,2]	3.13[b]	3.58[a]	3.61[a]	3.73[a]	0.065	0.001
Hb (g/dL)	7.0 – 13.0[1,2]	10.59[b]	10.91[b]	11.50[a]	11.58[a]	0.266	0.001
PCV (%)	22.0-35.0[1,2]	34.26[b]	34.01[b]	35.30[ab]	36.77[a]	0.828	0.005
MCV (fL)	90.0 – 140.0[1,2]	111.90[b]	110.6[c]	112.30[b]	118.1[a]	0.245	0.001
MCH (pg)	33.0 - 47.0[1,2]	35.82[a]	35.21[b]	34.94[b]	34.92[c]	0.129	0.001
MCHC (g/dL)	26.0 – 35.0[1,2]	30.90[c]	32.10[b]	32.59[a]	32.59[a]	0.076	0.001
Trb (10^9/L)	3.0-33.0[1,2]	27.49[b]	28.15[b]	28.43[b]	30.16[a]	0.549	0.001
WBC (10^9/L)	1.9 - 9.5[1]	8.98[a]	7.73[b]	7.45[c]	7.77[b]	0.074	0.001
Heterophils (%)	29.0 - 48.7[1]	42.31[a]	30.68[c]	30.75[c]	32.19[b]	0.566	0.001
Basophils (%)	0.0 -6.4[1]	0.70[a]	0.15[c]	0.45[b]	0.52[b]	0.036	0.001
Eosinophils (%)	0.0 -11.5[1,2]	8.24[a]	5.56[b]	3.82[c]	3.95[c]	0.203	0.001
Monocytes (%)	0.0 -6.5[1,2]	1.87[a]	1.64[a]	1.13[b]	1.05[b]	0.121	0.001
Lymphocytes (%)	26.9 - 70.6[1,2]	64.20[a]	40.32[c]	45.81[b]	45.22[b]	0.649	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$).
SEM = Standard error of means; RBC = Red blood cells; Hb = Haemoglobin; PCV = Packed cell volume;
MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin;
MCHC = Mean corpuscular haemoglobin concentration; Trb = Thrombocytes; WBC = White blood cells;
[1]Clinical Diagnostic Division (1990); [2]Bounous and Stedman (2000).

6.5.3. Interaction effect of noni fruit extract treatment and physiological stage on blood haematological indices.

The interaction between noni fruit extract and physiological stage on blood haematological parameters was found to be significant ($p < 0.05$) for all the haematological parameters determined (RBC, Hb, PCV, MCV, MCH, MCHC, Trb, WBC, heterophils, lymphocytes, monocytes, eosinophils, and basophils) see appendix 4, 5 and 6.

6.5.4 Effect of varying concentrations of Noni fruit extract on serum biochemical parameters of commercial laying birds

6.5.4.1 Effect of varying concentrations of Noni fruit extract on liver function indicators

The administration of noni fruit extract decreased ($p < 0.05$) AST, and ALT concentrations compared to the control T_1 (0 mg/ml) as shown in Table 6.4. The AST/ALT ratio was also lowest ($p < 0.05$) for the birds on T_3 (40 mg/ml). The treatment groups T_1 (0 mg/ml), T_2 (20 mg/ml) and T_3 (40 mg/ml) had similar ALP and GGT concentrations ($p > 0.05$). Birds on the control treatment T_1 had higher ($p < 0.05$) TP, GLB, TB and DB concentrations than those on noni fruit extract treatments T_2 and T_3, with those on T_3 recording the lowest values. However, the ALB and ALB/GLO ratio for birds on the noni fruit extract treatments (T_2 and T_3) were higher ($p < 0.05$) than their counterparts on the control treatment (T_1).

Table 6.4 Effect of Noni fruit extract on liver function indices

Parameters	Reference Range	Treatment (Noni fruit extract)			SEM	p-value
		T_1 (0mg/ml)	T_2 (20mg/ml)	T_3 (40mg/ml)		
AST **(U/L)**	9.0 - 49.0[1]	32.20[a]	25.75[b]	23.71[c]	0.390	0.001
ALT **(U/L)**	10.0 -109.0[1]	38.14[a]	30.64[b]	30.18[b]	0.440	0.001
AST/ALT	1.0 – 1.2[1]	0.84[a]	0.84[a]	0.79[b]	0.009	0.001
ALP **(U/L)**	1.0 – 114.0[1]	40.02	40.01	40.03	0.010	0.153
GGT **(U/L)**	3.0 – 19.0[1]	10.28	10.29	10.28	0.029	0.931
TP (g/l)	54.0 – 75.0[1]	68.33[a]	59.58[b]	56.58[c]	0.565	0.001
ALB (g/l)	23.0 – 31.0[1]	25.16[b]	28.42[a]	28.46[a]	0.052	0.001
GLB (g/l)	0.0 – 45.0[1,2]	43.17[a]	31.16[b]	28.11[c]	0.545	0.001
ALB/GLB	0.0 – 10.0[1,2]	0.58[c]	0.93[b]	1.01[a]	0.014	0.001
TB (µmol/L)	0.0 – 5.13[1]	3.24[a]	2.94[b]	2.84[c]	0.019	0.001
DB (µmol/L)	1.0 – 2.0[1]	1.32[a]	1.25[b]	1.22[c]	0.006	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$);
SEM = Standard error of means; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; AST/ALT = Aspartate Aminotransferase/Alanine Aminotransferase ratio; ALP = Alkaline Phosphatase; GGT = Gamma-Glutamyl Transferase; TP = Total Protein; ALB = Albumin; GLB = Globulin; ALB/GLB = Albumin/Globulin ratio; TB = Total Bilirubin; TD = Direct Bilirubin;
[1]Clinical Diagnostics Division (1990); [2]Harr (2002).

6.5.4.2 Effect of physiological stage on liver function indicators

Physiological stage significantly ($p < 0.05$) affected all serum biochemical liver function parameters measured except ALP and GGT (Table 6.5). The AST, ALT, TP, GLB, TB, and DB concentration during the pre-lay stage (week 16) was higher ($p < 0.05$) than concentrations at early-lay (week 22) peak-lay (week 30) and late-lay (week 48) stage. This trend was however, reversed, being higher ($p < 0.05$) for the AST/ALT ratio, ALB and ALB/GLB ratio in birds at the early (week 22), peak (week 30) and late-lay (week 48) stages than the pre-lay (week 16) stage.

Table 6.5 Effect of physiological stage on liver function indicators

Parameter	Reference range	Physiological stage				SEM	p-value
		Week 16 (pre-lay)	Week 22 (early-lay)	Week 30 (peak-lay)	Week 48 (late-lay)		
AST **(U/L)**	9.0 - 49.0[1]	29.52[a]	26.46[b]	26.45[b]	26.44[b]	0.450	0.001
ALT **(U/L)**	10.0 -109.0[1]	37.52[a]	31.48[b]	31.47[b]	31.47[b]	0.508	0.001
AST/ALT	1.0 – 1.2[1]	0.79[b]	0.84[a]	0.84[a]	0.84[a]	0.010	0.001
ALP **(U/L)**	1.0 – 114.0[1]	40.02	40.03	40.02	40.02	0.012	0.692
GGT **(U/L)**	3.0 – 19.0[1]	10.26	10.33	10.27	10.26	0.034	0.131
TP (g/l)	54.0 – 75.0[1]	64.35[a]	60.38[b]	60.65[b]	60.61[b]	0.652	0.001
ALB (g/l)	23.0 – 31.0[1]	26.99[b]	27.41[a]	27.49[a]	27.51[a]	0.061	0.001
GLB (g/l)	0.0 – 45.0[1,2]	37.36[a]	32.97[b]	33.16[b]	33.10[b]	0.629	0.001
ALB/GLB	0.0 – 10.0[1,2]	0.76[b]	0.87[a]	0.87[a]	0.87[a]	0.016	0.001
TB (µmol/L)	0.0 – 5.13[1]	3.12[a]	3.00[b]	3.00[b]	3.00[b]	0.022	0.001
DB (µmol/L)	1.0 – 2.0[1]	1.29[a]	5.56[b]	3.82[b]	3.95[b]	0.203	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$). SEM = Standard error of means;
AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; AST/ALT = Aspartate Aminotransferase/Alanine Aminotransferase ratio;
ALP = Alkaline Phosphatase; GGT = Gamma-Glutamyl Transferase; TP = Total Protein; ALB = Albumin; GLB = Globulin;
ALB/GLB = Albumin/Globulin ratio; TB = Total Bilirubin; TD = Direct Bilirubin; [1]Clinical Diagnostics Division (1990); [2]Harr (2002).

6.5.4.3 Effect of varying concentrations of Noni fruit extract on kidney function indicators

The kidney function status of birds administered with two levels of the noni fruit extract as indicated by the serum biochemical parameters are shown in Table 6.6. Birds administered with the noni fruit extract T_2 and T_3 had higher ($p < 0.05$) CRE concentrations than those on the control (T_1). Urea and UA concentrations decreased ($p < 0.05$) in birds on the noni fruit extract treatments (T_2 and T_3) compared to the control (T_1). Birds on the T_3 treatment had the lowest urea and UA concentrations. Calcium (Ca^{2+}) concentration decreased ($p < 0.05$) in birds on 20 mg/ml (T_2) compared to those on T_1 and T_3 groups. The sodium (Na^+) and chloride (Cl^-) levels in all 3 treatment groups were similar ($p > 0.05$). Potassium (K^+) concentrations however increased ($p < 0.05$) with increased concentration of noni fruit extract administration, with birds on T_3 recording the highest and those on the control (T_1) recording the lowest concentration.

Table 6.6: Effect of Noni fruit extract on kidney function indicators

Parameters	Reference Range	Treatment (Noni Fruit Extract)			SEM	p-value
		T_1 (0mg/ml)	T_2 (20mg/ml)	T_3 (40mg/ml)		
CRE (µmol/L)	0.9 - 1.8[1]	1.12[b]	1.15[a]	1.15[a]	0.162	0.001
UREA (mg/dL)	2.9 – 10.0[1]	4.66[a]	4.00[b]	3.58[c]	0.091	0.001
UA (mg/dL)	1.9 – 12.5[1]	3.3[a]	2.0[b]	2.0[c]	0.084	0.001
Ca^{2+} (mmol/L)	2.2 – 3.0[1]	2.79[a]	2.13[b]	2.78[a]	0.053	0.003
Na^+ (mmol/L)	139.0 155.0[1]	141.4	141.4	141.4	0.084	0.688
Cl^- (mmol/L)	108.0 – 124.0[1]	121.60	121.60	121.60	0.030	0.326
K^+ (mmol/L)	3.5 – 5.2[1]	3.85[c]	8.01[b]	9.20[a]	0.249	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$); SEM = Standard error of means; CRE = Creatinine; UA = Uric Acid; Ca^{2+} = Calcium; Na^+ = Sodium; Cl^- = Chloride; and K^+ = Potassium; [1]Clinical Diagnostics Division (1990).

6.5.4.4 Effect of physiological stage on kidney function indicators

The CRE concentration was higher ($p < 0.05$) at peak-lay (week 30) and late-lay (week 48) than at pre-lay (week 16), while the Urea, UA and Ca^{2+} concentrations were rather higher at pre-lay (week 16) than at early-lay (week 22), peak-lay (week 30) and late-lay (week 48) stages. The Na+ and Cl- concentrations were however not significantly influenced ($p > 0.05$) by the physiological stage (Table 6.7). The K+ concentrations increased ($p < 0.05$) with laying activity of the birds being higher at early-lay (weeks 22), peak-lay (week 30) and late-lay (week 48) stages than at pre-lay (week 16).

Table 6.7: Effect of Physiological stage on kidney function

Parameter	Reference range	Physiological stage				SEM	p-value
		Week 16 (pre-lay)	Week 22 (early-lay)	Week 30 (peak-lay)	Week 48 (late-lay)		
CRE (umol/L)	0.9 - 1.8[1]	1.13^{b}	1.14^{ab}	1.15^{a}	1.15^{a}	0.019	0.001
UREA (mg/dL)	2.9 – 10.0[1]	4.88^{a}	4.16^{b}	3.72^{c}	3.54^{c}	0.105	0.001
UA (mg/dL)	1.9 – 12.5[1]	2.7^{a}	2.1^{b}	2.1^{b}	2.1^{b}	0.097	0.001
Ca^{2+} (mmol/L)	2.2 – 3.0[1]	3.01^{a}	2.84^{b}	2.55^{c}	2.53^{c}	0.016	0.001
Na^{+} (mmol/L)	139.0 155.0[1]	141.60	141.50	141.10	141.10	0.098	0.087
Cl^{-} (mmol/L)	108.0 – 124.0[1]	121.60	121.70	121.60	121.60	0.035	0.080
K^{+} (mmol/L)	3.5 – 5.2[1]	5.43^{b}	7.53^{a}	7.56^{a}	7.56^{a}	0.288	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$);
SEM = Standard error of means; CRE = Creatinine; UA = Uric Acid; Ca^{2+} = Calcium; Na^{+} = Sodium; Cl^{-} = Chloride; K^{+} = Potassium; [1]Clinical Diagnostics Division (1990)

6.5.4.5 Effect of Noni fruit extract on serum lipid and metabolic profile of Birds

The effect of varying concentrations in noni fruit extract on serum lipid and metabolic profiles is shown in Table 6.8. The TCHOL, LDL, and VLDL concentrations decreased ($p < 0.05$) with increasing levels of noni fruit extract administration in the water, with T_3 having the lowest values compared to T_2, and T_1 (control). The HDL concentration, however, increased ($p < 0.05$) with increasing levels of noni fruit extract administration in the water of the birds with T_3 having the highest concentration compared with T_1 and T_2. The TG and GLU concentrations for the control (T_1) were higher ($p < 0.05$) than the noni fruit extract treatments T_2, and T_3.

Table 6.8: Effect of Noni fruit extract on serum lipid and metabolic profile indicators

Parameters	Reference Range	Treatment (Noni fruit extract)			SEM	p-value
		T_1 (0mg/ml)	T_2 (20mg/ml)	T_3 (40mg/ml)		
TCHOL (mmol/L)	3.34 – 7.7[1,2]	6.82[a]	5.32[b]	4.83[c]	0.095	0.001
HDL (mg/dL)	0.0 – 10.0[1,2]	1.29[c]	1.34[b]	1.36[a]	0.006	0.001
LDL (mmol/L)	0.0 – 10.0[1,2]	2.60[a]	2.37[b]	2.29[c]	0.016	0.001
VLDL (mmol/L)	0.0 – 10.0[1]	2.93[a]	1.61[b]	1.18[c]	0.080	0.001
TG (mmol/L)	0.2 – 2.8[1]	1.79[a]	1.49[b]	1.49[b]	0.025	0.001
GLU (mmol/L)	4.2 - 6.6[1]	4.60[a]	4.50[b]	4.48[c]	0.006	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$).
SEM = Standard error of means; TCHOL = Total Cholesterol; HDL = High-Density Lipoprotein; LDL = Low-Density Lipoprotein; VLDL = Very Low-Density Lipoprotein; TG = Triglycerides; GLU = Glucose (GLU); [1]Clinical Diagnostics Division (1990); [2]Bueno *et al*. (2017).

6.5.4.6 Effect of physiological stage on serum lipid and metabolic profile indicators

The levels of TCHOL, LDL, VLDL, TG and GLU were significantly ($p < 0.05$) influenced by the physiological stage being higher ($p < 0.05$) at pre-lay (week 16) than early-lay (week 22), peak-lay (week 30) and late-lay (week 48) stages (Table 6.9). The HDL concentration however increased with the egg-laying activity of the birds being higher ($p < 0.05$) at the early-lay (week 22), peak-lay (week 30) and late-lay (week 48) stages than the pre-lay stage.

Table 6.9: Effect of Physiological Stage on Serum Lipid and Metabolic Profile

Parameter	Ref. Range	Physiological stage				SEM	p-value
		Week 16 (pre-lay)	Week 22 (early-lay)	Week 30 (peak-lay)	Week 48 (late-lay)		
TCHOL (mmol/L)	3.34 – 7.7[1,2]	6.16[a]	5.49[b]	5.49[b]	5.49[b]	0.110	0.001
HDL (mg/dL)	0.0 – 10.0[1,2]	1.29[b]	1.34[a]	1.34[a]	1.35[a]	0.007	0.001
LDL (mmol/L)	0.0 – 10.0[1,2]	2.52[a]	2.35[c]	2.41[b]	2.40[b]	0.018	0.001
VLDL (mmol/L)	0.0 – 10.0[1]	2.35[a]	1.80[b]	1.74[b]	1.74[b]	0.093	0.001
TG (mmol/L)	0.2 – 2.8[1]	1.72[a]	1.56[b]	1.53[b]	1.55[b]	0.088	0.001
GLU (mmol/L)	4.2 - 6.6[1]	4.69[a]	4.47[b]	4.47[b]	4.47[b]	0.007	0.001

Means in the same row with different superscripts (a, b, c) are significantly different ($p < 0.05$) .
SEM = Standard error of means; TCHOL = Total Cholesterol; HDL = High-Density Lipoprotein;
LDL = Low-Density Lipoprotein; VLDL = Very Low-Density Lipoprotein; TG = Triglycerides;
GLU = Glucose (GLU); [1]Clinical Diagnostics Division (1990); [2]Bueno *et al*. (2017).

6.5.4.7 Interaction effect of noni fruit extract treatment and physiological stage on serum biochemical indices.

The interaction effect of noni fruit extract treatment, and physiological stage was significant ($p < 0.05$) for the following blood biochemical indices: AST, ALT, AST/ALT ratio, TP, ALB, GLB, ALB/GLB ratio, CRE, Urea, UA, TB, DB, Ca^{2+} and K^{+} (appendix 7, 8, 9, 11 and 12). Others were TCHOL, HDL, LDL, VLDL, TG, and GLU (appendix 10 and 13). The treatment by physiological stage interaction was not significant ($p > 0.05$) for ALP, GGT, Na^{+} and Cl^{-} (appendix 7, 9, 11 and 12).

6.6 Discussion

6.6.1. Effect of Noni fruit extract, and physiological stage of birds on blood haematological parameters

The increased level of red blood cells and haemoglobin in birds administered with the noni fruit extract (Table 6.1) could be attributed to the role of noni fruit extract in lowering lipid peroxide levels in the blood to protect red blood cells from osmotic fragility, oxidative stress and oxidative damage (Mhatre and Marar, 2016; Adrian *et al*., 2017). Also, the improved haemoglobin level in birds administered with noni fruit extract may be due to the noni fruit extract's biotin and folate content, which aids in promoting cell differentiation and red blood cell production (Scanes, 2022) compared to the control. The increased packed cell volume (PCV) level in birds on the noni fruit extract treatments may suggest an overall increase in red blood cells and an improved oxygen-carrying capacity.

Although the MCV and MCH levels were reduced in birds administered with noni fruit extract their levels were within the normal physiological range ruling out any issues of anaemia and iron deficiency. Although thrombocyte (Trb) levels decreased with increasing levels of noni fruit extract, the values were within the normal physiological range suggesting

no adverse effects of excessive bleeding associated with vitamin B_{12} deficiency. Compared to the control, the total WBC counts with its differentials (heterophils, basophils, eosinophils, monocytes and lymphocytes) decreased with noni fruit extract treatment (Table 6.1). This could be due to the immunomodulation properties of noni fruit extract (Almeida-Souza *et al.*, 2016; Diarra *et al.*, 2019) conferred by its flavonoids, phenolics and polysaccharides components (Lohani *et al.*, 2019).

In the current study, all values of haematological parameters determined on all treatments control T_1 (0 mg/ml), T_2 (20 mg/ml) and T_3 (40 mg/ml) were within the normal physiological range reported for poultry (Table 6.1; Clinical Diagnostics Division, 1990; Bounous and Stedman, 2000). The only exception was the slightly higher PCV levels in birds treated with the noni fruit extract. The above observations suggest that the administration of noni fruit extract did not adversely affect the haematology of the birds, but enhanced the production of haemoglobin for efficient transportation of oxygen and carbon dioxide, normal synthesis of red blood cells and production of enough white blood cells to defend the birds' body against infection adequately.

The increased level of red blood cells with the physiological stage (Table 6.2) could be attributed to the general increase demand for oxygen due to rapid growth from the pre-lay stage (week 16), which stabilised in mature hens (weeks 20, 30 and 48). Also higher oxygen-carrying capacity (higher Hb level) for increased metabolism and reproduction are required as physiological stage progresses from pre-lay to the laying period (Minias, 2015; Hong *et al.*, 2021). The increased level of PCV at advanced stage of production reflects the requirements for higher levels of red blood cells as the chicken approaches peak production periods. The increased MCV and MCHC levels with increased physiological stage may

probably be due to the efficiency of haemoglobin in red blood cells. Typically, there may be increases in these values as birds grow and their bodies adapt to the demands of laying. The decrease in MCH levels with age but within the normal physiological levels 33.0 - 47.7pg (Bounous and Stedman, 2000; Clinical Diagnostic Division, 1990) suggest no adverse effect on blood clotting processes before egg laying and throughout the laying activities of the hens.

Normal levels of WBC count with its differentials (heterophils, basophils, eosinophils, monocytes and lymphocytes) were maintained as physiological stage progressed suggesting no adverse effects of infection before egg laying and throughout the laying activities of the birds.

6.6.2. Effect of Noni fruit extract, and physiological stage of birds on serum liver function indicators

The activities of AST, ALT, ALP, and GGT in serum are used as indicators for liver health (Mian Ying *et al.*, 2002; Lin *et al.*, 2017). The reduced concentrations of AST, ALT and the AST/ALT ratio in the birds administered with noni fruit extract compared to the control which were within the normal physiological range (Table 6.4; Clinical Diagnostic Division, 1990) indicates the improvement in the birds liver function (absence of liver damage or dysfunction) as suggested by (Hong *et al.*, 2021; Abo-Ghanima *et al.*, 2023).

The normal levels of total protein, albumin, globulin and the albumin/globulin ratio in the present study (Table 6.4; Clinical Diagnostic Division, 1990; Harr, 2002) suggest the administration of the noni fruit extract did not adversely affect the nutritional and immune function of the birds. It also did not lead to liver or kidney problems in the birds. Normal levels of total protein and albumin have been reported to represent better nutritional status

(Scanes, 2022), while lower than normal globulin and albumin/globulin ratio are indicators of reduced immune function (Kokoré *et al.*, 2021; Scanes, 2022). The total bilirubin and direct bilirubin concentrations in birds administered with the noni fruit extract were lower compared to their counterparts on the control (0 mg/ml) treatment, although within the normal physiological range of the birds suggesting proper functioning of their liver.

The AST and ALT concentrations were within the normal physiological range (Table 6.4; Clinical Diagnostic Division, 1990) suggesting no liver problems during early-lay, peak-lay and late-lay periods (Paulo *et al.*, 2017).

Egg development requires supply of amino acids. The total protein and globulin concentrations reduced with physiological stage but remained in the normal physiological range for birds (Table 6.5; Clinical Diagnostics Division, 1990; Harr, 2002) suggesting sufficient levels to support growth and egg formation (Scanes, 2022) and immune status. Also the increased concentration of albumin, and the albumin/globulin ratio but within the normal physiological range (Table 6.5; Clinical Diagnostic Division, 1990; Harr, 2002) as birds move from the pre-laying period to the egg-laying periods suggest adequate supply of protein for egg development. Brandt *et al.* (1951) reported that high serum albumin levels within the normal physiological range in a laying hen indicate better overall health and nutritional status leading to increased egg production and reproductive performance. Furthermore, the concentration of total bilirubin was within the normal physiological range (Table 6.5; Clinical Diagnostic Division, 1990), an indication of no liver dysfunction during the various stages examined (pre-lay, peak and late-lay). The higher direct bilirubin levels than normal in the current study may suggest liver dysfunction or bile duct obstruction, which

is common in older hens with fatty liver syndrome or other liver diseases (Benzo *et al.*, 1986). However, no such symptoms were observed in the birds.

6.6.3. Effect of Noni fruit extract, and physiological status on kidney function of birds

Kidney function indicators such as creatinine (CRE), urea, uric acid (UA), sodium, potassium, and chloride levels are used to assess kidney health, potential muscle damage, and electrolyte balance (Benzo *et al.*, 1986; Lin *et al.*, 2017; Hagan *et al.*, 2022; Scanes, 2022).

The higher CRE concentration of birds administered with the noni fruit extract compared to the control may be due to the higher body mass and muscular activity of the birds on the noni fruit extract treatment (Sunder *et al*., 2011a, 2016). The levels were however within the normal physiological range reported for poultry (Table 6.6; Clinical Diagnostic Division, 1990) suggesting no adverse effects on the birds. The decreased urea and UA concentrations of birds administered with the noni fruit extract compared to the control may be due to a well-hydrated state or efficient renal function of the birds (Paulo *et al.*, 2017). This reduction but within the normal physiological range (Table 6.6; Clinical Diagnostic Division, 1990) demonstrated improved kidney function and protein catabolism associated with the administration of noni fruit extract (Al-Mamun, 2020; Mhatre and Marar, 2016). Although the administration of noni fruit extract reduced Ca^{2+} concentration in the birds compared to the control, the concentrations were within the normal physiological range (Table 6.6; Clinical Diagnostic Division, 1990) suggesting adequate levels for bone growth and development, egg production, and eggshell formation (Knowles *et al.*, 1935; Hester, 2017).

The similar Na^{+} and Cl^{-} concentrations among the control and various treatments suggest no adverse effect of noni fruit extract administration on the osmotic balance of the birds with

respect to their kidney function. Although levels of K^+ were elevated above normal in the birds administered with the noni treatments which may be attributed to the high potassium content of the noni fruit extract, the birds did not display any symptoms associated with hyperkalaemia (higher than normal potassium levels) such as depression and muscle weakness.

The CRE concentrations of birds increased with the physiological stage but remained within the normal physiological range for poultry (Table 6.7; Clinical Diagnostic Division, 1990). This may suggest reduced clearance and higher muscle mass as the birds advanced in physiological stage (Sunder *et al.*, 2011a; 2016). This agrees with the report of Minias (2015) who observed an increased serum creatinine level with increased body mass and muscular activity in birds.

The decreased urea and uric acid concentration with physiological stage but within the normal physiological range (Table 6.7; Clinical Diagnostic Division, 1990) may indicate normal kidney function (effective nitrogen excretion) as birds progress from the pre-lay to egg-laying periods (Al-Mamun, 2020).

The increased requirement for eggshell production from early-lay to the late-lay period may account for the reduced Ca^{2+} concentration with advancing physiological stage/age in concordance with the suggestion by Knowles *et al*. (1935) and Hester (2017) that serum concentrations of Ca^{2+} may be decreased during active eggshell formation than when shell secretion is not taking place. The Ca^{2+} concentration however remained within the normal physiological range suggesting no adverse effect on egg production and eggshell formation.

Physiological changes, including increased metabolic activity, environmental stressors or hormonal fluctuations during the laying cycle may have affected electrolyte balance leading

to elevated potassium levels. The laying birds were, however, not observed to display any symptoms associated with hyperkalaemia (higher than normal potassium levels) such as depression, or muscle weakness. The similar Na^+ and Cl^- concentrations at all physiological stages suggest a stable osmotic balance of the birds with respect to their kidney function.

6.6.4. Effect of Noni extract, and physiological stage on lipid profile and metabolic status

The reduction in the concentrations of TCHOL, LDL, VLDL and TG with increasing concentrations of noni fruit extract in the birds compared to those on the control but within the normal physiological range for poultry (Table 6.8; Clinical Diagnostic Division, 1990; Bueno *et al.*, 2017) may be due to the chemical components such as phenolic acids and flavonoids present in the noni fruit extract exhibiting antilipidemic activity (Mian-Ying *et al*., 2002; Pazos *et al.*, 2011; Sunder *et al.*, 2011a; Palu *et al*., 2012). For example, Sunder *et al.* (2011a) observed reduced blood cholesterol concentrations in the Nicobari Fowl when fed noni fruit extract. The authors explained that this was due to the anti-cholesterol activity of phytochemicals and a plant sterol (beta-sitosterol) present in the noni fruit extract. The antidyslipidemic activity tends to reduce lipid levels through inhibition of biosynthesis, absorption and secretion of lipids and hence the potential against cardiovascular diseases (Mandukhail *et al.*, 2010). Also, the presence of lignans in the noni fruit extract has been reported to prevent arteriosclerosis related to the oxidation of low-density lipoproteins (Kamiya *et al*., 2004). In the present study, HDL concentration increased in birds with increasing concentrations of noni fruit extract, above those on the control treatment. The values were however normal demonstrating the ability of the noni fruit extract to reduce the risk of cardiovascular diseases. The decrease in glucose concentration with increasing levels of noni fruit extract in the birds compared to those on the control but within normal levels

(Table 6.8; Clinical Diagnostic Division, 1990) for birds may be due to the hypoglycaemic activity of the noni fruit extract. For example, noni fruit extract was reported to decrease glucose levels in diabetic-induced rats because it probably potentiates the action of insulin directly or increases peripheral tissue sensitivity to insulin storage (Nayak and Mengi, 2010).

The decreased concentrations of TCHOL, LDL, VLDL, TG and GLU during the egg-laying stages (early-lay, peak-lay, and late-lay) compared to the pre-lay stage may be due to the increased demand for the egg-laying process (embryo and yolk formation) and metabolism (Oketch *et al*., 2023). The concentrations were within the normal physiological range for poultry (Table 6.9; Clinical Diagnostic Division, 1990; Bueno *et al*., 2017) indicating no adverse effect on the egg-laying processes.

6.7 Conclusion

1. Administration of noni fruit extract up to 40 mg/ml led to improvement in levels of haematological and biochemical indices in laying hens, which were within the normal levels for poultry demonstrating no adverse effects on physiology and health of the hens.
2. The haematological and serum biochemical parameters were positively influenced by the physiological stage of the birds (pre-lay, early-lay, peak-lay and late-lay). They however did not have adverse effects on the egg-laying process in the hens since their levels remained within the normal physiological range for poultry.

These findings imply that noni fruit extract holds promise as a natural feed additive for enhancing the health and productivity of laying hens.

CHAPTER 7

7.0 EXPERIMENT 4

INVESTIGATING THE ADMINISTRATION OF NONI FRUIT EXTRACT AT 16 OR 20 WEEKS OF AGE ON PRE-LAY SEXUAL DEVELOPMENT, EGG PRODUCTION AND BLOOD METABOLITE PROFILES OF LAYERS

7.1 Summary

Noni fruit extract is known for its antioxidant, anti-inflammatory, and immunomodulatory properties, which can enhance the birds' immune system, reduce stress, and improve the overall health of birds. The study aimed at investigating the effect of noni fruit extract administration at two physiological stages (week 16 or week 20 of age) to: improve growth rate and feed conversion ratio, enhance egg production and quality and promote sustainable and natural production methods. Birds placed on noni fruit extract at week 16 (T_2) had significantly ($p < 0.05$) delayed days-to-first-egg compared to the birds fed noni at week 20 (T_3) and the control group (T_1) but the control group dropped their eggs earlier than week 20 (T_3). Pullets fed noni fruit extract at week 16 (T_2) exhibited a significantly ($p < 0.05$) higher reduction in visceral fat, increased uterus weight, right tibia bone length and diaphysis diameter compared to the week 20 (T_3) and control (T_1) groups respectively at peak-of-lay and late lay. Administering noni fruit extract at week 16 (T_2) offered more protection from oxidative damage of the RBC and its components and conferred a strong immunomodulatory effect, liver, and kidney function and antidyslipidemic activity on the birds compared to the week 20 (T_3) and control (T_1) groups respectively at peak-of-lay and late lay. The study concluded that supplementing with 40 mg/ml noni fruit extract at week 16 resulted in improvements in body weight gain, feed conversion ratio, net feed efficiency index, egg

freshness, antidyslipidemic activity, and erythropoiesis and blood oxygen-carrying capacity as well as, liver and kidney function.

7.2 Introduction

The poultry industry plays a crucial role in animal agriculture, and it is vital to ensure the optimal health and productivity of birds for sustainable production (Henchion *et al.*, 2021). Commercial pullets, a widely favoured breed of laying hens, require precise management to achieve peak performance (Gezahegn *et al.*, 2016). Research suggests that using noni fruit extract as a natural feed additive may improve poultry health and productivity (Mian-Ying *et al.*, 2002; Sunder *et al.*, 2011a; Widjastuti *et al.*, 2019). However, the ideal age for administering noni fruit extract to maximise performance in commercial layer pullets remains unknown. Determining the best age for administering noni fruit extract is crucial to maximising its benefits for the health and productivity of commercial pullets. Noni fruit extract is known for its antioxidant, anti-inflammatory, and immunomodulatory properties, which can enhance the birds' immune system, reduce stress, and improve overall health (Krishnakumar *et al.*, 2015). By identifying the recommended age for administering noni fruit extract, poultry farmers and producers can maximize its use. This can lead to improved growth rate and feed efficiency, enhanced egg production and quality, and reduced mortality and morbidity rates. Additionally, it can provide valuable insights into the poultry industry and contribute to the development of sustainable production practices.

7.3 Objective

The study was conducted with the following objective:

To determine the effect of administering noni fruit extract at weeks 16 or 20 on pre-laying performance, selected internal organs, egg quality indicators, and haematological and serum biochemical profiles of commercial layers.

7.4 Material and methods

7.4.1 Experimental diets

The experimental diets administered to the birds was layer mash (see Table 3.1; Chapter 3).

7.4.2 Management of experimental birds

The 300 commercial pullets with a mean body weight of 1.3 ±0.233 kg were randomly assigned to the three experimental treatments with 20 pullets per treatment replicated five times in a completely randomised design. The pullets were housed in groups of 20 birds per replicate. The birds were allowed to acclimatise for 2 weeks and randomly allocated to three treatments. The control group (Treatment 1; T_1) received plain water (0 mg/ml noni fruit extract) from week 16 to the end of the experiment (week 48). Birds in Treatment 2 (T_2) received 40 mg/ml noni fruit extract at week 16. Those in Treatment 3 (T_3) started with plain water (0 mg/ml noni fruit extract) at week 16 and then placed on 40 mg/ml noni fruit extract at week 20. The guidelines for brown-layer farming (Growel, 2017) were followed. The trial ended at week 48 when the hen house egg production began to fall.

7.4.3 Data collection

The data collected for growth assessment were weekly feed intake, weekly water intake, body weight gain, age at first egg, and egg weight. Ten birds per treatment (2 birds per replicate) were randomly selected and euthanised by rapid cervical dislocation according to the protocol outlined by the American Veterinary Medicine Association (AVMA, 2020) to harvest tissues of the following internal organs: abdominal fat, uterus, and the right tibia bone. The measurement of the bone length and diaphysis diameter from the right tibia bone of each bird was recorded. The days to first egg were calculated as the average number of days an egg was first laid in all the replicates of a treatment group. Eggs were collected at week 22, week 30, and week 48 to represent the physiological stages; early-lay, peak-lay and late-lay. Two eggs per replicate per treatment were used for the assessment of their internal quality and their freshness during storage at each stage of lay. Data for egg quality assessment were albumen height (AH), Haugh Unit (HU), yolk index, yolk colour (YC) and the estimated eggshell thickness (EST).

Blood samples were collected after subjecting the birds to a 12-hour fast at weeks 22, 30 and 48 for haematological and serum biochemistry studies to assess the effect of noni fruit extract on the health status of the birds. The haematological parameters of the whole blood count measured were: Red blood cells (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), packed cell volume (PCV), and thrombocytes (Trb), white blood cells (WBC), heterophils, lymphocytes, monocytes, eosinophils and basophils. The parameters of the biochemical profile measured were total protein, albumin, globulin, creatinine, urea and uric acid. Others were total bilirubin, direct bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl

transferase, total cholesterol, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, triglycerides, glucose, calcium (Ca^{+2}), sodium (Na^{+}), chloride (Cl^{-}), and potassium (K^{+}). The albumin/globulin ratio (ALB/GLB), and AST/ALT ratio were calculated in Microsoft excel.

7.4.4 Statistical Analysis

The GenStat statistical software (VSN International, 2009) was used to conduct an analysis of variance to determine any significant difference among treatment means. The Student Newman-Keuls procedure was used to separate means.

The statistical model used to assess the pre-lay sexual development and laying performance parameters was as presented in equation 7.1:

$$Y_{ij} = \mu + T_i + e_{ij} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 7.1$$

Where:

Y_{ij} = The response variable

μ = Overall mean

T_i = Effect of the i^{th} treatment i = 1, 2, 3

e_{ij} = Random residual error term, assumed ≈ NID (0, $\sigma^2 e$)

The statistical models used to assess the effect of storage as well as the blood haematological and serum biochemical indices at 3 physiological stages (early-lay, peak-lay and late-lay) was as presented in equation 7.2 and 7.3

$$Y_{ijk} = \mu + S_i + P_j + e_{iik}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 7.2$$

$$Y_{ijk} = \mu + T_i + P_j + e_{iik}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 7.3$$

Where:

Y_{ijk} = The response variable

μ = Overall mean

S_i = i^{th} storage period i = 1, 2, 3, 4, 5

T_i = Effect of the i^{th} treatment i = 1, 2, 3

P_j = Effect of the j^{th} physiological stage j = 1, 2, 3

e_{ijk} = Random residual error term, assumed ≈ NID (0, $\sigma^2 e$)

The statistical model used to assess the selected internal organs, egg quality indicators was as presented in equation 7.4:

$$Y_{ijkl} = \mu + T_i + P_j + TP_{(ij)} + e_{iikl}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 7.4$$

Where:

Y_{ijkl} = The response variable

μ = Overall mean

T_i = Effect of the i^{th} treatment i = 1, 2, 3

P_j = Effect of the j^{th} physiological stage j = 1, 2, 3

$TP_{(ij)}$ = Effect of the interaction between the i^{th} treatment and j^{th} physiological stage (ij) = 1, 2, 3

e_{ijkl} = Random residual error term, assumed ≈ NID (0, $\sigma^2 e$)

7.5 Results

7.5.1. Effect of time of administering Noni fruit extract

7.5.1.1 Laying performance indices

The timing of noni fruit extract administration significantly ($p < 0.05$) affected the number of days to the first egg (Table 7.1). Birds that received noni fruit extract at week 16 took longer to lay their first egg than those that received noni fruit extract at week 20 and the control group. The days to first egg of the birds administered noni fruit extract at week 20

and that of the control was similar ($p > 0.05$). At early-lay the daily feed intake decreased in the birds, administered noni fruit extract. The birds administered noni fruit extract at week 16 had significantly ($p < 0.05$) lower feed intake compared to the week 20 group. However, the control group T_1 (0 mg/ml) had higher ($p < 0.05$) feed intake values than the treatment groups (T_2 and T_3, 40 mg/ml).

The body weights of the birds that received noni fruit extract at weeks 16 T_2 (40 mg/ml) or 20 T_3 (40 mg/ml) were similar ($p > 0.05$), but higher ($p < 0.05$) than the control T_1 (0 mg/ml) group. The egg mass (EM), percent hen day egg production (HDEP %) and the net feed efficiency index (NFEI) were significantly ($p < 0.05$) better in the treatment group that received noni fruit extract at week 16 (T_2) compared to the groups that received noni fruit extract at week 20 (T_3), and the control group (T_1) which were similar ($p > 0.05$). The values obtained for the feed conversion ratio (FCR) for all three groups were significantly ($p < 0.05$) different from each other. In early lay, FCR was better in the week 16 group (T_2) compared with week 20 (T_3), however, both T_2 and T_3 had better FCR than the control (T_1).

The effect of administering noni fruit extract at week 16 or 20 followed a similar trend at peak-lay and late-lay (Table 7.1). Compared to the control group T_1 (0 mg/ml), the daily feed intake was lower ($p < 0.05$) but similar in the noni-treated groups weeks 16 T_2 (40 mg/ml) or 20 T_3 (40 mg/ml). At peak-lay and late-lay the body weight was similar ($p > 0.05$) in the noni-treated groups but was higher ($p < 0.05$) compared to the control. The egg mass, HDEP% and NFEI were higher ($p < 0.05$) in the week 16 T_2 (40 mg/ml) group compared to the week 20 T_3 (40 mg/ml) and control T_1 (0 mg/ml) groups. The FCR was better in the birds that received noni fruit extract at week 16 compared to the birds that received the noni fruit extract at week 20 and the control respectively at both peak and late lay.

Table 7.1 Effect of Noni fruit extract administered at weeks 16 or 20 on laying performance indices at the physiological stages (e-lay, peak-lay and late-lay)

Data collection Time	Parameters	Treatment (Noni fruit Extract)			SEM	p-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
Early-Lay (week 22)	Days to 1st Egg	125.60[b]	128.60[a]	125.80[b]	0.187	0.001
	DFI (g)	102.00[a]	99.87[c]	101.23[b]	0.213	0.001
	BWt (kg)	1.803[b]	1.917[a]	1.907[a]	0.023	0.001
	Egg mass (g)	23.02[b]	29.32[a]	26.73[b]	0.041	0.001
	HDEP (%)	50.10[b]	56.05[a]	52.05[b]	0.084	0.001
	FCR	4.43[c]	3.41[a]	3.79[b]	0.006	0.001
	NFEI	63.21[b]	86.45[a]	64.08[b]	0.057	0.001
Peak-Lay (week 30)	DFI (g)	110.00[a]	109.50[b]	109.70[b]	0.213	0.001
	BWt (kg)	1.934[b]	2.036[a]	2.027[a]	0.023	0.001
	Egg mass (g)	39.96[c]	58.01[a]	52.65[b]	0.057	0.001
	HDEP (%)	79.78[c]	91.43[a]	87.25[b]	0.097	0.001
	FCR	2.75[c]	1.89[a]	2.08[b]	0.003	0.001
	NFEI	57.80[c]	74.55[a]	69.65[b]	0.052	0.001
Late-Lay (week 48)	DFI (g)	110.20[a]	109.80[b]	109.80[b]	0.213	0.001
	BWt (kg)	2.004[b]	2.105[a]	2.097[a]	0.023	0.001
	Egg mass (g)	41.40[c]	57.57[a]	50.90[b]	0.071	0.001
	HDEP (%)	69.61[c]	89.61[a]	82.86[b]	0.115	0.001
	FCR	2.66[a]	1.91[c]	2.16[b]	0.003	0.001
	NFEI	42.25[c]	58.26[a]	51.57[b]	0.064	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$); SEM. = Standard error of means; BWt = Body weight; HDEP % = Hen Day Egg Production; FCR = Feed conversion ratio; NEFI = Net Feed Efficiency Index

* birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment.

7.5.1.2 Internal organ indicators

The administration of noni fruit extract at weeks 16 or 20 significantly ($p < 0.05$) affected all the internal organ parameters sampled (Table 7.2). The birds that received 40 mg/ml noni fruit extract at week 16 (T_2) had significantly ($p < 0.05$) lower abdominal fat values than those that received 40 mg/ml noni fruit extract at week 20 (T_3) compared to the control group T_1 (0 mg/ml), respectively (Table 7.2).

Table 7.2 Effect of Noni fruit extract administered at week 16 or 20 and physiological stage (early-lay, peak-lay and late-lay) on internal organs of laying birds.

	Treatment (Noni Fruit Extract)				
Parameters	**(T_1,control) *(Week 16, 0mg/ml)**	**(T_2) (Week 16; 40mg/ml)**	**(T_3) (Week 20; 40mg/ml)**	**SEM**	**p-value**
Abdominal fat (g)	32.78^a	18.82^c	20.78^b	0.070	0.001
Uterus weight (g)	39.17^c	54.78^a	44.43^b	0.058	0.001
Ash (g)	2.65^c	3.40^a	3.33^b	0.022	0.001
RTL (mm)	112.25^b	122.70^a	119.02^b	0.142	0.001
RTDD	6.53^c	7.66^a	7.55^b	0.043	0.001
	Physiological Stage				
Parameters	**Early-Lay (Week 22)**	**Peak-Lay (Week 30)**	**Late-Lay (Week 48)**	**SEM**	**p-value**
Abdominal fat (g)	25.12^a	23.75^b	23.51^c	0.070	0.001
Uterus weight (g)	42.63^c	46.56^b	49.19^a	0.058	0.001
Ash (g)	3.25^a	3.17^b	2.97^c	0.022	0.001
RTL (mm)	116.51^c	118.22^b	119.23^a	0.0142	0.001
RTDD	6.41^c	7.60^b	7.74^a	0.043	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$);
SEM. = Standard error of means; RTDD = Right tibia bone diaphysis diameter.
* birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment

There were significant differences ($p < 0.05$) in the weight of the uterus (UWt) ash (mineral content), right tibia bone length (RTL) and right tibia bone diaphysis diameter (RTDD) among all three experimental groups (Table 7.2). The mean values for birds that received the noni fruit extract at week 16 were higher ($p < 0.05$) compared to the values of birds that received noni fruit extract at week 20 weeks and the control group T_1 (0 mg/ml noni fruit extract) respectively.

The abdominal fat weight and Ash (mineralisation of the right tibia bone) decreased significantly ($p < 0.05$) with physiological stage from earl-lay through peak-lay to late lay. The mean values of the uterus weight, right tibia bone length, and right tibia bone diaphysis diameter. increased ($p < 0.05$) with physiological stage with late-lay having higher ($p < 0.05$) values compared to peak-lay and late-lay respectively. (Table 7.2).

7.5.1.2.1 Interaction effect of noni fruit extract administration at week 16 or 20 and physiological stage on selected internal organ parameters.

The interaction between noni fruit extract and physiological stage on selected internal organ parameters was found to be significant ($p < 0.05$) for all the parameters abdominal fat, uterus weight, ash (mineral content of right tibia bone), right tibia bone length and diaphysis diameter (see appendix 14)

7.5.1.3 Egg quality indicators

The timing of administration of noni fruit extract at weeks 16 (T_2) or 20 (T_3) significantly ($p < 0.05$) affected the following internal egg quality parameters - albumen height (AH), Haugh unit (HU), yolk index (YI), and estimated eggshell thickness (EST) as shown in Table 7.3. The values obtained for the group administered with 40 mg/ml noni fruit extract at week 16

(T_2) were significantly ($p < 0.05$) better for AH, HU, and EST compared to those that received 40 mg/ml noni fruit extract at week 20 (T_3) and the control T_1 (0 mg/ml). The values of the yolk index for the noni-treated groups were similar ($p > 0.05$) but higher ($p < 0.05$) compared to the control (T_1) group.

The AH, HU and YI decreased with physiological stage from early-lay to late-lay. The albumin height increased from early-lay to peak-lay and decreased at late-lay. The YC increased with physiological stage with late-lay having the highest ($p < 0.05$) compared to peak-lay and early-lay respectively. The values of the estimated eggshell thickness decreased with physiological stage with week 48 having the lowest ($p < 0.05$).

Table 7.3 Effect of 40mg/ml Noni fruit extract administered at weeks 16 or 20 and physiological stages (early-lay, peak-lay and late-lay) on internal egg quality indices

Treatment (Noni Fruit Extract)					
Parameters	**(T_1,control) *(Week 16, 0mg/ml)**	**(T_2) (Week 16; 40mg/ml)**	**(T_3) (Week 20; 40mg/ml)**	**SEM**	**p-value**
Albumen height (mm)	4.45^{c}	5.75^{a}	5.40^{b}	0.102	0.001
Haugh Unit (mm)	66.85^{c}	74.78^{a}	73.86^{b}	0.708	0.001
Yolk index (mm)	0.37^{b}	0.40^{a}	0.39^{a}	0.005	0.001
Yolk colour	4.87	5.17	5.00	0.188	0.282
EST (mm)	0.43^{c}	0.48^{a}	0.46^{b}	0.003	0.001
	Physiological Stage				
Parameters	**Early-Lay (Week 22)**	**Peak-Lay (Week 30)**	**Late-Lay (Week 48)**	**SEM**	**p-value**
Albumen height (mm)	5.34^{a}	5.30^{b}	4.96^{c}	0.023	0.001
Haugh Unit (mm)	73.58^{a}	72.06^{b}	69.85^{c}	0.708	0.001
Yolk index (mm)	0.41^{a}	0.37^{b}	0.37^{b}	0.005	0.001
Yolk colour	4.77^{b}	4.43^{b}	5.83^{a}	0.188	0.001
EST (mm)	0.46^{a}	0.46^{a}	0.44^{b}	0.004	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$);
SEM. = Standard error of means; EST= Estimated Eggshell Thickness.
* birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment

7.5.1.3.1 Interaction effect of noni fruit extract administration on week 16 or 20 and physiological stage on egg quality indicators.

The interaction between noni fruit extract and physiological stage on egg quality indicators was found to be significant ($p < 0.05$) for all the parameters albumen height, Haugh unit, yolk index, yolk colour and estimated eggshell thickness (see appendix 15).

7.5.1.4 Effect of storage at physiological stages (early-lay, peak-lay and late-l Lay) on egg quality indicators

At early lay, the effect of storage on the mean values of the albumen height and Haugh unit declined ($p < 0.05$) from day 1 to week 4 of storage. The mean value of the yolk index (YI) was similar ($p > 0.05$) for day 1 and week 1 of storage and significantly declined in weeks 2, 3 and 4 respectively (Table 7.4). The mean value of the yolk colour for day 1 was significantly higher ($p < 0.05$) compared to the values for weeks 1 and 2 and weeks 3 and 4 respectively. However, the mean values for weeks 1 and 2 were similar ($p > 0.05$) but higher compared to those of weeks 3 and 4 which were also similar ($p > 0.05$). The mean value of the estimated eggshell thickness (EST) did not change during the 4-week storage period. The effect of storage on the mean values of the egg weight loss significantly ($p < 0.05$) increased from Day 1 to week 4 of storage.

At peak-lay, the mean values of the albumen height, Haugh unit and yolk index declined ($p < 0.05$) from day 1 to week 4 respectively (Table 7.4). The mean value of the yolk colour declined ($p < 0.05$) from day 1 to week 1 but was similar ($p > 0.05$) from weeks 2 to 4. The value of the estimated eggshell thickness was not affected by the storage period. The egg weight loss increased ($p < 0.05$) with increased storage period from day 1 to week 4 of storage.

At late-lay, the mean values of the AH and HU decreased ($p < 0.05$) with storage period from day 1 to week 4. The mean value of the yolk index of day 1 was significantly ($p < 0.05$) higher compared to weeks 1 and 2 and subsequently declined ($p < 0.05$) from week 3 to week 4. However, the mean values of the yolk index for weeks 1 and 2 were similar ($p > 0.05$). The yolk colour of day 1 and week 1 was similar ($p > 0.05$) but significantly, higher ($p < 0.05$) compared to weeks 2, 3 and 4, which were similar ($p > 0.05$). Storage did not affect eggshell thickness. The mean values of the egg weight loss increased significantly ($p < 0.05$) from day 1 to week 4 of storage (Table 7.4).

Table 7.4 Effect of storage over four weeks on egg quality indices at physiological stages early-lay, peak-lay and late-lay at 40mg/ml Noni fruit extract

Physiological stage	Parameters	Storage					SEM	P-value
		Day 1	Week 1	Week 2	Week 3	Week 4		
Early-Lay (week 22)	AH (mm)	5.64^{a}	4.70^{b}	3.98^{c}	3.37^{d}	2.97^{e}	0.114	0.001
	HU (mm)	77.95^{a}	70.95^{b}	65.30^{c}	57.98^{d}	51.64^{e}	1.799	0.001
	YI (mm)	0.40^{a}	0.39^{a}	0.35^{b}	0.30^{c}	0.24^{d}	0.011	0.001
	YC	4.00^{a}	3.00^{b}	3.00^{b}	2.67^{c}	2.67^{c}	0.091	0.001
	EST (mm)	0.46	0.46	0.46	0.46	0.46	0.001	0.999
	Wt. L (g)	0.00^{e}	0.47^{d}	0.79^{c}	1.28^{b}	1.71^{a}	0.042	0.001
Peak-Lay (week 30)	AH (mm)	6.11^{a}	4.97^{b}	4.91^{c}	3.83^{d}	2.83^{e}	0.017	0.001
	HU (mm)	78.37^{a}	69.43^{b}	63.77^{c}	58.75^{d}	45.98^{e}	0.388	0.001
	YI (mm)	0.42^{a}	0.36^{b}	0.33^{c}	0.28^{d}	0.21^{e}	0.002	0.001
	YC	4.03^{a}	3.67^{b}	2.67^{c}	2.67^{c}	2.67^{c}	0.052	0.001
	EST (mm)	0.47	0.47	0.47	0.47	0.47	0.000	0.999
	Wt. L (g)	0.00^{e}	0.52^{d}	1.08^{c}	1.39^{b}	1.83^{a}	0.051	0.001
Late-Lay (week 48)	AH (mm)	5.70^{a}	5.12^{b}	4.73^{c}	4.07^{d}	4.07^{e}	0.069	0.001
	HU (mm)	73.59^{a}	68.89^{b}	64.85^{c}	57.89^{d}	49.05^{e}	0.993	0.001
	YI (mm)	0.40^{a}	0.34^{b}	0.33^{b}	0.31^{c}	0.23^{d}	0.006	0.001
	YC	4.00^{a}	4.00^{a}	2.67^{b}	2.67^{b}	2.67^{b}	0.062	0.001
	EST (mm)	0.47	0.47	0.47	0.47	0.47	0.000	0.999
	Wt. L (g)	0.00^{e}	0.52^{d}	1.10^{c}	1.33^{b}	1.58^{a}	0.042	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$); SEM. = Standard error of difference of means.
AH =Albumen height; HU= Haugh Unit; YI = Yolk Index; YC= Yolk Colour; EST= Estimated Eggshell Thickness;
Wt. L. = Weight Loss in eggs,

7.5.1.5 Effect of 40mg/ml Noni fruit extract administration at weeks 16 or 20 on haematological indices at early-lay

The treatment had no significant effect ($p > 0.05$) on the mean values of the red blood cell (RBC) counts in all experimental groups (week 16,

Table 7.5 Effect of 40mg/ml Noni fruit extract administered at weeks 16 or 20 on haematological indices at early-lay

Parameters	Reference Range	Treatment (40mg/ml) Noni Fruit Extract			SEM	p-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
RBC (10^6µL)	2.5 -3.9 [1,2]	3.58	3.58	3.58	0.007	0.872
Hb (g/dL)	7.0 – 13.0[1,2]	10.20[c]	11.25[a]	10.82[b]	0.037	0.001
PCV (%)	22.0-35.0[1,2]	31.37[c]	35.23[a]	33.87[b]	0.108	0.001
MCV (fL)	90.0 – 140.0[1,2]	110.60[a]	110.54[b]	110.54[b]	0.027	0.047
MCH (pg)	33.0 - 47.0[1,2]	32.84[b]	32.91[a]	32.93[a]	0.011	0.001
MCHC (g/dL)	26.0 – 35.0[1,2]	31.92[b]	32.52[a]	31.93[b]	0.036	0.001
Trb (10^9/L)	3.0-33.0[1,2]	30.48[a]	28.03[b]	30.38[a]	0.052	0.001
WBC (10^9/L)	1.9 - 9.5[1]	8.01	7.93	8.00	0.107	0.714
Heterophil (%)	29.0 - 48.7[1]	31.11[a]	30.53[b]	30.50[b]	0.156	0.001
Basophil (%)	0.0 -6.4[1]	0.40[a]	0.33[b]	0.39[a]	0.009	0.001
Eosinophil (%)	0.0 -11.5[1,2]	6.21[a]	5.23[b]	5.31[b]	0.086	0.001
Monocyte (%)	0.0 -6.5[1,2]	2.52[a]	1.175[b]	2.37[a]	0.048	0.001
Lymphocyte (%)	26.9 - 70.6[1,2]	44.01[a]	38.53[b]	40.70[b]	1.151	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$); SEM = Standard error of means;
RBC = Red blood cells; Hb = Haemoglobin; PCV = Packed cell volume; MCV = Mean corpuscular volume;
MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; Trb = Thrombocytes;
WBC = White blood cells; [1]Clinical Diagnostic Division (1990); [2]Bounous and Stedman (2000);
* birds on control received 0 mg/ml Noni fruit extract from week 16 to the end of the experiment.

Week 20, and the control). However, the mean values of haemoglobin concentration (Hb) and packed cell volume (PCV) differed significantly ($p < 0.05$) in week 16, week 20, and the control (Table 7.5). Furthermore, the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were similar ($p > 0.05$) in week 16 and week 20, but varied significantly ($p < 0.05$) with the control group, which had lower total haemoglobin concentrations and a smaller fraction of blood consisting of red blood cells. The mean haemoglobin concentration per red blood cell (MCHC) and thrombocyte (Trb) levels, also known as 'platelets' in mammalian blood were similar ($p > 0.05$) in week 20 and the control. However, MCHC was significantly higher ($p < 0.05$) and Trb significantly lower ($p < 0.05$) compared to the week 16 group. The mean white blood cell (WBC) counts were similar ($p > 0.05$) for week 16, week 20, and the control group respectively. The mean levels of heterophils, eosinophils, and lymphocytes were significantly higher ($p < 0.05$) in the control group but those of week 16 and week 20 were similar ($p > 0.05$). The mean basophil and monocyte values were significantly lower ($p < 0.05$) in week 16 and similar ($p > 0.05$) in week 20 and the control.

7.4.1.6 Effect of 40mg/ml Noni fruit extract administration at weeks 16 or 20 on haematological indices at peak-lay

The treatment showed no significant effect ($p > 0.05$) on the mean RBC counts in all experimental groups at peak-lay (Table 7.6). Additionally, the treatment did not have a significant effect ($p > 0.05$) on the MCV, MCH, and MCHC. The mean values of Hb, PCV, and Trb in week 16 and week 20 were similar ($p > 0.05$). Furthermore, the mean values for Hb and PCV were higher ($p < 0.05$) and that of Trb was lower than that in control as shown in Table 7.6.

Table 7.6 Effect of 40mg/ml Noni fruit extract administered at weeks 16 or 20 on haematological indices at peak-lay

Parameters	Reference Range	Treatment (40 mg/ml) Noni fruit extract			SEM	P-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
RBC (10^6µL)	2.5 -3.9 [1,2]	3.68	3.68	3.68	0.017	0.919
Hb (g/dL)	7.0 – 13.0[1,2]	10.35[b]	12.44[a]	10.40[a]	0.446	0.001
PCV (%)	22.0-35.0[1,2]	31.74[b]	38.16[a]	38.04[a]	1.170	0.001
MCV (fL)	90.0 – 140.0[1,2]	115.10	116.57	115.20	0.411	0.373
MCH (pg)	33.0 - 47.0[1,2]	32.91	32.85	32.91	0.025	0.280
MCHC (g/dL)	26.0 – 35.0[1,2]	32.59	32.59	32.59	0.023	0.989
Trb (10^9/L)	3.0-33.0[1,2]	30.75[a]	30.53[b]	30.52[b]	0.046	0.001
WBC (10^9/L)	1.9 - 9.5[1]	8.06[a]	7.42[b]	7.45[b]	0.099	0.001
Heterophil (%)	29.0 - 48.7[1]	33.66[a]	30.53[b]	30.50[b]	0.541	0.001
Basophil (%)	0.0 -6.4[1]	0.61[a]	0.38[b]	0.38[b]	0.009	0.001
Eosinophil (%)	0.0 -11.5[1,2]	5.15[a]	3.13[b]	3.14[b]	0.086	0.001
Monocyte (%)	0.0 -6.5[1,2]	1.06	1.04	1.03	0.023	0.352
Lymphocyte (%)	26.9 - 70.6[1,2]	46.85[a]	45.34[c]	46.24[a]	0.295	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$).
SEM – Standard error of means. RBC = Red blood cells; Hb = Haemoglobin; PCV = Packed cell volume;
MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration;
Trb = Thrombocytes; WBC = White blood cells; [1]Clinical Diagnostic Division (1990); [2]Bounous and Stedman (2000).
* Birds on control received 0 mg/ml Noni fruit extract from week 16 to the end of the experiment.

The mean values of white blood cell (WBC) count, heterophil, basophil and eosinophil in week 16 and week 20 were similar ($p > 0.05$) but lower than those in the control. The mean values for lymphocytes in week 20 and the control were similar ($p > 0.05$) but higher ($p < 0.05$).

7.5.1.7 Effect of 40mg/ml of Noni fruit extract administration at week 16 or 20 on haematological indices at late-lay

The mean values of RBC, MCV, and MCHC were not influenced ($p > 0.05$) by the treatment. The mean values of Hb, PCV, MCH, and Trb for weeks 16 or 20 were similar ($p > 0.05$). The mean value of Trb was higher ($p < 0.05$) in the control group compared to those of weeks 16 or 20, but the mean values of Hb, PCV, and MCH were lower ($p < 0.05$) in the control group compared to those of the treatment groups (Table 7.7).

The mean values of WBC, heterophil, basophil, and eosinophil for weeks 16 or 20 were similar ($p > 0.05$) but lower ($p < 0.05$) compared to those of the control. The mean values for the lymphocytes for week 20 and the control were similar ($p > 0.05$) but lower ($p < 0.05$) compared to that of week 16. The mean values for the monocytes were not significantly ($p > 0.05$) affected by the treatment.

Table 7.7 Effect of 40mg/ml Noni fruit extract administered at weeks 16 or 20 on haematological indices at late-lay

Parameters	Reference Range	Treatment (40mg/ml) Noni Fruit Extract			SEM	p-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
RBC (10^6µL)	2.5 -3.9 [1,2]	3.64	3.63	3.63	0.007	0.325
Hb (g/dL)	7.0 – 13.0[1,2]	10.85[b]	12.64[a]	12.65[a]	0.034	0.001
PCV (%)	22.0-35.0[1,2]	33.31[b]	38.47[a]	38.60[a]	0.113	0.001
MCV (fL)	90.0 – 140.0[1,2]	118.07	118.06	118.06	0.411	0.373
MCH (pg)	33.0 - 47.0[1,2]	32.89[b]	32.83[a]	32.92[a]	0.016	0.001
MCHC (g/dL)	26.0 – 35.0[1,2]	32.59	32.59	32.59	0.034	0.998
Trb (10^9/L)	3.0-33.0[1,2]	30.98[a]	30.52[b]	30.52[b]	0.017	0.001
WBC (10^9/L)	1.9 - 9.5[1]	8.31[a]	7.54[b]	7.61[b]	0.139	0.001
Heterophil (%)	29.0 - 48.7[1]	35.59[a]	30.54[b]	30.70[b]	0.784	0.001
Basophil (%)	0.0 -6.4[1]	0.60[a]	0.47[b]	0.47[b]	0.009	0.001
Eosinophil (%)	0.0 -11.5[1,2]	5.39[a]	3.23[b]	3.23[b]	0.039	0.001
Monocyte (%)	0.0 -6.5[1,2]	1.13	1.13	1.13	0.013	0.973
Lymphocyte (%)	26.9 - 70.6[1,2]	49.05[a]	43.44[b]	47.73[a]	2.009	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$). SEM – Standard error of means. RBC = Red blood cells, Hb = Haemoglobin; PCV = Packed cell volume; Mean corpuscular volume; MCH = Mean corpuscular haemoglobin;
MCHC = Mean corpuscular haemoglobin concentration; Trb = Thrombocytes; WBC = White blood cells; [1]Clinical Diagnostic Division (1990); [2]Bounous and Stedman (2000) * Birds on control received 0 mg/ml Noni fruit extract from week 16 to the end of the experiment.

7.4.1.8 Effect of 40mg/ml Noni fruit extract administration at weeks 16 or 20 on liver function indices at early-lay

At early lay the mean values of AST, ALT, AST/ALT ratio, TP, total bilirubin TB, and DB in week 20 and the control were similar ($p > 0.05$) but higher compared to that of week 16, except AST/ALT ratio, which was significantly ($p < 0.05$) lower in week 16 (Table 7.8). The mean values of ALP and GGT for weeks 16, 20, and the control group were not significantly ($p > 0.05$) different.

Globulin (GLB), ALB/GLB ratio for Weeks 16, 20, and the control were significantly ($p < 0.05$) different. The mean values for the ALB for weeks 16 or 20 were similar ($p > 0.05$), and the mean values of GLB and ALB/GLB ratio were significantly ($p < 0.05$) different in all three experimental groups.

Table 7.8 Effect 40mg/ml Noni fruit extract administered at weeks 16 or 20 on liver function biochemical indices at early-lay

Parameters	Reference Range	Treatment (40 mg/ml) Noni Fruit Extract			SEM	p-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
AST **(U/L)**	9.0 - 49.0[1]	32.12[a]	23.62[b]	32.13a	0.014	0.001
ALT **(U/L)**	10.0 -109.0[1]	38.17[a]	28.14[b]	38.12[a]	0.013	0.001
AST/ALT	1.0 – 1.2[1]	0.84[b]	0.89[a]	0.84b	0.001	0.001
ALP **(U/L)**	1.0 – 114.0[1]	40.03	40.02	40.02	0.020	0.901
GGT **(U/L)**	3.0 – 19.0[1]	10.34	10.37	10.33	0.060	0.846
TP (g/l)	54.0 – 75.0[1]	68.46[a]	56.36[b]	68.41[a]	0.018	0.001
ALB (g/l)	23.0 – 31.0[1]	25.42[b]	28.38[a]	28.39[a]	0.042	0.001
GLB (g/l)	0.0 – 45.0[1,2]	43.03[a]	27.98[c]	40.02[b]	0.051	0.001
ALB/GLB	0.0 – 10.0[1,2]	0.59[c]	1.01[a]	0.71[b]	0.002	0.001
TB (µmol/L)	0.0 – 5.13[1]	3.24[a]	2.84[b]	3.24[a]	0.003	0.001
DB (µmol/L)	1.0 – 2.0[1]	1.32[a]	1.22[b]	1.32[a]	0.004	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$). SEM Standard error of means.
AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; AST/ALT = Aspartate Aminotransferase/Alanine Aminotransferase ratio, ALP = Alkaline Phosphatase; GGT = Gamma-Glutamyl Transferase; TP = Total Protein; ALB = Albumin; GLB = Globulin; ALB/GLB = Albumin/Globulin ratio; TB = Total Bilirubin; TD = Direct Bilirubin [1]Clinical Diagnostics Division (1990); [2]Harr (2002). * Birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment.

7.4.1.9 Effect of 40mg/ml Noni Fruit extract administration at weeks 16 or 20 on liver function indices at peak-lay

The mean values of AST, ALT, AST/ALT ratio, TP, ALB, GLB, TD, and DB were similar ($p > 0.05$) in weeks 16 or 20. However, the mean values of AST, ALT, TP, GLB, TB, and DB were significantly higher ($p < 0.05$) in the control group compared to Weeks 16 or 20. Additionally, the values of ALB and ALB/GLB ratio were significantly ($p < 0.05$) lower in the control group.

Table 7.9 Effect 40mg/ml Noni fruit extract administered at weeks 16 or 20 on liver function indices at peak-lay

Parameters	Reference Range	Treatment (40mg/ml) Noni Fruit Extract			SEM	p-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
AST **(U/L)**	9.0 - 49.0[1]	32.13[a]	23.63[b]	23.62[b]	0.004	0.001
ALT **(U/L)**	10.0 -109.0[1]	38.14[a]	28.15[b]	28.14[b]	0.012	0.001
AST/ALT	1.0 – 1.2[1]	0.84[a]	0.83[b]	0.83[b]	0.000	0.001
ALP **(U/L)**	1.0 – 114.0[1]	40.02	40.01	40.03	0.019	0.521
GGT **(U/L)**	3.0 – 19.0[1]	10.27	10.25	10.26	0.058	0.972
TP (g/l)	54.0 – 75.0[1]	68.29[a]	56.82[b]	56.82[b]	0.020	0.001
ALB (g/l)	23.0 – 31.0[1]	25.47[b]	28.48[a]	28.44[a]	0.031	0.001
GLB (g/l)	0.0 – 45.0[1,2]	42.82[a]	28.34[b]	28.34[b]	0.031	0.001
ALB/GLB	0.0 – 10.0[1,2]	0.59[b]	1.00[a]	1.00[a]	0.001	0.001
TB (µmol/L)	0.0 – 5.13[1]	3.24[a]	2.84[b]	2.84[b]	0.002	0.001
DB (µmol/L)	1.0 – 2.0[1]	1.32[a]	1.23[b]	1.31[a]	0.007	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$) SEM = Standard error of means. AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; AST/ALT = Aspartate Aminotransferase/Alanine Aminotransferase ratio, ALP = Alkaline Phosphatase; GGT = Gamma-Glutamyl Transferase; TP = Total Protein; ALB = Albumin; GLB = Globulin; ALB/GLB = Albumin/Globulin ratio; TB = Total Bilirubin; TD = Direct Bilirubin [1]Clinical Diagnostics Division (1990); [2]Harr (2002).* Birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment.

7.4.1.10 Effect of 40 mg/ml of Noni fruit extract administration at weeks 16 or 20 on liver function indices at late-lay

The mean values of AST, ALT, AST/ALT ratio, TP, ALB, GLB, ALB/GLB ratio, TD, and DB were similar ($p > 0.05$) in weeks 16 or 20. However, the mean values of ALB, ALB/GLB ratio were significantly ($p < 0.05$) lower in in the control,

Table 7.10 Effect of 40mg/ml Noni fruit extract administered at weeks 16 or 20 on liver function biochemical indices at late-lay

Parameters	Reference Range	Treatment (40mg/ml) Noni Fruit Extract Late Lay			SEM	P-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
AST **(U/L)**	9.0 - 49.0[1]	32.12[a]	23.62[b]	23.63[b]	0.004	0.001
ALT **(U/L)**	10.0 -109.0[1]	38.14[a]	28.14[b]	28.14[b]	0.018	0.001
AST/ALT	1.0 – 1.2[1]	0.84[a]	0.83[b]	0.83[b]	0.000	0.001
ALP **(U/L)**	1.0 – 114.0[1]	40.04	40.00	40.01	0.018	0.143
GGT **(U/L)**	3.0 – 19.0[1]	10.25	10.25	10.28	0.051	0.784
TP (g/l)	54.0 – 75.0[1]	68.22[a]	56.80[b]	56.81[b]	0.013	0.001
ALB (g/l)	23.0 – 31.0[1]	25.54[b]	28.56[a]	28.56[a]	0.020	0.001
GLB (g/l)	0.0 – 45.0[1,2]	42.68[a]	28.24[b]	28.25[b]	0.026	0.001
ALB/GLB	0.0 – 10.0[1,2]	0.60[b]	1.01[a]	1.01[a]	0.001	0.001
TB (µmol/L)	0.0 – 5.13[1]	3.24[a]	2.84[b]	2.84[b]	0.003	0.001
DB (µmol/L)	1.0 – 2.0[1]	1.32[a]	1.23[b]	1.23[a]	0.007	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$). SEM= Standard error of means.
AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase, AST/ALT = Aspartate Aminotransferase/Alanine Aminotransferase ratio, ALP = Alkaline Phosphatase; GGT = Gamma-Glutamyl Transferase; TP = Total Protein; ALB = Albumin; GLB = Globulin; ALB/GLB = Albumin/Globulin ratio; TB = Total Bilirubin; TD = Direct Bilirubin [1]Clinical Diagnostics Division (1990); [2]Harr (2002). * Birds on control received 0 mg/ml Noni fruit extract from week 16 to the end of the experiment..

while AST, ALT, AST/ALT ratio, TP, ALB, GLB, ALB/GLB ratio, TD were significantly ($p < 0.05$) higher in the control (**T_1**) group (Table 7.10).

7.4.1.11 Effect of 40mg/ml of Noni fruit extract administration on kidney and lipid metabolites profile at early-lay

The mean values of creatinine (CRE), urea (U), uric acid (UA), calcium ions (Ca^{+2}) and potassium ions (K^+) were similar ($p > 0.05$) in week 20 and the control group (Table 7.11). The mean values for CRE, Ca^{2+} and K^+ in week 16 group were higher than those in week 20 and the control. However, the mean values for urea and UA were significantly ($p < 0.05$) lower in week 16 group as compared to week 20 and the control groups. The mean values for Sodium (Na^+) and Chloride (Cl^-) ions were not significantly ($p > 0.05$) different in all the experimental groups.

The mean values of the total cholesterol (TCHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), triglycerides (TG) and glucose (GLU) were similar ($p > 0.05$) in week 20 and control group. The mean values of the lipid profile for week 16 were significantly ($p < 0.05$) lower except HDL, which had a significantly ($p < 0.05$) higher value than week 20 and the control group.

Table 7.11 Effect 40mg/ml Noni fruit extract administered at weeks 16 or 20 on kidney and lipid profile indices at early-lay

Parameters	Reference Range	Treatment (40 mg/ml) Noni Fruit Extract			SEM	P-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
CRE (umol/L)	0.9 - 1.8[1]	1.12[b]	1.15[a]	1.12[b]	0.000	0.001
UREA (mg/dL)	2.9 – 10.0[1]	4.74[a]	3.87[b]	4.39[a]	0.013	0.001
UA (umol/L)	1.9 – 12.5[1]	3.31[a]	1.55[b]	3.31[a]	0.094	0.001
Ca^{+2} (mmol/L)	2.2 – 3.0[1]	2.82[b]	2.86[a]	2.82[b]	0.002	0.001
Na^{+} (mmol/L)	139.0 - 155.0[1]	141.80	141.50	141.60	0.215	0.460
Cl^{-} (mmol/L)	108.0 – 124.0[1]	121.60	121.70	121.60	0.076	0.923
K^{+} (mmol/L)	3.5 – 5.2[1]	3.85[b]	9.37[a]	3.85[b]	0.009	0.001
TCHOL (mmol/L)	3.34 – 7.7[1,2]	6.83[a]	4.82[b]	6.82[a]	0.005	0.001
HDL (mg/dL)	0.0 – 10.0[1,2]	1.30[b]	1.35[a]	1.31[b]	0.003	0.001
LDL (mmol/L)	0.0 – 10.0[1,2]	2.61[a]	2.22[b]	2.61[a]	0.004	0.001
VLDL (mmol/L)	0.0 – 10.0[1]	2.91[a]	1.25[b]	2.92[a]	0.014	0.001
TG (mmol/L)	0.2 – 2.8[1]	1.73[a]	1.48[b]	1.72[a]	0.002	0.001
GLU (mmol/L)	4.2 - 6.6[1]	4.56[a]	4.42[b]	4.56[a]	0.001	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$).SEM = Standard error of means; CRE = Creatinine; UA = Uric Acid; Ca^{2+} = Calcium; Na^{+} = Sodium; Cl^{-} = Chloride; and K^{+} = Potassium; TCHOL = Total Cholesterol; HDL = High-Density Lipoprotein LDL = Low-Density Lipoprotein; VLDL = Very Low-Density Lipoprotein; TG = Triglycerides; GLU = Glucose (GLU).
[1]Clinical Diagnostics Division (1990); [2]Bueno *et al*. (2017). *Birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment.

7.4.1.12 Effect of 40 mg/ml of Noni fruit extract administration at weeks 16 or 20 on kidney and lipid profile at peak-lay

The average levels of CRE, urea, UA, Ca^{+2}, and K^{+} for weeks16 or 20 were found to be similar ($p > 0.05$) as shown in Table 7.12. In the control group (T_1), the average values for CRE and K+ were lower than in the weeks 16 or 20 groups. However, the average urea, UA, and Ca^{2+} levels for week 16 were significantly higher ($p < 0.05$) compared to week 20 and the control group. The average levels for Na^{+} and Cl^{-} ions did not show significant differences ($p > 0.05$) across all experimental groups.

Similarly, the average values of TCHOL, HDL, LDL, VLDL, TG, and GLU were also similar ($p > 0.05$) for weeks16 or 20. For control group, the average lipid profile values were significantly ($p < 0.05$) higher, except HDL, which had a significantly ($p < 0.05$) lower value compared to week 16 and control group.

Table 7.12 Effect of 40mg/ml Noni fruit extract administered at weeks 16 or 20 on kidney and lipid profile at peak-lay

Parameters	Reference Range	Treatment (40 mg/ml) Noni Fruit Extract			SEM	P-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
CRE (umol/L)	0.9 - 1.8[1]	1.12[b]	1.16[a]	1.16[a]	0.001	0.001
UREA (mg/dL)	2.9 – 10.0[1]	4.27[a]	3.45[b]	3.44[b]	0.008	0.001
UA (umol/L)	1.9 – 12.5[1]	3.31[a]	1.54[b]	1.54[b]	0.065	0.001
Ca^{+2} (mmol/L)	2.2 – 3.0[1]	2.8[a]	2.4[b]	2.4[b]	0.001	0.001
Na^{+} (mmol/L)	139.0 - 155.0[1]	141.2	141.3	141.3	0.119	0.838
Cl^{-} (mmol/L)	108.0 – 124.0[1]	121.6	121.6	121.5	0.058	0.257
K^{+} (mmol/L)	3.5 – 5.2[1]	3.80[b]	9.40[a]	9.40[a]	0.003	0.001
TCHOL (mmol/L)	3.34 – 7.7[1,2]	6.83[a]	4.82[b]	4.82[b]	0.005	0.001
HDL (mg/dL)	0.0 – 10.0[1,2]	1.31[b]	1.35[a]	1.35[a]	0.001	0.001
LDL (mmol/L)	0.0 – 10.0[1,2]	2.61[a]	2.31[b]	2.31[b]	0.003	0.001
VLDL (mmol/L)	0.0 – 10.0[1]	2.90[a]	1.15[b]	1.13[b]	0.006	0.001
TG (mmol/L)	0.2 – 2.8[1]	1.68[a]	1.45[b]	1.45[b]	0.001	0.001
GLU (mmol/L)	4.2 - 6.6[1]	4.56[a]	4.42[b]	4.42[b]	0.000	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$), SEM. – Standard error of means, CRE = Creatinine, UA = Uric Acid, Ca^{2+} = Calcium, Na^{+} = Sodium, Cl^{-} = Chloride, and K^{+} = Potassium.. TCHOL = Total Cholesterol, HDL = High-Density Lipoprotein LDL = Low-Density Lipoprotein, VLDL = Very Low-Density Lipoprotein, TG = Triglycerides, GLU = Glucose (GLU), [1]Clinical Diagnostics Division (1990), [2]Bueno *et al*. (2017). * Birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment.

7.4.1.13 Effect of 40mg/ml Noni fruit extract administration at weeks 16 or 20 on kidney and lipid profile at late-lay

The mean values of CRE, urea, UA, Ca^{2+}, and K^{+} in weeks 16 or 20 were similar ($p > 0.05$) as shown in Table 7.13. The mean values for CRE and K^{+} were lower ($p < 0.05$) in the control group compared to those of weeks 16 or 20. However, the mean values for urea, UA, and Ca^{2+} levels in the control group were significantly ($p < 0.05$) lower compared to those weeks 16 or 20. The average levels for Na^{+} and Cl^{-} did not show significant differences ($p > 0.05$) across all experimental groups.

The mean values of TCHOL, HDL, LDL, VLDL, TG, and GLU in weeks 16 or 20 had similar ($p > 0.05$) levels but lower compared to the control, except HDL, which had a significantly ($p < 0.05$) lower value in the control group compared to weeks 16 or 20.

Table 7.13 Effect of Noni fruit extract administered at weeks 16 or 20 on kidney and lipid serum biochemical indices at late-lay

Parameters	Reference Range	Treatment (40 mg/ml) Noni fruit extract			SEM	p-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
CRE (umol/L)	0.9 - 1.8[1]	1.22[b]	1.16[a]	1.16[a]	0.001	0.001
UREA (mg/dL)	2.9 – 10.0[1]	4.18[a]	3.24[b]	3.25[b]	0.007	0.001
UA (umol/L)	1.9 – 12.5[1]	3.31[a]	1.54[b]	1.54[b]	0.010	0.001
Ca^{+2} (mmol/L)	2.2 – 3.0[1]	2.73[a]	2.43[b]	2.42[b]	0.587	0.001
Na^{+} (mmol/L)	139.0 - 155.0[1]	141.07	141.14	141.15	0.031	0.123
Cl^{-} (mmol/L)	108.0 – 124.0[1]	121.66	121.61	121.68	0.040	0.222
K^{+} (mmol/L)	3.5 – 5.2[1]	3.86[b]	9.41[a]	9.40[a]	0.004	0.001
TCHOL (mmol/L)	3.34 – 7.7[1,2]	6.82[a]	4.83[b]	4.82[b]	0.006	0.001
HDL (mmol/L)	0.0 – 10.0[1,2]	1.31[b]	1.35[a]	1.35[a]	0.003	0.001
LDL (mmol/L)	0.0 – 10.0[1,2]	2.58[a]	2.32[b]	2.31[b]	0.012	0.001
VLDL (mmol/L)	0.0 – 10.0[1]	2.93[a]	1.16[b]	1.16[b]	0.014	0.001
TG (mmol/L)	0.2 – 2.8[1]	1.68[a]	1.48[b]	1.48[b]	0.001	0.001
GLU (mmol/L)	4.2 - 6.6[1]	4.56[a]	4.42[b]	4.42[b]	0.001	0.001

Means in the same row with different superscripts are significantly different ($p < 0.05$); SEM. – Standard error of means; CRE = Creatinine; UA = Uric Acid; Ca^{2+} = Calcium; Na^{+} = Sodium; Cl^{-} = Chloride; and K^{+} = Potassium; TCHOL = Total Cholesterol; HDL = High-Density Lipoprotein LDL = Low-Density Lipoprotein; VLDL = Very Low-Density Lipoprotein; TG = Triglycerides; GLU = Glucose; [1]Clinical Diagnostics Division (1990); [2]Bueno *et al*. (2017). *Birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment.

7.6 Discussion

7.6.1. Effect of 40 mg/ml of Noni fruit extract administration at weeks 16 or 20 on laying performance indices

According to Guni *et al.*, (2021), an important economic factor that determines the days-to-first-egg is the age at which chickens attain sexual maturity. The age at first egg is dependent on the body weight of birds of the same breed or variety, as birds with higher body weights laid their eggs earlier than those with lower body weights (Olawumi, 2011). Olawumi (2011) reported that body weight also affects the age at which they reach peak egg production and their overall performance. In this study (Table 7.1) the birds placed on noni fruit extract at week 16 had significantly delayed days-to-first-egg. However, their body weight was significantly higher at 50 % laying (week 22; early-lay) compared to the birds fed noni at week 20 or the control group (0 mg/ml) that had similar body weights but dropped their eggs earlier than the week 16 treatment group. On the contrary, Churchil *et al.* (2019) observed a significant reduction in the days-to-first egg in noni-fed layer type Japanese quails as compared to the control. However, the mean days to first egg in the noni group of the Japanese quails was not significantly different from each other at 38.2 ± 0.49 days at 2 ml/l and 39.00 ± 0.71 days at 1 ml/l, while the control group 0 ml/l was significantly different from the noni groups at 40.60 ± 0.51 days. Although in the current study the week 16 group (T_2) delayed reaching sexual maturity they had significantly higher egg mass, percent hen day egg production and net feed efficiency index, and better feed conversion ratio at early-lay compared to the week 20 (T_3) and control (T_1) groups. This more than compensated economically for the approximately three days delay in egg production to the advantage of the poultry farmer.

At peak and late-lay, there was a significantly higher body weight, egg mass, and better feed conversion ratio per egg mass, additionally, there was a higher percentage of hen day egg

production and net feed efficiency index in the week 16 (T_2) group compared to the week 20 (T_3) and the control (T_1) groups (Table 7.1). This may be attributed to the early administration as well as the main active substances found in noni fruit, such as polysaccharide, scopoletin, ascorbic acid, β-carotene, L-arginine, proxeronine, and proxeroninase. These active substances are closely related to the metabolic activities that support the addition of weight and eventually result in a high percentage of carcass, and egg production (Ali *et al.*, 2016; Widjastuti *et al.*, 2019). Thus, administering noni fruit extract to pullets at week 16 exhibited superior laying performance and body weight at the peak of lay and late lay.

In this study, pullets fed noni fruit extract at week 16 exhibited a significantly higher visceral fat reduction compared to those with noni fruit extract from week 20 and the control group respectively at peak and late-lay. The reduced visceral fat may contribute to improved egg production, as the birds will experience reduced egg impaction, and less straining at lay and lay bigger eggs as the noni will channel most of the triglycerides and LDL into egg yolk formation (Scanes, 2022).

According to Nishioka (2007), noni fruit extract reduces adipose tissue weight and plasma triglyceride levels in animals, as well as improves glucose tolerance without any harmful side effects. Similarly, Mandukhail *et al.* (2010) found that ethanolic extracts from noni fruit, leaves, and roots could lower triglyceride levels, total cholesterol, and LDL levels while increasing high-density lipoprotein levels. They concluded that the extracts' anti-dyslipidemic activity was due to inhibiting lipid biosynthesis, adsorption, and secretion, making it beneficial for managing visceral fat deposition and serum lipid levels. Jambocus *et al.* (2017) also reported positive effects of noni leaf extracts on obesity, showing inhibition

of pancreatic and lipoprotein activity, leading to improved lipid profiles, reduced LDL levels, and visceral fat deposition in male Sprague-Dawley rats.

The abdominal fat results obtained in this study (Table 7.2) align with the findings of Widjastuti *et al.* (2019), who observed a significant reduction of abdominal fat in Sentul Debu chickens fed noni fruit extract.

The increased rate of sexual development of the uteri of birds begins around 16 weeks of age, with the uterus gradually increasing in size until it reaches its maximum level by week 18, subsequently, the rate of increase decreases until the birds start laying eggs (Dumoulin, 2018; Yin *et al.*, 2020). The uterus weight in this study increased progressively in all the experimental groups and was significantly higher in the week 16 group compared to week 20 or the control group (Table 7.2), which may be attributed to the beneficial nutraceutical components of noni fruit extract (Saki *et al.*, 2014). The increased weight of the uterus with physiological production stage in this study was enhanced by introducing the birds to the noni fruit extract at week 16 (Table 7.2). This is evident from the superior uterus weights obtained for the week 16 group of birds from early-lay to late-lay. Hafez and Kamar (1955) reported that the size and weight of the hen's ovary and oviduct are influenced by both age and the reproductive phase. (Barua, 2021) reported that the length and weight of the oviduct segment of Bangladesh native chickens progressively increased in rate up to 10 months of age and then decreased at 11 months.

In this study, the noni fruit extract administered at week 16 coincided with the development of the medullary bone (Fleming *et al.*, 1998) shown in Figure 2.1 in Chapter 2 (Dumoulin, 2018). Therefore, the significant increase in right tibia bone density (mineralisation), bone length and diaphysis of the birds in week 16 as compared to week 20 and the control group

suggests that administering 40 mg/ml of noni fruit extract at week 16 enhanced medullary bones development in the right tibia bone of the birds. The enhanced medullary bone development may strengthen the tibia bones by preventing paralysis and fracture, and promote eggshell formation for the production of thicker-shelled eggs, less porous shells that will result in eggs with longer shelf lives (Zhang and Coon, 1997; Dumoulin, 2018). This aligns with Javid *et al.* (2022) who reported the effects of prebiotics and probiotics on the morphometric characteristics of tibia bones in broilers. In their study, the supplemented groups displayed significant differences in morphometric parameters, such as tibia bone length, weight, thickness of lateral and medial walls, tibiotarsal index of bone, and bone ash percentage, in comparison to the control group that received antibiotics. Similar results were obtained in this study using noni fruit extract, which is a phytogenic.

Hussain *et al.* (2016), reported that noni fruit extract promotes tissue regeneration (neo-angiogenesis) by increasing the proliferation rate of bone marrow mesenchymal stem cells (BMSC). Noni fruit extract also upregulated genes associated with bone formation, including osteocalcin and runt-related transcription factor-2, as well as the enzyme alkaline phosphatase (ALP). Norton *et al.* (1996), also observed that bones grow not only longer but also wider in diaphysis diameter which is attributed to the action of osteoclasts, which break down old bone in the right tibia cavity, while simultaneously generating new bone tissue beneath the periosteum through intramembranous ossification.

7.6.2. Effect of 40mg/ml Noni fruit extract administration at weeks 16 or 20 on egg quality indicators

The egg quality parameters in this study were higher for the birds that received noni treatment at week 16 (T_2). This may be due to the main nutraceutical components (flavonoids, phenolics, iridoids and rich mineral content) of noni fruit extract (Asmara *et al.*, 2019). Although the values of the internal egg quality parameters generally decreased with increasing physiological stage as the birds progressed through the laying period they remained higher in the week 16 group compared to those receiving noni fruit extract at week 20 (T_3) and the control group (T_1) respectively. Except for the Haugh unit of the week 20 group at late lay that was empirically higher than that of the week 16 group but was similar ($p > 0.05$). This may be due to the effect of the noni fruit extract, which appeared more evident in older hens during longer laying periods.

The physiological stage and laying period negatively affected egg quality, although the early application of noni fruit extract enhanced and preserved the freshness of the eggs, effectively slowing the degradation process during storage. Zita *et al*. (2012), reported that the internal quality of an egg progressively declines in various quality parameters as the laying period progresses. Williams (1992) and Osei-Amponsah *et al.*(2014) also reported that irrespective of chicken ecotype, the main factors that lead to egg degradation during storage were temperature and age of the bird which affects the internal quality of the egg as well as eggshell characteristics.

7.6.3. Effect of four-week storage period at physiological stage (early-lay, peak-lay and late-lay) on egg quality indicators

Over the four-week storage period in this study, the egg weight loss was as a result the loss of moisture and carbon dioxide from egg whites (albumen) during storage, which led to a reduction in the albumen height, Haugh units and yolk index. Moreover, the deterioration of egg quality during storage causes the vitelline membrane to weaken and stretch, thus increasing the water content in the egg yolk, resulting in a flatter yolk, and raising the empirical value of the yolk index (Scott and Silversides, 2000). The permeability of the vitelline membrane increases as well causing a mixing of the albumen proteins and the yolk contents resulting in a pale yolk and mottling (Chukwuka *et al.*, 2011). Eke (2013) reported a decrease in egg weight and all other egg quality indices such as the Haugh unit and yolk index within a four-week storage period under tropical ambient conditions of 32± 2ºC.

The rate of decrease in Haugh unit and yolk index (egg freshness) with storage time in this study was significantly reduced as the Haugh unit remained in the B grade (regular) at early, peak and late laying at the end of the four-week storage period. The yolk index, however, reduced to the C grade (poor) at early, peak and late lay by the fourth week respectively. The better performance of the Haugh unit may be attributed to the polysaccharide and mineral components of the noni fruit extract that can balance the colloid osmotic and homoeostatic environment of the egg (Lohani *et al*., 2019). This also agrees with Moon *et al.* (2021), who reported no significant ($p > 0,05$) change in the Haugh unit at the end of 4 weeks compared to the control when they supplemented a phytogenic blend (a mixture containing fermented *Schisandra chinensis* pomace, fermented *Pinus densiflora* needle extract, and *Allium tuberosum* powder in the ratio of 2:2:1). Suggesting that the phytogenic blend had the property to slow down the degradation rate of eggs stored at tropical ambient temperature.

The estimated eggshell thickness in this study was not affected by the 4-week storage period. However, Olomu (2021) and Camargo *et al.* (2022) reported that prolonged storage of eggs, depending on factors such as temperature, humidity, and the movement of carbon dioxide from the albumen to the shell, caused a reduction in eggshell thickness with long storage times of up to 28 days. Camargo *et al.*(2022) reported a negative influence by storage duration on eggshell thickness of 7µm between 3 days and 28 days, which they attributed to the shrinkage of the inner egg membrane and or cuticle. Camargo *et al.*, (2022) may have observed the reductions in eggshell thickness because they took their measurements in micrometres (µm) while measurements in this study were done in millimetres (mm) thus explaining the no influence on eggshell quality observed. Callejo *et al*.(2010) and Maciel *et al.* (2011) did not observe any changes in eggshell thickness during storage, which aligns with the results of this study.

7.6.4. Effect of 40mg/ml Noni fruit extract administration at week 16 or 20 on haematological indicators

The full blood count (FBC) provides a pathological reflection of the status of the exposed animals to toxicants and other conditions (Seo and Lee, 2022). Blood constituents change with the physiological status of an animal. These changes are important in assessing the response of farm animals to various physiological situations and health (Etim, 2014). In this study, there was no significant difference in RBC counts suggesting that the effect of time of administering 40 mg/ml noni fruit extract on all the physiological stages did not influence RBC counts and the values remained in the normal physiological range for chicken (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000). This suggested that exposing the birds to noni fruit extract from week 16 or week 20 did not have any negative health

implications on the birds as they approached their peak and late laying period (Table 7.5, 7.6 and 7.7). However, the components of the RBC (Hb, PCV, MCV, MCH, MCHC and Trb) for weeks 16 or 20 (T_2 and T_3) were similar ($p > 0.05$) but differed significantly ($p < 0.05$) compared to the control (T_1). Except for the Trb values at early lay where the values for week 20 and the control were similar but higher than that of the week 16 group. All the values were in the normal physiological range for chicken (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000). Addass *et al.* (2012) reported that there were lower RBC values and its differentials at a younger age, as observed in this study.

In the early laying period, PCV and Hb among all the treatment groups were significant but were similar at peak and late lay in the noni groups. MCV and MCH values for week 16 (T_2) and week 20 (T_3) were similar but higher than the control (T_1) at early lay suggesting an increased size in the RBC but were not significantly different at peak and late lay though the values at peak lay were higher than early lay and late lay. This may have been due to the increased demand for oxygen supply during the increased metabolic activities at peak lay and the enhancing effect of the noni fruit extract resulting in the increased value of MCV and MCH. This may explain why the blood parameters related to oxygen transport change during the laying period as the physiological stage or reproduction status influences some haematological parameters. MCH is dependent on Hb hence their similar fluctuation trend during the laying period. The values of MCHC were similar for week 20 (T_3) and the control (T_1) groups but significantly different compared to week 16 (T_2) at early lay. However, at peak and late lay the MCHC values were similar for weeks 16 or 20 and the control groups suggested that the time of administration of noni fruit extract did not influence the MCHC values similar to the report by Chinenye *et al.* (2017). All the values of the RBC differentials were in their normal physiological ranges thus ruling out factors such as debilitating diseases,

and kidney failure that could negatively affect the full blood count test results as reported by Elagib and Ahmed (2011) and Etim (2014). All of which may be attributed to beneficial effect of early administration of the noni fruit extract. The study observed that noni administration had an anti-thrombocytosis effect, as evidenced by a significant decrease in thrombocyte count at early lay (Table 7.5) in week 16 (T_2) but similar to week 20 (T_3) at peak and late lay (Table 7.6 and 7.7). These values were lower than the higher values observed in the control group (T_1) across all physiological stages. However, the values for Trb for weeks 16, 20 and control remained in the normal physiological range of 3.0-33.0 x 10^9/L for chicken (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000).

The values of the WBC differentials; heterophil, eosinophil, and lymphocytes in weeks 16 or 20 (T_2 and T_3) were significantly lower than the control T_1 at early lay (Table 7.5) though they all were in their normal physiological ranges of 29.0-48.70 %, 0.00-11.50 % and 26.9-70.6 % respectively (Clinical Diagnostic Division, 1990; Bounous and Stedman, 2000). The levels of Basophil were similar in week 20 (T_3) and the control (T_1) group compared to week 16 (T_2) group at early lay. During peak and late lay, the basophil levels were similar in weeks 16 (T_2) and 20 (T_3), but lower compared to the control (T_1) group (Table 7.6 and 7.7). These values were within the normal physiological range of 0.00 - 6.40 % as reported by Kokoré *et al*. (2021) suggesting that the noni fruit extract played an immunomodulatory role due to the presence and activities of its phytochemical and polysaccharides components.

The Monocyte levels in the week 20 (T_3) and the control (T_1) groups during early lay (Table 7.5) were significantly higher compared to those of the week 16 (T_2) group. However, at peak and late lay, the Monocyte levels were similar for all the experimental groups (T_1, T_2 and T_3) but remained within the normal physiological range (Table 7.6 and 7.7), indicating

no chronic or sub-acute infection. The timing of administering the noni fruit extract had a significant effect, as the values of week 16 (T_2) remained consistently lower compared to those of week 20 (T_3) and control (T_1) groups during early, peak, and late lay. It should be noted that an increase in the concentration levels of white blood cells and its differentials beyond the normal physiological range indicates the presence of pathological processes in the body of the birds (Kuzmina *et al.*, 2021). Bovi *et al.* (2023) also reported the therapeutic properties of noni fruit extract in modulating the behaviour and phenotype of monocytes and macrophage cells in vitro. Their results showed that noni fruit extract inhibited the production of nitric oxide (NO) and inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-4, while stimulating the production of the anti-inflammatory cytokine IL-10 in monocytes thus conferring an improved health status on the birds.

7.6.5. Effect of 40mg/ml Noni fruit extract administration at weeks 16 or 20 on serum biochemical profile

7.6.5.1 Liver function indices

Lower values of AST and ALT indicate less muscle damage better liver health and less liver damage. ALT is more specific to liver injury (Abo *et al.*, 2023) thus a lower AST/ALT ratio value indicate less liver damage and fewer liver problems (Hong *et al.*, 2021; Azi *et al.*, 2023). In this study the AST, ALT, AST/ALT ratio, TP, TB, and DB in the control (T_1) and week 20 (T_3) groups were significantly higher compared to the week 16 (T_2; Table 7.8). Decreasing values within the normal reference range (Clinical Diagnostic Division, 1990), of serum TB and DB in commercial poultry may indicate improved liver function and health, as well as, reduced haemolysis (red blood cell breakdown), enhanced bilirubin excretion and clearance (Benzo *et al.*, 1986). Alkaline phosphate (ALP) and GGT showed no significant

($p > 0.05$) differences in all groups (Table 7.8, 7.9 and 7.10), while GLB and ALB/GLB ratio differed among all groups at early lay (Table 7.8). During peak and late laying (Table 7.9 and 7.10), the values of AST, ALT, TP, GLB, TB, and DB for the control group (T_1) was significantly ($p < 0.05$) higher compared to the weeks 16 or 20 (T_2 and T_3) groups. The ALB and ALB/GLB ratios were lower but all the values were within the normal physiological range of 23.0 - 31.0 g/L, and 10.0 -109.0 UL (Clinical Diagnostics, 1990) respectively as reported by Kokoré *et al.* (2021). Thus, indicating that administering the noni fruit extract at week 16 did not lead to stress or other liver health issues in the laying hens.

According to Sturkie and Newman (1951) and Scanes (2022) lower levels of total protein in the serum of laying hens below 54.0 g/L indicate hypoproteinemia, which may suggest issues such as malnutrition, liver or kidney disease, or blood loss. Lower globulin levels below 0.0 g/L may imply impaired immune function or vitamin deficiencies. The total protein and ALB/GLB ratio in this study remained in the normal physiological range (Clinical Diagnostics, 1990) ruling out hyperproteinemia, which may indicate dehydration, inflammation, or organ (liver) disease (Table 7.8, 7.9 and 7.10). Sunder *et al*., (2016) also reported lower values for the level of total serum protein and albumin in the noni-fed group

Serum globulin levels higher than 4.5 g/dL (45 g/L) may indicate hyperglobulinemia, which may suggest issues like inflammation, infections, autoimmune disorders, or cancer (Mian-Ying and Su, 2001). The administration of noni fruit extract at week 16 tempered any negative reactions that could occur (Table 7.8, 7.9 and 7.10). The physiological stress and the demands of the physiological stages were modulated by the noni fruit extract thereby improving the health and productivity of laying birds. This agrees with Sunder *et al*, (2011a)

who reported that feeding of noni fruit extract at 1.5 ml/bird /day enhanced growth, production and immune response in Nicobari fowl.

The albumin levels obtained in this study but were within the normal physiological range for chicken (Table 7.8 7.9 and 7.10) indicating normal liver function. Brandt *et al.* (1951) reported that high serum albumin levels within the normal physiological range in a laying hen's serum can indicate better overall health, and nutritional status which results in increased egg production and reproductive performance.

7.6.5.2 Kidney and lipid profile indicators

A decrease in serum urea may indicate improved kidney function, increased glomerular filtration rate, reduced protein catabolism, decreased muscle breakdown, increased feed intake, or improved nutrition. Conversely, an increase in serum urea may suggest reduced kidney function. At the early-lay stage, the levels of creatinine (CRE), calcium ions (Ca^{2+}), and potassium ions (K^+) were similar in the week 20 (T_2) and control (T_1) groups compared to the week 16 (T_2) group which had higher values (Table 11) . However, the higher ($p < 0.05$) values for CRE and K^+ in the experimental groups weeks 16 (T_2) and 20 (T_3) compared to the control (T_1) group at peak-lay and late-lay (Table 7.12 and 7.13), may be due to the increased muscle mass induced by the noni fruit extract. The high potassium content of the noni fruit extract played a critical role in maintaining fluid balance and osmotic pressure within cells and tissues, as well as improved nerve impulse transmission, and muscle movement and coordination.

Echols (2006) reported that K^+ aids in the regulation of muscle contractions, influencing overall locomotion and physical performance and maintains the acid-base equilibrium in the body, which is vital for various metabolic processes. Calcium was higher in the week 16

(T_2) group at early lay compared to the week 20 and control (T_1) groups that were similar. This may be due to enhanced calcium absorption from the ileum of the bird, influenced by the noni fruit extract and augmented by the calcium and mineral-rich content of the noni fruit extract. This mineral component of the noni fruit extract is essential for eggshell formation and the coagulation of blood following injury, as well as for muscle contraction and relaxation, which affect the overall mobility and physical activity of birds (Benzo *et al.*, 1986). However, the calcium levels at peak-lay and late-lay were similar for the weeks 16 (T_2) and 20 (T_3) groups but lower compared to the control (T_1) group (Table 7.12 and 7.13). This may be due to the active uptake of calcium from the serum (Hester, 2017) for eggshell formation, which is enhanced by the noni fruit extract for stronger and thicker eggshell formation (Rath *et al.*, 2000; Hussain *et al.*, 2016). This agrees with Sunder *et al.* (2013) who reported a higher ($p < 0.05$) mean concentration of calcium deposition in eggshell and yolk in the noni-fed group.

Urea and Uric Acid (UA) were notably lower in the week 16 (T_2) and week 20 (T_3) groups compared to the control (T_1) group at the early-lay, peak-lay and late-lay (Table 7.11, 7.12 and 7.13) but the values were in the normal physiological range for chicken (Clinical Diagnostics Division, 1990). This may have been as a result of the influence of the noni fruit extract, indicating an effective renal function allowing for an efficient nitrogen and water excretion (Scanes, 2022), ruling out increased dietary protein intake generating higher amounts of nitrogenous waste or dehydration for higher levels and inadequate protein intake or malnutrition for lower levels (Lin *et al.,* 2017),

Additionally, there were no significant differences in the levels of Sodium (Na^+) and Chloride (Cl^-) ions across all experimental groups (Table 7.11, 7.12 and 7.13), indicating

further protection rendered by the noni fruit extract against lipid peroxidation that assisted in regulating the osmotic pressure of the blood through balancing its electrolytes (Mian-Ying *et al.,* 2013).

Noni fruit produces antioxidants such as scopoletin, nitric oxide, vitamin C and vitamin A, and has the efficacy to increase the secretion of bile and NO (Nitrite Oxide) that can stimulate the excretion of cholesterol through faeces (Krishnakumar *et al*., 2015). This may have resulted in the low levels of total cholesterol, LDL and VLDL in the noni-fed groups (week 16; T_2 and week 20; T_3) compared to the control (T_1). The mean values of the lipid profile were similar in the week 16 (T_2) and week 20 (T_3) groups, with the control (T_1) showing significantly lower levels, except for HDL, which was notably higher for weeks 16 (T_2) and 20 (T_3) compared to the control (T_1) group (Table 7.12 and 7.13). This may be due to the phenolic compound, chlorogenic acid, found in noni fruit extract, reported to have the capability of decreasing plasma and hepatic lipid by inhibiting fatty acids and cholesterol biosynthesis (Lin *et al*., 2017). Consequently, daily faecal lipid and bile acid output increases. This may have influenced superior values observed for week 16 (T_2) in this study (Table 7.11 7.12 and 7.13). However, it is noteworthy that low HDL on the other hand can be caused by increased oestrogen levels (Regar *et al.*, 2019).

Dietary supplementation of noni fruit extract at week 16 or 20 reduced blood glucose levels but within the normal physiological range (Table 7.11, 7.12 and 7.13) which agrees with results reported by Mhatre and Marar (2016). That noni fruit extract inhibited the toxic effects (hypoinsulinemia and hepatotoxicity) of methotrexate thus restoring elevated glucose levels to normal physiological levels in adult male albino rats of Wistar strain. The metabolism of carbohydrates is assessed by the decreased glucose levels in the serum of

chicken which indicates its increased consumption as an energy component for metabolic processes associated with the intensive growth and production of layers (Kuzmina *et al.*, 2021). Pu *et al.* (2004) reported that the hypoglycaemic effects of the two anthraquinonoid molecules, damnacanthal-3-O-beta-D-primeveroside and lucidin 3-O-beta-D-primeveroside were responsible for the glucose-lowering effect of noni fruit extract.

7.6 Conclusion

- The timing of administering 40 mL/mL of Noni fruit extract significantly affects the pre-laying performance, internal organs, egg quality, and blood metabolite profiles of the birds.
- Administering noni fruit extract at week 16 appeared to be more beneficial in terms of body weight gain, feed conversion, egg freshness, erythropoiesis, blood oxygen capacity, immunomodulation, and organ function compared to administration at week 20 or in control groups.
- Noni fruit extract is a promising natural feed additive for enhancing the health and productivity of laying hens, especially in older birds. However, it is important to administer the extract before the laying period to effectively support the development of the reproductive system and improve laying performance.

CHAPTER 8

8.1 General discussion

Noni fruit extract yield and biochemical properties varied significantly across fermentation stages. Extract yields increased to 580 ml/kg at weeks 8 and 12, compared to 397 ml/kg at week 2 and 440 ml/kg at week 4. The lower yields at earlier stages were due to less fruit tissue disintegration, but extended fermentation broke cellular structures down, releasing vital compounds. Nelson and Elevitch (2006) reported that yield outcomes depended on extraction methods like pressing or blending and factors such as fruit ripeness and processing temperature. The 40-50 % fruit extract recovery from original fruit weight by Nelson and Elevitch (2006) points to opportunities for maximising extraction, especially through processing residual pulp for additional fluids. The increase in the antioxidant properties of the noni fruit extract at weeks 8 and 12 compared to weeks 2 and 4, may be attributed to the growth of microorganisms, particularly Acetobacter and Gluconobacter, which transformed the flavonoids present in the noni fruit extract thereby increasing the concentration of antioxidants like quercetin and kaempferol, as reported by Zhang *et al*. (2021) and Jakfar *et al.* (2023). The consistent augmentation of bioactive compounds across various maturation stages, as reported by Almeida *et al.* (2019) and Thomson (2011), highlights the important role of fermentation in maximizing the health benefits derived from mature noni fruit.

Administering noni fruit extract in drinking water revealed a noteworthy trend regarding feed intake. An increase in the concentration of noni fruit extract has been associated with a decrease in feed intake, attributed to the appetite-suppressing properties of the extract (Singh, 2012; Aroche *et al.*, 2018; Asmara *et al.*, 2019). This suggests that noni fruit extract may influence feeding behaviours by stimulating the secretion of cholecystokinin (CCK), a hormone that plays a crucial role, in regulating digestive processes and signalling feelings of

fullness (Pu *et al.*, 2004). Consequently, this modulation can lead to slower gastric emptying and reduced feed consumption. Despite the decrease in feed intake, the results in this study indicated increased weight gain in birds consuming noni fruit extract. This may probably have been due to the nutrient-rich profile of noni fruit, extract (amino acids, vitamins, minerals, coenzymes, polysaccharides and alkaloids) which enhanced metabolic activity, cell and tissue growth including protein accretion during muscle development. These observations align with earlier studies (Sunder *et al.*, 2011a; 2011b; 2013; 2015a; 2016; Dumoulin, 2018; Javid *et al.,* 2022) indicating significant weight gain, increased uterus development, and bone morphometric characteristics that may be linked to enhanced nutrient availability and assimilation. The increased intake of noni fruit extract resulted in a reduction in abdominal fat in the current study suggesting that it may possess anti-obesity properties, which decreased adipose tissue accumulation and improved lipid profiles without any adverse effects. Notably, the serum triglyceride levels remained within the normal physiological range, indicating that energy availability was not compromised (Nishioka, 2007; Clinical Diagnostics Division, 1990; Jambocus *et al.* 2017).

The maturity of the chickens' reproductive organ initiates around 16 weeks, peaking by week 18 although uterine size continues to increase till week 40 as observed by Barua (2021). . The day-to-first egg is an important metric for assessing sexual maturity in chickens (Guni *et al.*, 2021). Olawumi (2011) noted that heavier birds generally lay eggs earlier while Bornstein *et al.* (1984) reported that a high degree of fatness due to an accelerated rate of fat accumulation appeared to be associated with the start of ovulation and may shorten the transition period from pullet to layer. The current study found that noni-treated birds with higher weights but less abdominal fat deposits due to the effect of administration of noni fruit extract, experienced a three-day delay in reaching sexual maturity. However, this delay was

compensated for by the larger egg size and weight observed in the noni-treated groups T_2 (20 mg/ml) and T_3 (40 mg/ml) compared to the control group T_1 (0 mg/ml). This effect could be attributed to the continued stimulation of uterine development and protein accretion by proxeronine, and the anti-dyslipidemia activity of the noni fruit extract (Ali *et al.*, 2016; Dumoulin, 2018; Yin *et al*., 2020).

During the 4-week storage period, eggs lost weight in the control and noni fruit extract treatments due to the loss of moisture and carbon dioxide from the albumen, resulting in decreased Haugh units, and yolk index. This deterioration compromised the vitelline membrane, leading to increased water content in the yolk and a flatter yolk, which decreased the yolk index (Scott and Silversides, 2000). Furthermore, the increased permeability of the vitelline membrane facilitated the mixing of albumen proteins and yolk contents, resulting in paler yolk colour and mottling (Chukwuka *et al.*, 2011a). Despite the decline in Haugh units it retained a B grade (regular) status in the noni fruit extract treatment in the current study. This positive outcome observed in the Haugh unit could be attributed to the polysaccharide and mineral components present in noni fruit extract, which effectively aided in preserving the egg's internal colloid homeostatic balance (Lohani *et al.*, 2019).

The outcome of the haematological parameters in this study has highlighted the important role of FBC (Full Blood Count) as an indicator of physiological responses in commercial laying birds, especially when considering the dietary intervention using noni fruit extract. While no significant changes were observed in the overall red blood cell (RBC) counts in response to the noni fruit extract, its ability to affect the RBC differential parameters suggests it may enhance avian health and productivity. These findings are consistent with previous research (Elagib and Ahmed, 2011; Graczyk, 2016; Sidharthan, 2023) indicating that

environmental and dietary factors can influence blood parameters, particularly during the birds' reproductive cycle when physiological demands are heightened. The increased Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) during peak laying periods, highlight the importance of adequate oxygen transportation for supporting reproductive performance. This suggest the potential application of noni fruit extract, not only in maintaining health but also in improving laying efficiency during critical periods. Additionally, the observed anti-thrombocytosis effect could have implications for managing blood viscosity and overall avian health, although the underlying mechanisms have yet to be fully understood. The observed decrease in white blood cell differentials which fell within the normal physiological reference range for poultry suggests a potential immunomodulatory effect, indicating enhanced immune response or reduced stress. The current study emphasises that while certain parameters may fluctuate significantly in response to treatment, it is crucial to keep overall values within physiological norms to ensure the flock's well-being and productivity. This finding is particularly significant in poultry production, as stress can markedly influence egg production and overall health of birds. The reduction in concentration of liver enzymes, particularly AST and ALT, in noni-fed groups but within the normal physiological range reported for poultry indicated improved liver health, supporting previous studies (West *et al.*, 2009; Mhatre and Marar, 2016; Tellez, 2018; Lohani *et al*., 2019;) on noni fruit extract's hepatoprotective effects.

Maintained levels of total protein and albumin suggest that noni supplementation aids nutritional balance and immune function without causing hyperproteinemia. Additionally, the current study shows that noni extract may enhance kidney function, as evidenced by decreased urea and uric acid levels, promoting renal efficiency. The positive impact on lipid profiles, with lower total cholesterol and higher HDL levels, suggests that incorporating noni

into the diet could enhance nutrient absorption and mitigate metabolic disorders in commercial laying birds and poultry in general. Additionally, administering noni fruit extract at week 16 was more beneficial in terms of body weight gain, feed conversion ratio, net feed efficiency index, egg freshness, erythropoiesis and blood oxygen-carrying capacity, immunomodulation, and liver and kidney function as well as, antidyslipidemic activity, compared to administering at week 20. Overall, the findings position noni fruit extract as a beneficial dietary supplement for commercial laying hens.

CHAPTER 9

9.1 General conclusion

- Feeding noni fruit extract improved pre-lay sexual development performance (final body weight, weight gain), some internal organs affecting egg production (weight of uterus, medullary bone growth and mineralisation characteristics) during early-lay period in commercial layer pullets with the 40mg/ml concentration achieving the most desirable results.
- Administration of noni fruit extract in drinking water improved egg production performance (egg mass, HDEP %, FCR, NFEI), and egg quality characteristics (albumin height, Haugh unit, estimated eggshell thickness, yolk index, yolk colour and egg mass) during the early to late lay period with the 40 mg/ml showing more promise.
- Inclusion of noni fruit extract in drinking water did not adversely affect the physiology and health of the birds. The haematological and serum biochemical profile determined remained within the normal physiological reference range for poultry, improvements were observed in erythropoiesis, blood oxygen carrying capacity, liver and kidney function, and antidyslipidemic activity.

Overall, the results show that noni fruit extract is a potential beneficial feed supplement that could enhance the productivity and health of poultry.

9.2 Recommendations

Based on the results from the studies in this thesis, the following are recommended for future studies:

First, comprehensive studies should be conducted to clarify the biochemical and physiological mechanisms through which noni fruit extract exerts its beneficial effects on laying hens, especially regarding its antioxidant properties and the maintenance of egg quality.

Second, the effects of varying dosages of noni fruit extract beyond the 40 mg/ml concentration should be explored to determine the best range for maximising health benefits and production performance without negative impacts.

Third, the possible interactions of noni fruit extract supplementation with other feed components or medications on the overall health, reproductive performance, and longevity of laying hens at different physiological stages should be investigated.

Fourth, research should be expanded to evaluate if noni fruit extract supplementation also affects the growth performance and meat quality of layer chickens, thereby providing a comprehensive view of its benefits.

Finally, an economic analysis should be performed to determine the cost-effectiveness of incorporating noni fruit extract as a commercial product into poultry diets compared to traditional feed ingredients, including its impact on overall on-farm profitability.

These recommendations should further establish the role of noni fruit extract in sustainable and health-focused poultry production.

10.0 REFERENCES

Abd El-Hack, M. E., Alagawany, M., Mahrose, K. M., Arif, M., Saeed, M., Arain, M. A., Soomro, R. N., Siyal, F. A., Fazlani, S. A. & Fowler, J. (2019). Productive performance, egg quality, heamatological parameters and serum chemistry of laying hens fed diets supplemented with certain fat-soluble vitamins, individually or combined, during summer season. *Animal Nutrition*, *5*(1), 49–55. https://doi.org/10.1016/j.aninu.2018.04.008

Abdel-Wareth, A. A. A. (2016). Effect of dietary supplementation of thymol, synbiotic and their combination on performance, egg quality and serum metabolic profile of Hy-Line Brown hens. *British Poultry Science*, *57*(1), 114–122. https://doi.org/10.1080/00071668.2015.1123219

Abdel-Wareth, A. A. A. & Lohakare, J. D. (2014). Effect of dietary supplementation of peppermint on performance, egg quality, and serum metabolic profile of Hy-Line Brown hens during the late laying period. *Animal Feed Science and Technology*, *197*, 114–120. https://doi.org/10.1016/j.anifeedsci.2014.07.007

Abdel-Wareth, A. A. A. & Lohakare, J. D. (2020). Productive performance, egg quality, nutrients digestibility, and physiological response of bovans brown hens fed various dietary inclusion levels of peppermint oil. *Animal Feed Science and Technology*, *267*(April), 114554. https://doi.org/10.1016/j.anifeedsci.2020.114554

Abdelli, N., Solà-Oriol, D. & Pérez, J. F. (2021). Phytogenic feed additives in poultry: Achievements, prospective and challenges. *Animals*, *11*(12). https://doi.org/10.3390/ani11123471

Abel, C. & Busia, K. (2005). An exploratory ethnobotanical study of the practice of herbal medicine by the Akan peoples of Ghana. *Alternative Medicine Review*, *10*(2), 112–122.

Abo-Al-Ela, H. G., El-Kassas, S., El-Naggar, K., Abdo, S. E., Jahejo, A. R. & Al Wakeel, R. A. (2021). Stress and immunity in poultry: light management and nanotechnology as effective immune enhancers to fight stress. *Cell Stress and Chaperones*, *26*(3), 457–472. https://doi.org/10.1007/s12192-021-01204-6

Abo-Ghanima, M. M., Abd El-Hack, M. E., Al-Otaibi, A. M., Nasr, S., Almohmadi, N. H., Taha, A. E., Jaremko, M. & El-Kasrawy, N. I. (2023). Growth performance, liver and kidney functions, blood hormonal profile, and economic efficiency of broilers fed different levels of threonine supplementation during feed restriction. *Poultry Science*, *102*(8), 1–8. https://doi.org/10.1016/j.psj.2023.102796

Abou Assi, R., Darwis, Y., Abdulbaqi, I. M., khan, A. A., Vuanghao, L. & Laghari, M. H. (2017). Morinda citrifolia (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials. *Arabian Journal of Chemistry*, *10*(5), 691–707. https://doi.org/10.1016/J.ARABJC.2015.06.018

Addass, P. A., David, D. L., Edward, A., Zira, K. E. & Midau, A. (2012). Effect of age, sex and management system on some haematological pparaeters of intensively and semi-intensiely kept chicken in Mubi, Adamawa State, Nigeria. *Iranian Journal Of Applied Animal Science*, *2*(3), 277–282.

Adei, D. & Asante, B. K. (2012). The challenges and prospects of the poultry industry in Dormaa district. *Journal of Science and Technology (Ghana)*, *32*(1). https://doi.org/10.4314/just.v32i1.11

Adriani, L., Indrayati, N., Rusmana, D., Hernawan, E. & Rochana, A. (2017). Effect of noni (Morinda citrifolia) fruit flour on antioxidant status and hematological indices of laying Japanese quail. *International Journal of Poultry Science*, *16*(3), 93–97. https://doi.org/10.3923/ijps.2017.93.97

Agyarko, C. (2013). *Effect of Direct-Fed Microbial (DFM) As Administered In Water on the Health and Performance of Poultry in Hot Humid Environment*. Kwame Nkrumah University of Science and Technology Kumasi.

Ahlawat, S. P. S., Sunder, J., Kundu, A., Chatterjee, R. N., Rai, R. B., Kumar, B., Senani, S., Saha, S. K. & Yadav, S. P. (2004). Use of RAPD-PCR for genetic analysis of Nicobari fowl of Andamans. *British Poultry Science*, *45*(2), 194–200. https://doi.org/10.1080/00071660410001715795

Akbari Moghaddam Kakhki, R., Golian, A. & Zarghi, H. (2016). Effect of digestible methionine + cystine concentration on performance, egg quality and blood metabolites in laying hens. *British Poultry Science*, *57*(3), 403–414. https://doi.org/10.1080/00071668.2016.1173199

Al-Mamun. (2020). Blood Biochemical Parameters in chickens: A review. *Journal of Poultry Science*.

Al-Obaidi, F. A., Al-Shadeedi, S. M. J. & Al-Dalawi, R. H. (2011). Quality, chemical and microbial characteristics of table eggs at retail stores in Baghdad. *International Journal of Poultry Science*, *10*(5), 381–385. https://doi.org/10.3923/ijps.2011.381.385

AL-Saegh, H. A. H. (2018). Hemato-Biochemical and Histopathological Changes Caused by Coccidiosis in broilers. *Kufa Journal For Veterinary Medical Sciences*, *9*(1). https://doi.org/10.36326/kjvs/2018/v9i14092

Algenstaedt, P., Stumpenhagen, A. & Westendorf, J. (2018). The Effect of Morinda citrifolia L. fruit juice on the blood sugar level and other serum parameters in patients with diabetes Type 2. *Evidence-Based Complementary and Alternative Medicine*, *2018*. https://doi.org/10.1155/2018/3565427

Ali, M., Kenganora, M. & Manjula, S. N. (2016). Health benefits of morinda citrifolia (Noni): A review. *Pharmacognosy Journal*, *8*(4), 321–334. https://doi.org/10.5530/PJ.2016.4.4

Almeida-Souza, F., de Souza, C. da S. F., Taniwaki, N. N., Silva, J. J. M., de Oliveira, R. M., Abreu-Silva, A. L. & Calabrese, K. da S. (2016). Morinda citrifolia Linn. fruit (Noni) juice induces an increase in NO production and death of Leishmania amazonensis amastigotes in peritoneal macrophages from BALB/c. *Nitric Oxide - Biology and Chemistry*, *58*, 51–58. https://doi.org/10.1016/j.niox.2016.06.004

Almeida, É. S., de Oliveira, D. & Hotza, D. (2019). Properties and applications of Morinda citrifolia (Noni): A review. *Comprehensive Reviews in Food Science and Food Safety*, *18*, 883–909. https://doi.org/10.1111/1541-4337.12456

Amad, A. A., Männer, K., Wendler, K. R., Neumann, K. & Zentek, J. (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poultry Science*, *90*(12), 2811–2816. https://doi.org/10.3382/ps.2011-01515

Ames, B. N., Shigenaga, M. K. & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(17), 7915–7922. https://doi.org/10.1073/pnas.90.17.7915

Amoah, K. O., Boateng, M., Atuahene, P. Y., Frimpong, Y. O. & Okai, D. B. (2022). Incidence of blood and meat spots in eggs from a commercial poultry farm. *Ghana Journal of Science*, *63*(1), 90–96. https://doi.org/10.4314/gjs.v63i1.7

Anene, D. O., Akter, Y., Thomson, P. C., Groves, P. & O'shea, C. J. (2020). Variation and association of hen performance and egg quality traits in individual early-laying isa brown hens. *Animals*, *10*(9), 1–14. https://doi.org/10.3390/ani10091601

Antwi, D., Apike, I. A., Antwi, Adwoa Anima L., Frimpong Manso, J., Samed Muntaka, A. & Owusu-Bio, K. M. (2025). Reducing food losses in agri-food supply chains; a multi-faceted approach. *International Journal of Logistics Research and Applications*, 1–20. https://doi.org/10.1080/13675567.2025.2450368

Applegate, T. J., Klose, V., Steiner, T., Ganner, A. & Schatzmayr, G. (2010). Probiotics and phytogenics for poultry: Myth or reality? *Journal of Applied Poultry Research*, *19*(2), 194–210. https://doi.org/10.3382/japr.2010-00168

Aroche, R., Martínez, Y., Ruan, Z., Guan, G., Waititu, S., Nyachoti, C. M., Más, D. & Lan, S. (2018). Dietary inclusion of a mixed powder of medicinal plant leaves enhances the feed efficiency and immune function in broiler chickens. *Journal of Chemistry*, *2018*. https://doi.org/10.1155/2018/4073068

Ashworth, M., Small, B., Oldfield, L., Evans, A., Greenhalf, W., Halloran, C. & Costello, E. (2021). The holding temperature of blood during a delay to processing can affect serum and plasma protein measurements. *Scientific Reports*, *11*(1), 1–11. https://doi.org/10.1038/s41598-021-85052-5

Asmara, I. Y., Widjastuti, T. & Setiawan, I. (2019). The effect of Morinda citrifolia L . (Noni) in drinking water on egg production and egg quality of Sentul Chickens. *World Scientific News*, *136*(October), 122–133.

Assefa, A. & Tesfaye, B. (2022). Serum Biochemistry and Haematological Indices of Broiler Chickens Fed Graded Levels of Frog (Rana esculata) Meal as Replacement to Fish Meal. *Internationl Journal of Agriculture and Biosciences*, *8*(2), 89–98.

Assimakopoulos, S. F. (2011). Enterocytes' tight junctions: From molecules to diseases. *World Journal of Gastrointestinal Pathophysiology*, *2*(6), 123. https://doi.org/10.4291/wjgp.v2.i6.123

AVMA. (2020). *American Veterinary Medical Association guidelines for the euthanasia of animals: 2020 edition*.

Ayunda, N. M., Zulharmita, A. Z. & Rivai, H. (2020). Review of phytochemical and pharmacological activities of Noni (Morinda citrifolia L.). *Scholars Academic Journal of Pharmacy*, *9*(12), 340–346. https://doi.org/10.36347/sajp.2020.v09i12.003

Azi, M. M. N., Bidura, G. N. G., Astawa, P. A. & Siti, N. W. (2023). Growth and blood lipid profile of native chickens given noni juice (Morinda citifolia) via drinking water. *World Journal of Biology Pharmacy and Health Sciences*, *14*(3), 011–015. https://doi.org/10.30574/wjbphs.2023.14.3.0230

Barua, H. (2021). *Study on Anatomy of Chicken Oviduct*. Chattogram Veterinary and Animal Sciences University.

Bauer, H., Zweimueller-Mayer, J., Steinbacher, P., Lametschwandtner, A. & Bauer, H. C. (2010). The dual role of zonula occludens (ZO) proteins. *Journal of Biomedicine and Biotechnology*, *2010*(Figure 1). https://doi.org/10.1155/2010/402593

Benzo, C. A., Brown, K. I., Duke, G. E., Fedde, M. R., Glick, B., Griminger, P., Harvey, A. L., Harvey, S., Hazelwood, R. L., Johnson, A. L., Kare, M. R., Kenny, A. D., Mason, J. R., Marshall, L., Meyer, D. B., Meyer, D. C., Ringer, R. K., Scanes, C. G., Sturkie, P. O., … Whittow, G. C. (1986). Avian physiology. In P. D. Sturkie (Ed.), *Annual review of physiology* (4th ed., Vol. 37). https://doi.org/10.1146/annurev.ph.37.030175.002301

Berardinelli, A., Ragni, L., Giunchi, A., Gradari, P. & Guarnieri, A. (2008). Physical-mechanical modifications of eggs for food-processing during storage. *Poultry Science*, *87*(10), 2117–2125. https://doi.org/10.3382/ps.2007-00216

Berger, J. & Moller, D. E. (2002). The Mechanism of Action of PPARs. *Annual Review of Medicine*, *53*, 409–435. https://doi.org/https://doi.org/10.1146/annurev.med.53.082901.104018

Bhatia, R., Thapliyal, K. & Kumar, D. (2015). Phytochemical and therapeutic aspects of Morinda citrifolia L. (Noni plant): A review. *Research Journal of Pharmacognosy and Phytochemistry*, *7*(3), 167. https://doi.org/10.5958/0975-4385.2015.00028.x

Biovet, S. A. (2022). *Poultry Production In Ghana*. Veterinaria Digital S.A. https://www.veterinariadigital.com/en/articulos/poultry-production-in-ghana/

Blonska, M., Bronikowska, J., Pietsz, G., Czuba, Z. P., Scheller, S. & Krol, W. (2004). Effects of ethanol extract of propolis (EEP) and its flavones on inducible gene expression in J774A.1 macrophages. *Journal of Ethnopharmacology*, *91*(1), 25–30. https://doi.org/10.1016/J.JEP.2003.11.011

Bluett, J., Bond, D., Scott, N. & Birrell, F. (2021). Methotrexate-Information-Booklet-April2021. *Versus Arthritis*.

Bornstein, S., Plavnik, I. & Lev, Y. (1984). Body weight and/or fatness as potential determinants of the onset of egg production in broiler breeder hens. *British Poultry Science*, *25*(3), 323–341. https://doi.org/10.1080/00071668408454873

Bounous, D. & Stedman, N. (2000). Normal avian hematology: Chicken and Turkey. In B. F. Feldman, l. J. G. Zink & N. C. Jain (Eds.), *Schalm's veterinary hematology* (pp. 1147–1154). Wiley.

Bovi, W. G., Desidério, C. S., Trevisan, R. O., Santos, M. M., de Oliveira Silva, M., da Silva, M. V., Júnior, V. R., Bucek, E. U. & Oliveira, C. J. F. (2023). Exploring the potential immunomodulatory properties of Noni juice on human monocytes and macrophages. *Journal of Biologically Active Products from Nature*, *13*(2), 167–178. https://doi.org/10.1080/22311866.2023.2225480

Brand-Williams, W., Cuvelier, M. E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Brandt, L. W., Clegg, R. E. & Andrews, A. C. (1951). The effect of age and degree of maturity on the serum proteins of the chicken. *The Journal of Biological Chemistry*, *191*(1), 105–111. https://doi.org/10.1016/s0021-9258(18)50956-x

Bueno, J. P. R., Nascimento, M. R. B. de M., Martins, J. M. da S., Marchini, C. F. P., Gotardo, L. R. M., Ribeiro de Sousa, G. M., Mundim, A. V., Guimarães, E. C. & Rinaldi, F. P. (2017). Effect of age and cyclical heat stress on the serum biochemical profile of broiler chickens. *Semina:Ciencias Agrarias*, *38*(3), 1383–1392. https://doi.org/10.5433/1679-0359.2017v38n3p1383

Burkea, J. D. & Young, H. A. (2019). IFN-γ: A cytokine at the right time, is in the right place. *Semin Immunol*, *43*, 1–19. https://doi.org/10.1016/j.smim.2019.05.002.IFN-

Callejo, R. A., Cardoso, W. M. & Daza, A. A. (2010). Effect of bird age and storage system on physical properties of eggs from brown laying hens. *PUBVET*, *4*(37), 142.

Camargo, S. M. P., Oliveira, N. F. de, Cordeiro, D. A., Oliveira, H. F. de, Carvalho, D. P., Rocha, C. H. R., Racanicci, A. M. C., Carvalho, F. B. de & Stringhini, J. H. (2022). Environment type and storage period on eggshell quality of laying hens at different ages. *Ciência Rural*, *52*(1), 1–6. https://doi.org/10.1590/0103-8478cr20200908

Chan-Blanco, Y., Vaillant, F., Perez, A. M., Belleville, M.-P., Z ´u ˜niga, C. & Brat, P. (2007). The ripening and aging of noni fruits (Morinda citrifolia L.): microbiological flora and antioxidant compounds. *Journal of the Science OfFood and Agriculture*, *87*, 1710–1716. https://doi.org/10.1002/jsfa

Chaves Hernández, A. J. (2014). Poultry and Avian Diseases. *Encyclopedia of Agriculture and Food Systems*, *4*, 504–520. https://doi.org/10.1016/B978-0-444-52512-3.00183-2

Chearskul, S., Kooptiwut, S., Chatchawalvanit, S., Onreabroi, S., Churintrapun, M., Saralamp, P. & Soonthornchareonnon, N. (2004). Morinda citrifolia has very weak estrogenic activity in vivo. *Thai Journal of Physiological Sciences*, *17*(1), 22–29.

Chelakkot, C., Ghim, J. & Ryu, S. H. (2018). Mechanisms regulating intestinal barrier integrity and its pathological implications. *Experimental & Molecular Medicine*, *50*(103), 1–9. https://doi.org/10.1038/s12276-018-0126-x

Cheng, Y., Li, P., Hu, B., Xu, L., Liu, S., Yu, H., Guo, Y., Xie, Y., Yao, W. & Qian, H. (2021). Correlation analysis reveals the intensified fermentation via Lactobacillus plantarum improved the flavor of fermented noni juice. *Food Bioscience*, *43*(June), 101234. https://doi.org/10.1016/j.fbio.2021.101234

Chikumba, N., Swatson, H. & Chimonyo, M. (2013). Haematological and serum biochemical responses of chickens to hydric stress. *Animal*, *7*(9), 1517–1522. https://doi.org/10.1017/S175173111300102X

Chinenye, G., Chidera, C., Iyidiobi, S., Oscar, I., Didigwu, C. & Author, C. (2017). Haematological Parameters Dynamics of Developing Gallus Gallus Domesticus. *Animal Research International*, *14*(2), 2769–2776.

Chukwuka, O. K., Okoli, I. C., Okeudo, N. J., Udedibie, A. B. I., Ogbuewu, I. P., Aladi, N. O., Iheshiulor, O. O. M. & Omede, A. A. (2011). Egg quality defects in poultry management and food safety. In *Asian Journal of Agricultural Research* (Vol. 5, Issue 1, pp. 1–16). https://doi.org/10.3923/ajar.2011.1.16

Chunhieng, T., Hay, L. & Montet, D. (2005). Detailed study of the juice composition of noni (Morinda citrifolia) fruits from Cambodia. *Fruits*, *60*(1), 13–24. https://doi.org/10.1051/fruits:2005008

Churchil, R. R., Omprakash, A. V., Kanagaraju, P., Daffine, S. & Pandian, C. (2019). Effect of Noni (morinda citrifolia) fruit juice on production performance of layer type Japanese quails. *Indian Veterinary Journal*, *96*(5), 14–16.

Cimrin, T., Avsaroglu, M., Tunca, R. I., Kandir, S. & Ayasan, T. (2019). Effects of the dietary supplementation of layer diets with natural and synthetic antioxidant additives on yolk lipid peroxidation and fatty acid composition of eggs stored at different temperatures and duration. *Brazilian Journal of Poultry Science*, *21*(2). https://doi.org/10.1590/1806-9061-2018-0991

Clinical Diagnostic Division, . (1990). *Veterinary Reference Guide: A Summary of Reference Intervals for use with KODAK AKTACHEM Products*. Eastman Kodak Company, Rochester.

College of Agriculture and Natural Resources, K. (2016). *Baseline Survey Report*.

Creeden, J. F., Gordon, D. M., Stec, D. E. & Hinds, T. D. (2021). The Pathology of Low Bilirubin Levels. *Endocrinology and Metabolism*, *320*(2), E191–E207.

Deng, L. Z., Mujumdar, A. S., Zhang, Q., Yang, X. H., Wang, J., Zheng, Z. A., Gao, Z. J. & Xiao, H. W. (2019). Chemical and physical pretreatments of fruits and vegetables: Effects on drying characteristics and quality attributes–a comprehensive review. *Critical Reviews in Food Science and Nutrition*, *59*(9), 1408–1432. https://doi.org/10.1080/10408398.2017.1409192

Deng, S., West, B. J., Palu, 'Afa K. & Jensen, C. J. (2011). Determination and comparative analysis of major iridoids in different parts and cultivation sources of Morinda citrifolia. *Phytochemical Analysis*, *22*(1), 26–30. https://doi.org/10.1002/pca.1246

Deng, S., West, B. J., Palu, 'Afa K. & Jensen, C. J. (2012). Phytochemical, antioxidant and toxicological investigation of Morinda citrifolia L. blossoms. *ISRN Analytical Chemistry*, *2012*, 1–5. https://doi.org/10.5402/2012/160871

Diarra, S., Amosa, F. & Lameta, S. (2019). Potential of Morinda (Morinda citrifolia L.) products as alternative to chemical additives in poultry diets. *Egyptian Journal of Veterinary Sciences*, *50*(1), 37–45. https://doi.org/10.21608/ejvs.2019.7469.1062

Ding, X., Yu, Y., Su, Z. & Zhang, K. (2017). Effects of essential oils on performance, egg quality, nutrient digestibility and yolk fatty acid profile in laying hens. *Animal Nutrition*, *3*(2), 127–131. https://doi.org/10.1016/j.aninu.2017.03.005

Du, X., Qin, P., Liu, Y., Amevor, F. K., Shu, G., Li, D. & Zhao, X. (2022). Effects of key farm management practices on pullets welfare—A Review. *Animals*, *12*(6), 1–14. https://doi.org/10.3390/ani12060729

Dumoulin, P. G. (2018). *The Importance of Bodyweight Management*. Hendrick Genetics ISA. https://www.isa-poultry.com/en/news/bodyweight-management/

Dussossoy, E., Brat, P., Bony, E., Boudard, F., Poucheret, P., Mertz, C., Giaimis, J. & Michel, A. (2011). Characterization, anti-oxidative and anti-inflammatory effects of Costa Rican noni juice (Morinda citrifolia L.). *Journal of Ethnopharmacology*, *133*(1), 108–115. https://doi.org/10.1016/j.jep.2010.08.063

Echols, M. S. (2006). Evaluating and treating the kidneys. *Clinical Avian Medicine*, 451–491.

Eke, M. O., Olaitan, N. I. & Ochefu, J. H. (2013). Effect of Storage Conditions on the Quality Attributes of Shell (Table) Eggs. *Nigerian Food Journal*, *31*(2), 18–24. https://doi.org/10.1016/s0189-7241(15)30072-2

El-Sabrout, K., Aggag, S. & Mishra, B. (2022). Advanced practical strategies to enhance table egg production. *Scientifica*, *2022*(Figure 1). https://doi.org/10.1155/2022/1393392

Elagib, H. A. A. & Ahmed, A. D. A. (2011). Comparative study on haematological values of blood of indigenous chickens in Sudan. In *Asian Journal of Poultry Science* (Vol. 5, Issue 1, pp. 41–45). https://doi.org/10.3923/ajpsaj.2011.41.45

Erhabor, O., Muhammad, H. A., Muhammad, K., Onwuchekwa, C. & B, E. N. (2021). Interpretation of Full Blood Count Parameters in Health and Disease. *Haematology International Journal*, *5*(1), 1–25. https://doi.org/10.23880/hij-16000180

Etim, N. N. (2014). Haematological Parameters and Factors Affecting Their Values. *Agricultural Science*, *2*(1), 37–47. https://doi.org/10.12735/as.v2i1p37

European Commission. (2006). Ban on antibiotics as growth promoters in animal feed enters into effect. *Regulation*, *December 2005*, 1.

FAO. (2023). *World Food and Agriculture – Statistical Yearbook 2023*. https://doi.org/10.4060/cc8166en

Filipović, N., Stojević, Z., Milinković-Tur, S., Ljubić, B. B. & Zdelar-Tuk, M. (2007). Changes in concentration and fractions of blood serum proteins of chickens during fattening. *Veterinarski Arhiv*, *77*(4), 319–326.

Firdous, D. H. & Singh, B. J. (2023). *Periosteum (Human Anatomy): Image, Functions, Diseases and Treatments*. Lybrate.Com. lybrate.com/topic/periosteum-image

Fleming, R. H., Mccormack, H. A. & Whitehead, C. C. (1998). Bone structure and strength at different ages in laying hens and effects of dietary particulate limestone, vitamin K and ascorbic acid. *British Poultry Science*, *39*(3), 434–440. https://doi.org/10.1080/00071669889024

Fulton, R. M. (2018). Causes of Normal Mortality in Commercial Egg-Laying Chickens. *Avian Diseases*, *61*(3), 289–295. https://doi.org/https://doi.org/10.1637/11556-120816-RegR

Gandi, B. R., Barnabas, J. C., Mallam, I., Aliyu, Z. I., Kaboshio, K. Y. & Yayi, J. (2009). Effects of storage duration and season on egg quality parameters of laying chicken. *Nigerian Journal of Animal Science and Technology*, *1*(2), 63–70.

Gavril, R. & Usturoi, M. G. (2012). Effect of storage time and temperature on hen egg quality. *Lucrări Ştiinţifice - Seria Zootehnie*, *57*, 221–228.

Gezahegn, T., Ashenafi, M. & Berhan, T. (2016). Evaluation of the egg production performance in Bovans brown and Koekoek Chicken Breeds under varied seasons and feeding regimes in South Wollo zone, Ethiopia. *Global Veterinaria*, *17*(4), 318–324. https://doi.org/10.5829/idosi.gv.2016.17.04.103154

Grabner, G. F., Xie, H., Schweiger, M. & Zechner, R. (2021). Lipolysis: cellular mechanisms for lipid mobilization from fat stores. *Nature Metabolism*, *3*(11), 1445–1465. https://doi.org/10.1038/s42255-021-00493-6

Graczyk, S. (2016). Examinations of hematological and metabolic changes mechanisms of acute stress in turkeys. *Electronic Journal of Polish Agricultural Universities (EJPAU) : Veterinary Medicine*, *6*(1).

Growel. (2017). *Brown Layer Poultry Farming Guide*. VDocuments Mexico. https://vdocuments.mx/brown-layer-poultry-farming-guide.html

Guni, F. S., Mbaga, S. H., Katule, A. M. & Goromela, E. H. (2021). Performance evaluation of Kuroiler and Sasso chicken breeds reared under farmer management conditions in highland and lowland areas of Mvomero district, Eastern Tanzania. *Tropical Animal Health and Production*, *53*(2), 53–59. https://doi.org/10.1007/s11250-021-02693-4

Guo, M., Mao, B., Ahmed Sadiq, F., Hao, Y., Cui, S., Yi, M., Hong, Q., Lee, Y. K. & Zhao, J. (2020). Effects of noni fruit and fermented noni juice against acute alcohol induced liver injury in mice. *Journal of Functional Foods*, *70*(December 2019), 103995. https://doi.org/10.1016/j.jff.2020.103995

Gyan, A. B. K. (2015). *The effect of Dfm (Re-3, Re-3 Plus And P3) on the growth performance, carcass characteristics, microbiological and haemato-biochemical indices of broiler chickens*. Kwame Nkrumah University of Science and Tecnology, Kumasi.

Hafez, E. S. E. & Kamar, G. A. R. (1955). Developmental Changes in the Reproductive Organs of the Domestic Fowl. *Poultry Science*, *34*(5), 1002–1010. https://doi.org/10.3382/ps.0341002

Hagan, J. K. & Apori, S. O. (2013). The egg production performance of three layer strains kept under intensivve system in te hot andd umid tropics. *Scientific Journal of Animal Science*, *2*(6), 144–149.

Hagan, J. K., Hagan, B. A., Ofori, S. A. & Etim3, N. . (2022). Haematological and serum biochemical proiles of two broiler strains fed rations with varying levels of palm kernel oil reidue. *Ghanaian Journal of Animal Science*, *13*(2), 30–41.

Halliwell, B., Zentella, A., Gomez, E. O. & Kershenobich, D. (1997). Antioxidants and human disease: A general introduction. *Nutrition Reviews*, *55*(1 II), S44–S49. https://doi.org/10.1111/j.1753-4887.1997.tb06100.x

Harnsoongnoen, S. & Jaroensuk, N. (2021). The grades and freshness assessment of eggs based on density detection using machine vision and weighing sensor. *Scientific Reports*, *11*(1), 1–12. https://doi.org/10.1038/s41598-021-96140-x

Harr, K. E. (2002). Clinical chemistry of companion avian species: A review. *Veterinary Clinical Pathology*, *31*(3), 140–151. https://doi.org/10.1111/j.1939-165X.2002.tb00295.x

Harvey, J. & Bruss, M. (1997). Clinical biochemistry of domestic animals. In A. H. Meek (Ed.), *Canadian Vetinary ournal* (5th ed., Vol. 39, Issue January, pp. 306–307). Academiic Press. https://doi.org/10.1016/B978-012396305-5/50032-4

Hasan, A. & Aylin, A. O. (2009). *Effect of storage time, temperature and hen age on egg quality in free-range layer hens*. *8*(10), 1953–1958.

Haugh, H. (1937). The Haugh Unit for Measuring Egg Quality. *The U.S. Egg & Poultry Magazine*, 552–555, 572–573.

Heinicke, R. M. (1985). The pharmacologically active ingredient of Noni. *Pacific Tropical Botanical Garden Bulletin*, *15*(1), 10–14.

Henchion, M., Moloney, A. P., Hyland, J., Zimmermann, J. & McCarthy, S. (2021). Review: Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. *Animal*, *15*, 100287. https://doi.org/10.1016/j.animal.2021.100287

Heryanto, R., Arlianti, T., Wahyuni, S. & Purwiyanti, S. (2023). Observation and morphological character of Noni (Morinda citrifolia L.) in Ciampea, Bogor Regency. *E3S Web of Conferences*, *373*, 1–7. https://doi.org/10.1051/e3sconf/202337303017

Hester, P. Y. (2017). Improving Egg Production and Hen Health with Calcium. In *Egg Innovations and Strategies for Improvements*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-800879-9.00030-5

Hong, D., Liyun, C., Fuwei, L. I., Qiaoxian, Y., Dehe, W., Rongyan, Z. & Hui, C. (2021). Research Note: Effect of age on hematological parameter and reference intervals for commercial Lohmann silver layer. *Poultry Science*, *100*(12), 101497. https://doi.org/10.1016/j.psj.2021.101497

Howarth, A. G., Hughes, M. R. & Stevenson, B. R. (1992). Detection of the tight junction-associated protein ZO-1 in astrocytes and other nonepithelial cell types. *American Journal of Physioloogy: CELL PHYSIOLOGY*, *262*(2), C461–C469. https://doi.org/https://doi.org/10.1152/ajpcell.1992.262.2.C461

Hsu, C.-L. & Yen, G.-C. (2007). Effects of Flavonoids and Phenolic Acids on the Inhibition of Adipogenesis in 3T3-L1 Adipocytes. *Journal o Agricultural Food Chemistry*, *55*(21), 8404–8410. https://doi.org/https://doi.org/10.1021/jf071695r

Huang, H. L., Liu, C. T., Chou, M. C., Ko, C. H. & Wang, C. K. (2015). Noni (Morinda citrifolia L.) Fruit Extracts Improve Colon Microflora and Exert Anti-Inflammatory Activities in Caco-2 Cells. *Journal of Medicinal Food*, *18*(6), 663–673. https://doi.org/10.1089/jmf.2014.3213

Hussain, S., Tamizhselvi, R., George, L. & Manickam, V. (2016). Assessment of the role of noni (Morinda citrifolia) juice for inducing osteoblast differentiation in isolated rat bone marrow derived mesenchymal stem cells. *International Journal of Stem Cells*, *9*(2), 221–229. https://doi.org/10.15283/ijsc16024

Inada, A. C., Figueiredo, P. S., dos Santos-Eichler, R. A., Freitas, K. de C., Hiane, P. A., de Castro, A. P. & Guimarães, R. de C. A. (2017). Morinda citrifolia Linn. (noni) and its potential in obesity-related metabolic dysfunction. *Nutrients*, *9*(6), 1–29. https://doi.org/10.3390/nu9060540

Jacob, J. P., Miles, R. D. & Mather, F. Ben. (2000). Egg Quality 1. *Animal Science*, 1–12.

Jakfar, J., Husin, H., Nasution, F. & Ahmadi, A. (2023). Development of sour flavored Noni juice using lactic acid compound as a fermentation media and optimation. *Aceh International Journa of Science Technology*, *12*(3), 345–357. https://doi.org/10.13170/aijst.12.3.34068

Jambocus, N. G. S., Ismail, A., Khatib, A., Mahomoodally, F., Saari, N., Mumtaz, M. W. & Hamid, A. A. (2017). Morinda citrifolia l. leaf extract prevent weight gain in sprague-dawley rats fed a high fat diet. *Food and Nutrition Research*, *61*(1). https://doi.org/10.1080/16546628.2017.1338919

Jang, E. (2022). Correlation between internal and external egg quality indicators in the early phase of Hy-Line Brown laying hens. *Korean Journal of Poultry Science*, *49*(2), 53–60.

Javid, M. A., Masood, S., Zaneb, H., Rehman, H. U., Nazir, M. M., Waqas, M. Y., Asif, M., Basit, M. A., Abbas, G. & Sufyan, A. B. (2022). Effect of Dietary Growth Promoter Supplementations on the Serum Biochemistry and Morphometric Characteristics of Tibia Bone in Broiler Chicken. *Revista Brasileira de Ciencia Avicola / Brazilian Journal of Poultry Science*, *24*(4). https://doi.org/10.1590/1806-9061-2021-1454

Johnson, A. S. & Merritt, E. S. (1955). Heritability of Albumen Height and Specific Gravity of Eggs from White Leghorns and Barred Rocks and the Correlations of These Traits with Egg Production. *Poultry Science*, *34*(3), 578–587. https://doi.org/10.3382/ps.0340578

Kamiya, K., Tanaka, Y., Endang, H., Umar, M. & Satake, T. (2004). Chemical constituents of Morinda citrifolia fruits inhibit copper-induced low-density lipoprotein oxidation. *Journal of Agriculture and Food Research*, *5843–5848*.

Karásková, K., Suchý, P. & Straková, E. (2015). Current use of phytogenic feed additives in animal nutrition: A review. *Czech Journal of Animal Science*, *60*(12), 521–530. https://doi.org/10.17221/8594-CJAS

Kelly, L. M. & Alworth, L. C. (2013). Techniques for collecting blood from the domestic chicken. *Lab Animal*, *42*(10), 359–361. https://doi.org/10.1038/laban.394

Kim, D. O., Jeong, S. W. & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, *81*(3), 321–326. https://doi.org/10.1016/S0308-8146(02)00423-5

Kim, D. O., Lee, K. W., Lee, H. J. & Lee, C. Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *Journal of Agricultural and Food Chemistry*, *50*(13), 3713–3717. https://doi.org/10.1021/jf020071c

Knowles, H. R., Hart, E. B. & Halpin, J. G. (1935). The Variation in the Calcium Level of the Blood of the Domestic Fowl. *Poultry Science*, *14*(2), 83–89. https://doi.org/10.3382/ps.0140083

Kokoré, A. B., Bleyere, N. M., Kamagate, S. & Yapo, P. A. (2021). Blood biochemical parameters exploration in broilers and local chickens in Korhogo, Côte d'Ivoire. *American Journal of Food and Nutrition*, *9*(2), 82–86. https://doi.org/10.12691/ajfn-9-2-4

Konsue, N., Yimthiang, S. & Kwanhian, W. (2018). Effect of fermentation conditions of noni (Morinda citrifolia L.) juice on glutathione content and lipid oxidation in vero cells. *International Food Research Journal*, *25*(4), 1534–1540.

Krishnakumar, N. M., Latha, P. G., Suja, S. R. & Rajasekharan, S. (2015). A Review on the Ethnomedicinal, Therapeutic and Nutraceutical Importance of 'Noni' (Morinda citrifolia L.). *International Journal of Medicinal Plants and Natural Products (IJMPNP)*, *1*(3), 1–14.

Krisnaningsih, A., Leondro, H., Lija, F. & Setiawan, A. A. (2023). External and internal qualities of chicken eggs early production at various storage times at room temperature. *Jurnal Ilmu Dan Teknologi Hasil Ternak*, *18*(1), 64–72. https://doi.org/10.21776/ub.jitek.2023.018.01.7

Kumar, M., Dahiya, S. P. & Ratwan, P. (2019). Backyard poultry farming in India: A tool for nutritional security and women empowerment. *Biological Rhythm Research*, *52*(10), 1476–1491. https://doi.org/https://doi.org/10.1080/09291016.2019.1628396

Kumar, N. & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, *24*, e00370. https://doi.org/10.1016/j.btre.2019.e00370

Kumari, A., Tripathi, U. K., Maurya, V. & Kumar, M. (2020). Internal quality changes in eggs during storage. *International Journal of Science, Environment and Technology*, *9*(4), 615–624.

Kuzmina, N. N., Yu Petrov, O., Semenov, V. G. & Boronin, V. V. (2021). The effect of an antioxidant on the hematological profile of birds. *IOP Conference Series: Earth and Environmental Science*, *935*(1). https://doi.org/10.1088/1755-1315/935/1/012015

Lee, M. H., Cho, E. J., Choi, E. S. & Sohn, S. H. (2016). The effect of storage period and temperature on egg quality in commercial eggs. *Korean Journal of Poultry Science*, *43*(1), 31–38. https://doi.org/10.5536/kjps.2016.43.1.31

Liao, A. P., Petrof, E. O., Kuppireddi, S., Zhao, Y., Xia, Y., Claud, E. C. & Sun, J. (2008). Salmonella type III effector AvrA stabilizes cell tight junctions to inhibit inflammation in intestinal epithelial cells. *PLoS ONE*, *3*(6). https://doi.org/10.1371/journal.pone.0002369

Lin, H. C., Tsai, S. H., Chen, C. S., Chang, Y. C., Lee, C. M., Lai, Z. Y. & Lin, C. M. (2008). Structure-activity relationship of coumarin derivatives on xanthine oxidase-inhibiting and free radical-scavenging activities. *Biochemical Pharmacology*, *75*(6), 1416–1425. https://doi.org/10.1016/j.bcp.2007.11.023

Lin, Y. L., Lin, H. W., Chen, Y. C., Yang, D. J., Li, C. C. & Chang, Y. Y. (2017). Hepatoprotective effects of naturally fermented noni juice against thioacetamide-induced liver fibrosis in rats. *Journal of the Chinese Medical Association*, *80*(4), 212–221. https://doi.org/10.1016/j.jcma.2016.10.014

Lohani, M., Majrashi, M., Govindarajulu, M., Patel, M., Ramesh, S., Bhattacharya, D., Joshi, S., Fadan, M., Nadar, R., Darien, B., Maurice, D. V., Kemppainen, B. & Dhanasekaran, M. (2019). Immunomodulatory actions of a Polynesian herb Noni (Morinda citrifolia) and its clinical applications. *Complementary Therapies in Medicine*, *47*(June), 102206. https://doi.org/10.1016/j.ctim.2019.102206

Lu, X., Kang, X., Zhan, L., Lv, C., Fan, Z., Wang, Y., Ali, R., Lv, C., Li, S. & Mu, J. (2014). Dai Huang Fu Zi Tang could ameliorate intestinal injury in a rat model of hemorrhagic shock by regulating intestinal blood flow and intestinal expression of p-VASP and ZO-1. *BMC Complementary and Alternative Medicine*, *14*, 1–9. https://doi.org/10.1186/1472-6882-14-80.

Luján, L., Lidianys, M., Iloki Assanga, Simon Bernard; Rivera-Castañeda, E. G., Gil-Salido, Armida Andrea; Acosta-Silva, Ana Lilian; Meza-Cueto, C. Y. & Rubio-Pino, J. L. (2014). Nutritional and Phenolic Composition of Morinda Citrifolia L. (Noni) Fruit at Different Ripeness Stages and Seasonal Patterns Harvested in Nayarit, Mexico. *International Journal of Nutrition and Food Sciences*, *3*(5), 421. https://doi.org/10.11648/j.ijnfs.20140305.19

Maciel, W. C., Daza, A. A., Callejo, R. A., Teixeira, R. S. de C. & Carbó, C. B. (2011). Effect of hen age, egg weight and storage system on physical properties of egg from white-egg laying hens. *PUBVET*, *5*(32).

Mandukhail, S. U. R., Aziz, N. & Gilani, A. H. (2010). Studies on antidyslipidemic effects of Morinda citrifolia (Noni) fruit, leaves and root extracts. *Lipids in Health and Disease*, *9*, 2–7. https://doi.org/10.1186/1476-511X-9-88

Marquez, U. M. L. and Sinnecker, P. (2008). Chlorophylls in foods: Sources and stability. In C. Socaciu (Ed.), *Food Colorants: Chemical and Functional Properties* (pp. 195–212). CRC Press, Boca Raton, FL.

Más-Toro, D., Martínez-Aguilar, Y., Rodríguez-Bertot, R., Betancur-Hurtado, C. & Rosabal-Nava, O. (2015). Effect of dietary supplementation with Morinda citrifolia on productivity and egg quality of laying hens. *Ciencia Y Agricultura*, *12*(2), 7. https://doi.org/10.19053/01228420.4348

Mensah, E. P., Bannor, R. K., Oppong-Kyeremeh, H. & Kyire, S. K. C. (2022). An assessment of postharvest losses to support innovation in the egg value chain in Ghana. *African Journal of Science, Technology, Innovation and Development*, *14*(4), 947–959. https://doi.org/10.1080/20421338.2021.1920679

Mhatre, B. A. & Marar, T. (2016). Protective effect of Morinda citrifolia L. (fruit extract) on methotrexate-induced toxicities—hematological and biochemical studies . *Cogent Biology*, *2*(1), 1207879. https://doi.org/10.1080/23312025.2016.1207879

Mian-Ying, W., Lutfiyya, M. N., Weidenbacher-Hoper, V., Anderson, G., Su, C. X. & West, B. J. (2009). Antioxidant activity of noni juice in heavy smokers. *Chemistry Central Journal*, *3*(1), 1–5. https://doi.org/10.1186/1752-153X-3-13

Mian-Ying, W., Nowicki, D., Anderson, G., Jensen, J. & West, B. (2008). Liver protective effects of Morinda citrifolia (Noni). *Plant Foods for Human Nutrition*, *63*(2), 59–63. https://doi.org/10.1007/s11130-008-0070-3

Mian-Ying, W. & Su, C. (2001). Cancer preventive effect of Morinda citrifolia (Noni). *Annals of the New York Academy of Sciences*, *952*, 161–168. https://doi.org/10.1111/j.1749-6632.2001.tb02737.x

Mian-ying, W., West, B. J., Jensen, C. J., Nowicki, D., Chen, S. U., Palu, A. K. & Anderson, G. (2002). *Morinda citrifolia (Noni): A literature review and recent advances in Noni research 1*. *23*(1 2), 1127–1141.

Mian-Ying, W., West, B. J., Jensen, C. J., Nowicki, D., Su, C., Palu, A. K. & Anderson, G. (2002). Morinda citrifolia (Noni): A literature review and recent advances in Noni research. *Acta Pharmacologica Sinica*, *23*(12), 1127–1141.

Minias, P. (2015). The use of haemoglobin concentrations to assess physiological condition in birds: A review. *Conservation Physiology*, *3*(1). https://doi.org/10.1093/conphys/cov007

Mintah, S. O., Archer, M.-A., Asafo-Agyei, T., Ayertey, F., Jnr, P. A.-A., Boamah, D. & Barimah, K. B. (2022). Medicinal plant use in Ghana: Advancement and challenges. *American Journal of Plant Sciences*, *13*(03), 316–358. https://doi.org/10.4236/ajps.2022.133020

Mohd Zin, Z., Abdul-Hamid, A. & Osman, A. (2002). Antioxidative activity of extracts from Mengkudu (Morinda citrifolia L.) root, fruit and leaf. *Food Chemistry*, *78*(2), 227–231. https://doi.org/10.1016/S0308-8146(01)00402-2

Mohd Zin, Z., Abdul Hamid, A., Osman, A., Saari, N. & Misran, A. (2007). Isolation and identification of antioxidative compound from fruit of mengkudu (Morinda citrifolia L.). *International Journal of Food Properties*, *10*(2), 363–373. https://doi.org/10.1080/10942910601052723

Moon, S. G., Lee, S. K., Lee, W. Do, Niu, K. M., Hwang, W. U., Oh, J. S., Kothari, D. & Kim, S. K. (2021). Effect of dietary supplementation of a phytogenic blend containing Schisandra chinensis, Pinus densiflora, and Allium tuberosum on productivity, egg quality, and health parameters in laying hens. *Animal Bioscience*, *34*(2), 285–294. https://doi.org/10.5713/ajas.20.0552

Moore, G., Knight, G. & Blann, A. (2016). *Fundamentals of Biomedical Science: HAEMATOLOGY* (2nd ed.). Oxford University Press.

Moreno, M. & Wiegand, T. J. (2014). Blood. *Encyclopedia of Toxicology: Third Edition*, *1*, 526–532. https://doi.org/10.1016/B978-0-12-386454-3.00683-7

Morrison, S. & McGee, S. L. (2015). 3T3-L1 adipocytes display phenotypic characteristics of multiple adipocyte lineages. *Adipocyte*, *4*(4), 295–302. https://doi.org/10.1080/21623945.2015.1040612

Motshakeri, M. & Ghazali, H. M. (2015). Nutritional, phytochemical and commercial quality of Noni fruit: A multi-beneficial gift from nature. *Trends in Food Science and Technology*, *45*(1), 118–129. https://doi.org/10.1016/j.tifs.2015.06.004

Mottet, A. & Tempio, G. (2017). Global poultry production: current state and future outlook and challenges. *World's Poultry Science Journal*, *73*(2), 245–256. https://doi.org/10.1017/S0043933917000071

Muralidharan, P. & Srikanth, J. (2010). Anti epileptic activity of Morinda citrifolia linn fruit extract. *E-Journal of Chemistry*, *7*(2), 612–616. https://doi.org/10.1155/2010/795804

Nabel, C. L. (2016). *Digital egg tester DET6500*. NaNABEL Co.,Ltd. 86 Morimoto-Cho Nishikujo Minami-Ku, Kyoto 601-8444 Japan. https://digitaleggtester.com/en/

Nadia, N. A. A., Bushra, S. R. Z., Layla, A. F. & Fira, M. A. (2012). Effect of coating materials (gelatin) and storage time on internal quality of chicken and quail eggs under refrigeration storage. *Poultry Science*, *32*(1), 107–115.

Net Feed Efficiency Index − As a measure of production efficiency in layers, 12 22 − 26 (testimony of R Narahari, D Kothandaraman, P Venugopalan, K Kumararaj).

Narahari, D Kothandaraman, P Venugopalan, K Kumararaj, R. (1983). Net Feed Efficiency Index - As a measure of production efficiency in layers. *Cheiron TANUVAS*, *12*(1), 22–26.

Natheer, S. E. (2012). Evaluation of antibacterial activity of Morinda citrifolia, Vitex trifolia and Chromolaena odorata. *African Journal of Pharmacy and Pharmacology*, *6*(11). https://doi.org/10.5897/ajpp11.435

Nayak, S. & Mengi, S. (2010). Immunostimulant activity of Noni (Morinda citrifolia) on T and B lymphocytes. *Pharmaceutical Biology*, *48*, 724–731. https://doi.org/https://doi.org/10.3109/13880200903264434

NCCIH. (2015). Noni. *Natural Medicines Website*, *20*(10), 17684–17719.

Nelson, S. C. (2006). Morinda citrifolia (noni). In C. R. Elevitch (Ed.), *Traditional Trees of Pacific Islands: Their Culture, Environment, and Use* (Issue April, pp. 1–18).

Nelson, S. C. & Elevitch, C. R. (2006). *Noni : the complete guide for consumers and growers*.

Newton, K. (2002). Production of noni juice and powder in Samoa. *Hawai'i Noni Conference*, 29–32.

Nishioka, A. (2007). *Health effects of Morinda citrifolia (noni) on lipid and glucose metabolism*. University of Hawaii at Manoa.

Noonan, M. (2018). *What is a Phytogenic Feed Additive?* (No. 4). Juky Newsletter. https://www.usyaks.org/wp-content/uploads/2021/05/What-Iis-a-Phytogenic-Feed-Additive.pdf

Norton, K. I., Wagreich, J. M., Granowetter, L. & Martignetti, J. A. (1996). Diaphyseal medullary stenosis (sclerosis) with bone malignancy (malignant fibrous histiocytoma): Hardcastle syndrome. *Pediatric Radiology*, *26*(9), 675–677. https://doi.org/10.1007/BF01356833

Nti, F. K. (2018). *Economic Analysis of Ghana's Chicken Industry*. KANSAS STATE UNIVERSITY Manhattan, Kansas.

Oketch, E. O., Wickramasuriya, S. S., Oh, S., Choi, J. S. & Heo, J. M. (2023). Physiology of lipid digestion and absorption in poultry: An updated review on the supplementation of exogenous emulsifiers in broiler diets. *Journal of Animal Physiology and Animal Nutrition*, *107*(6), 1429–1443. https://doi.org/10.1111/jpn.13859

Okiki, P. & Ahmed, O. (2017). Preservation of Quality of Table Eggs Using Vegetable Oil and Shea Butter. *International Letters of Natural Sciences*, *63*(May), 27–33. https://doi.org/10.18052/www.scipress.com/ilns.63.27

Olawumi, S. O. (2011). Study on Pre-laying Characteristics of Three Breeds of Commercial Layers in the Derived Savannah Zone of Nigeria. *Pakistan Journal of Biological Sciences*, *14*(23), 1061–1056. https://doi.org/10.3923/pjbs.2011.1061.1065

Olgun, O. (2016). The effect of dietary essential oil mixture supplementation on performance, egg quality and bone characteristics in laying hens. *Annals of Animal Science*, *16*(4), 1115–1125. https://doi.org/10.1515/aoas-2016-0038

Olomu, J. M. (2021). Effects of Traditional Methods of Storage on Egg Quality. *Nigerian Journal of Animal Production*, *2*(2), 182–187. https://doi.org/10.51791/njap.v2i2.2155

Onumah, E. E. & Ayeduvor, S. (2023). Dynamics of the Poultry Market in Ghana. *Journal of Agricultural Sciences (Belgrade)*, *68*(4), 489–506. https://doi.org/10.2298/JAS2304489O

Onyango, E. M., Hester, P. Y., Stroshine, R. & Adeola, O. (2003). Bone densitometry as an indicator of percentage tibia ash in broiler chicks fed varying dietary calcium and phosphorus levels. *Poultry Science*, *82*(11), 1787–1791. https://doi.org/10.1093/ps/82.11.1787

Orban, Z., Remaley, A. T., Sampson, M., Trajanoski, Z. & Chrousos, G. P. (1999). The differential effect of food intake and β-adrenergic stimulation on adipose-derived hormones and cytokines in man. *Journal of Clinical Endocrinology and Metabolism*, *84*(6), 2126–2133. https://doi.org/10.1210/jcem.84.6.5747

Osei-Amponsah, R., Kayang, B. B., Manu, H. & Naazie, A. (2014). Egg quality traits of local Ghanaian chickens and influence of storage period. *Animal Genetic Resources/Ressources Génétiques Animales/Recursos Genéticos Animales*, *55*(February 2017), 93–99. https://doi.org/10.1017/s2078633614000290

Özkan, G. & Bilek, S. E. (2015). Enzyme-assisted extraction of stabilized chlorophyll from spinach. *Food Chemistry*, *176*, 152–157. https://doi.org/10.1016/j.foodchem.2014.12.059

Ozougwu, J. C. (2017). Physiology of the liver. *International Journal of Research in Pharmacy and Biosciences*, *4*(8), 13–24. https://doi.org/10.1016/0002-9343(54)90342-3

Palu, A. K., Brown, A., Deng, S., Kaluhiokalani, N. & West, B. J. (2012). The effects of noni (morinda citrifolia l.) fruit juice on cholesterol levels: A mechanistic investigation and an open label pilot study. *Journal of Applied Pharmaceutical Science*, *2*(9), 025–030. https://doi.org/10.7324/JAPS.2012.2905

Pandy, V., Bonam, S. R., Raya, A. R. & Nadendla, R. R. (2020). Morinda citrifolia Linn. (Noni) fruit, antioxidant effects, and neuroprotection. In *Oxidative Stress and Dietary Antioxidants in Neurological Diseases*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-817780-8.00019-0

Paulo, J., Bueno, R., Regina, M., Mattos, B. De, Machado, J., Ferreira, C., Marchini, P., Ruggeri, L., Gotardo, M., Miranda, G. & Sousa, R. De. (2017). Effect of age and cyclical heat stress on the serum biochemical profile of broiler chickens Influência da idade e do estresse cíclico de calor no perfil bioquímico sérico em frangos de corte Resumo. *Semina: Ciencias Agrarias*, *38*(3), 1383–1392. https://doi.org/10.5433/1679-0359.2017v38n3p1383

Pazos, D. C., Jiménez, F. E., Garduño, L., López, V. E. & Cruz, M. C. (2011). Hypolipidemic effect of seed oil of Noni (Morinda citrifolia). *Natural Product Communications*, *6*(7), 1005–1008. https://doi.org/10.1177/1934578x1100600722

Perić, L., Đukić Stojčić, M. & Bjedov, S. (2017). The Effect of Storage and Age of Hens on the Quality of Table Eggs. *Advanced Research in Life Sciences*, *1*(1), 64–67. https://doi.org/10.1515/arls-2017-0011

Phillips, C. J. C., Hosseintabar-Ghasemabad, B., Gorlov, I. F., Slozhenkina, M. I., Mosolov, A. A. & Seidavi, A. (2023). Immunomodulatory effects of natural feed additives for meat chickens. *Life*, *13*, 1–16.

Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*, *63*(7), 1035–1042. https://doi.org/10.1021/np9904509

Pomaah, A. N., Abdallah, N., Kursun, K. & Baylan, M. (2023). Egg Production and Consumption: A Case Study in Teshie Municipality, Ghana Adelaide. *Osmaniye Korkut Ata Üniversity Journal of The Institute of Science and Technology*, *3*(1), 29–35.

Pu, H. F., Huang, W. J., Tseng, W. M., Wang, S. W., Liu, Y. W., Doong, M. L. & Wang, P. S. (2004). Effects of juice from Morinda citrifolia (Noni) on gastric emptying in male rats. *Chinese Journal of Physiology*, *47*(4), 169–174.

Puva, N. Č. A., Ev, V. S. Ć., Glamo, D. Č. I. Ć., Levi, J. Ć., Peri, L. Ć., Ev, V. S. Ć. & Mili, D. Ć. (2013). Beneficial effects of phytoadditives in broiler nutrition. *World Poultry Science*, *69*(March), 27–34. https://doi.org/10.1017/S0043933913000032

Qi, L., Zhao, M. cheng, Li, Z., Shen, D. hong & Lu, J. (2020). Non-destructive testing technology for raw eggs freshness: a review. *SN Applied Sciences*, *2*(6), 1–9. https://doi.org/10.1007/s42452-020-2906-x

Ramji, D. P. & Foka, P. (2002). CCAAT/enhancer-binding proteins: Structure, function and regulation. *Biochemical Journal*, *365*(3), 561–575. https://doi.org/10.1042/BJ20020508

Rath, N. C., Huff, G. R., Huff, W. E. & Balog, J. M. (2000). Factors Regulating Bone Maturity and Strength in Poultry. *Poultry Science*, *79*(7), 1024–1032. https://doi.org/10.1093/ps/79.7.1024

Regar, M. N., Tulung, B., Londok, J. J. M. R., Moningkey, S. A. E. & Tulung, Y. R. L. (2019). Blood lipid profile of broiler chicken as affected by a combination of Feed restriction and different crude fiber sources. *IOP Conference Series: Earth and Environmental Science*, *387*(1), 1–5. https://doi.org/10.1088/1755-1315/387/1/012053

Reichmann, K. G. & Connor, J. K. (1977). Influence of dietary calcium and phosphorus on metabolism and production in laying hens. *British Poultry Science*, *18*(6), 633–640. https://doi.org/10.1080/00071667708416414

Ricke, S. C., Dittoe, D. K. & Richardson, K. E. (2020). Formic acid as an antimicrobial for poultry production: A review. *Frontiers in Veterinary Science*, *7*(September). https://doi.org/10.3389/fvets.2020.00563

Roberts, H. (2001). Accra. A way forward for mental health care in Ghana? *Lancet*, *357*(9271), 1859. https://doi.org/10.1016/S0140-6736(00)05020-0

Rosen, E. D., Walkey, C. J., Puigserver, P. & Spiegelman, B. M. (2000). Transcriptional regulation of adipogenesis. *Genes & Development*, *14*, 1293–1307. https://doi.org/10837022

Saki, A. A., Aliarabi, H., Hosseini Siyar, S. A., Salari, J. & Hashemi, M. (2014). Effect of a phytogenic feed additive on performance, ovarian morphology, serum lipid parameters and egg sensory quality in laying hen. *Veterinary Research Forum : An International Quarterly Journal*, *5*(4), 287–293.

Salleh, M. N., Runnie, I., Roach, P. D., Mohamed, S. & Abeywardena, M. Y. (2002). Inhibition of low-density lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in HepG2 cells by tropical plant extracts. *Journal of Agricultural and Food Chemistry*, *50*(13), 3693–3697. https://doi.org/10.1021/jf011593f

Samarasinghe, G. H. S. A., Illeperuma, D. C. K. & Gunathilake, K. D. P. P. (2023). An assessment of the bioactive compounds and the antioxidant, anti-inflammatory, and antidiabetic potential of hydro-methanolic extracts derived from Fresh Noni (Morinda citrifolia L.) fruits growing in Sri Lanka. In M. Iriti (Ed.), *Biology and life sciences forum* (pp. 10–13). MDPI. https://doi.org/10.3390/ Foods2023-15095

Samarasiri, M. H., Chandrasiri, T. A., Wijesinghe, D. B. & Gunawardena, S. P. (2019). Antioxidant capacity and total phenolic content variations against Morinda citrifolia L. fruit jjuice production methods. *ETP International Journal of Food Engineering*, *5*(4), 293–299. https://doi.org/10.18178/ijfe.5.4.293-299

Samarasiri, M. H., Chandrasiri, T. A., Wijesinghe, D. B. & Gunawardena, S. P. (2022). *Antioxidant capacity with physical property variations of Morinda citrifolia L. jjuice in traditional fermentation*. 17. https://doi.org/10.3390/iocag2022-12177

Sang, S., Cheng, X., Zhu, N., Stark, R. E., Badmaev, V., Ghai, G., Rosen, R. T. & Ho, C. T. (2001). Flavonol glycosides and novel iridoid glycoside from the leaves of Morinda citrifolia. *Journal of Agricultural and Food Chemistry*, *49*(9), 4478–4481. https://doi.org/10.1021/jf010492e

Sarfo, G. K., Hamidu, J. A., Larbi, A. & Donkoh, A. (2019). Effect of direct fed microbial in layer diets, on egg laying performance and health response of indigenous Ghanaian Guinea fowls (Numida meleagris). *Poultry Science*, *98*(1), 227–235. https://doi.org/10.3382/ps/pey352

Scanes, C. G. (2022). Chapter 17 - Blood. In *Sturkie's Avian Physiology* (Seventh, pp. 293–326). Elsevier Inc. https://doi.org/10.1016/B978-0-12-819770-7.00044-X

Scott, T. A. & Silversidest, F. G. (2000). The effect of storage and strain of hen on egg quality. *Poultry Science*, *79*(12), 1725–1729. https://doi.org/10.1093/ps/79.12.1725

Selaledi, L. A., Hassan, Z. M., Manyelo, T. G. & Mabelebele, M. (2020). The current status of the alternative use to antibiotics in poultry production: An African perspective. *Antibiotics*, *9*(9), 1–18. https://doi.org/10.3390/antibiotics9090594

Seo, I. H. & Lee, Y. J. (2022). Usefulness of complete blood count (CBC) to assess cardiovascular and metabolic diseases in clinical settings: A comprehensive literature review. *Biomedicines*, *10*(11). https://doi.org/10.3390/biomedicines10112697

Shane. (2024). *Per Capita Egg Consumption by Country*. World Population Review. https://worldpopulationreview.com/country-rankings/egg-consumption-by-country

Shoeb, A., Alwar, M. C., Shenoy, P. J. & Gokul, P. (2016). Effect of morinda citrifolia (Noni) fruit juice on high fat diet induced dyslipidemia in rats. *Journal of Clinical and Diagnostic Research*, *10*(4), FF06-FF10. https://doi.org/10.7860/JCDR/2016/17900.7650

Sidharthan, C. (2023). *What is Hematology ?* News Medical: Life Sciences. https://www.news-medical.net/health/What-is-Hematology.aspx#4

Silveira, P., DaMatta, R. A. & Dagosto, M. (2009). Hematological changes of chickens experimentally infected with Plasmodium (Bennettinia) juxtanucleare. *Veterinary Parasitology*, *162*(3–4), 257–262. https://doi.org/10.1016/j.vetpar.2009.03.013

Silversides, F. G. & Scott, T. A. (2001). Effect of storage and layer age on quality of eggs from two lines of hens. *Poultry Science*, *80*(8), 1240–1245. https://doi.org/10.1093/ps/80.8.1240

Sina, H., Dramane, G., Tchekounou, P., Assogba, M. F., Chabi-Sika, K., Boya, B., Socohou, A., Adjanohoun, A. & Baba-Moussa, L. (2021). Phytochemical composition and in vitro biological activities of Morinda citrifolia fruit juice. *Saudi Journal of Biological Sciences*, *28*(2), 1331–1335. https://doi.org/10.1016/j.sjbs.2020.11.059

Singh, B. & Sharma, R. A. (2020). Indian Morinda species: A review. *Phytotherapy Research*, *34*(5), 924–1007. https://doi.org/10.1002/ptr.6579

Singh, R. (2012). Morinda citrifolia L. (Noni): A review of the scientific validation for its nutritional and therapeutic properties. *Journal of Diabetes and Endocrinology*, *3*(6), 77–91. https://doi.org/10.5897/jde10.006

Singh, Y. N., Ikahihifo, T., Panuve, M. & Slatter, C. (1984). Folk medicine in tonga. A study on the use of herbal medicines for obstetric and gynaecological conditions and disorders. *Journal of Ethnopharmacology*, *12*(3), 305–329. https://doi.org/10.1016/0378-8741(84)90060-6

Sonaiya, E. B. (2007). Family poultry, food security and the impact of HPAI. *World's Poultry Science Journal*, *63*(1), 132–138. https://doi.org/doi:10.1017/S0043933907001353

Sousa, B. C. De, Miguel, C. B., Francisco, W., Machado, J. R., Vinicius, M., Alvares, T., Lazo-chica, J. E., Degasperi, P., Bucek, E. U., José, C., Oliveira, F., Coutinho, B., Miguel, C. B., Francisco, W., Machado, J. R., Vinicius, M., Alvares, T., Emilio, J., Degasperi, P., … Bucek, E. U. (2017). Effects of short-term consumption of Morinda citrifolia (Noni) fruit juice on mice intestine , liver and kidney immune modulation. *Food and Agriculture Immunology*, *0105*. https://doi.org/10.1080/09540105.2017.1306492

Stadelman, W. J. & Cotterill, O. J. (Eds.). (2013). *Egg Science and Technology 4th Edition* (Fuorth). Routledge 2 Park Square, Milton Park, Abingdon, Oxon 0X14 4RN.

Stevenson, B. R., Siliciano, J. D., Mooseker, M. S. & Goodenough, D. A. (1986). Identification of ZO-I : A High Molecular Weight Polypeptide Associated with the Tight Junction (Zonula Occludens) in a Variety of Epithelia. *The Journal of Cell Biology*, *103*(September), 35–37. https://doi.org/https://doi.org/10.1083/jcb.103.3.755

Stuart, T. D., Hirst, P. M. & Warrington, I. J. (1988). Influence of orientation and position of fruiting laterals on canopy light penetration, yield, and fruit quality of 'Granny Smith'' Apple'. *Journal of American Society of Horticultural Science*, *113*(5), 693–699.

Sunder, J. (2014). Effect of Morinda citrifolia fruit juice and lactobacillus acidophilus on broiler duodenal morphology. *Advances in Animal and Veterinary Sciences*, *2*(3S), 28–30. https://doi.org/10.14737/journal.aavs/2014/2.3s.28.30

Sunder, J., Jeyakumar, S., Sujatha, T. & Kundu, A. (2013). Effect of feeding of morical (Morinda citrifolia) based herbal supplement on production and egg quality in Nicobari fowl. *Journal of Medicinal Plants Research*, *7*(40), 2999–3002. https://doi.org/10.5897/JMPR12.1120

Sunder, J., Jeyakumar, S., Sujatha, T. & Kundu, A. (2015b). Grommune: Morinda citrifolia-based herbal tonic for growth and immunity for commercial broilers. *Journal of Applied Animal Research*, *43*(2), 137–140. https://doi.org/10.1080/09712119.2014.928628

Sunder, J., Kundu, A., Singh, D. R., Jeyakumar, S. & Srivastava, R. C. (2011a). Effect of feeding of Morinda citrifolia fruit juice on growth, production and immunity of Nicobari fowl. *Indian Journal of Animal Sciences*, *81*(12), 1255–1258.

Sunder, J., Singh, D. R., Jeyakumar, S., Kundu, A. & De, A. K. (2011b). Antibacterial activity in solvent extract of different parts of Morinda citrifolia plant. *Journal of Pharmaceutical Sciences and Research*, *3*(8), 1404–1407.

Sunder, J., Sujatha, T. & Kundu, A. (2016). Effect of Morinda citrifolia in growth, production and immunomodulatory properties in livestock and poultry: a review. *Journal of Experimental Biology and Agricultural Sciences*, *4*(3S), 249–265. https://doi.org/10.18006/2016.4(3s).249.265

Sunder, J., Tamilvanan, S. & Kundu, A. (2015a). Efficacy of feeding of Morinda citrifolia fruit juice and Lactobacillus acidophilus in broiler. *Asian Journal of Animal and Veterinary Advances*, *10*(8), 352–359. https://doi.org/10.3923/ajava.2015.352.359

Tabong, P. T. N., Bawontuo, V., Dumah, D. N., Kyilleh, J. M. & Yempabe, T. (2018). Premorbid risk perception, lifestyle, adherence and coping strategies of people with diabetes mellitus: A phenomenological study in the Brong Ahafo Region of Ghana. *PLoS ONE*, *13*(6), 1–19. https://doi.org/10.1371/journal.pone.0198915

Taylor, J. (2023). *5 Simple Ways to tell if an egg is good or bad*. Healthline. https://www.healthline.com/nutrition/tell-if-eggs-are-bad

Tellez, G. (2018). *Effects of Noni-supplemented diet on intestinal tight jjunction proteins and stress biomarkers in heat-stressed broiler chickens*. 41.

Thomas, L. (2024). Haematology. In *Clinical Laboratory Diagnostics*.

TNAU. (2012). *Layer Performance Indices*. Expert System.

Toghyani, M., Toghyani, M., Shivazad, M., Gheisar, A. & Bahadoran, R. (2012). Effects of Heat Stress on Growth Performance, Carcass Traits, and Meat Lipid Oxidation of Broiler Chicks without Any Adverse Impacts on Blood Constituents. *Biological Trace Element Research*, *146*, 171–180.

Torshizi, M. A. K., Moghaddam, A. R., Rahimi, S. & Mojgani, N. (2010). Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. *British Poultry Science*, *51*(2), 178–184. https://doi.org/10.1080/00071661003753756

Tóthová, C., Nagy, O. & Kovac, G. (2016). Serum proteins and their diagnostic utility in veterinary medicine: A review. *Veterinarni Medicina*, *61*(9), 475–496. https://doi.org/10.17221/19/2016-VETMED

Tóthová, C., Sesztáková, E., Bielik, B. & Nagy, O. (2019). Changes of total protein and protein fractions in broiler chickens during the fattening period. *Veterinary World*, *12*(4), 598–604. https://doi.org/10.14202/vetworld.2019.598-604

Tramunt, B., Montagner, A., Tan, N. S., Gourdy, P., Rémignon, H. & Wahli, W. (2021). Roles of estrogens in the healthy and diseased oviparous vertebrate liver. *Metabolites*, *11*(8). https://doi.org/10.3390/metabo11080502

USDA. (2000). *USDA Egg- Grading Manual Agricultural Handbook No. 75*.

van de Braak, T. (2023). Blood and Meat Spots Found in Eggs. In *Hendrix Genetics: Layers* (p. 1,2). https://doi.org/10.3382/ps.0240363

Verma, R. K. (2014). An ethnobotanical study of plants used for the treatment of livestock diseases in Tikamgarh District of Bundelkhand, Central India. *Asian Pacific Journal of Tropical Biomedicine*, *4*(Suppl 1), S460–S467. https://doi.org/10.12980/APJTB.4.2014C1067

Veterinary Services Directorate, L.-A. (2017). *National Poultry Vaccination Schedule*.

Villafuerte, F. C., Simonson, T. S., Bermudez, D. & Leóon-Velarde, F. (2022). High-altitude erythrocytosis: mechanisms of adaptive and maladaptive responses. *Physiology*, *37*(4), 1–12. https://doi.org/10.1152/physiol.00029.2021

VSN International, L. (2009). *GenStat for Windows* (12th Edition.). Hemel Hempstead UK. Web page: Genstat.co.uk.

Vuilleumier, J. P. (1969). The 'Roche Yolk Colour Fan'—An Instrument for Measuring Yolk Colour. *Poultry Science*, *48*(3), 767–779. https://doi.org/10.3382/ps.0480767

Wardiny, T. . M. & Sinar, T. E. A. (2013). The Effect of Morinda Citrifolia L Leaf Extract on Quality of Quail Egg. *International Journal of Agriculture and Food Science Technology*, *4*(6), 607–612.

Werman, H. A. & Brown, C. G. (1986). White blood cell count and differential count. *Emergency Medicine Clinics of North America*, *4*(1), 41–58. https://doi.org/10.1016/s0733-8627(20)30981-0

West, B. J., Jensen, C. J., Westendorf, J. & White, L. D. (2006). A safety review of noni fruit juice. *Journal of Food Science*, *71*(8), 100–106. https://doi.org/10.1111/j.1750-3841.2006.00164.x

West, Brett. J., Deng, S., Isami, F., Uwaya, A. & Jensen, C. J. (2018). The potential health benefits of noni juice: A review of human intervention studies. *Foods*, *7*(4), 1–22. https://doi.org/10.3390/foods7040058

West, Brett J., Deng, S. & Jensen, C. J. (2011). Nutrient and phytochemical analyses of processed noni puree. *Food Research International*, *44*(7), 2295–2301. https://doi.org/10.1016/j.foodres.2010.09.038

West, Brett J., White, L. D., Jensen, C. J. & Palu, A. K. (2009). A double-blind clinical safety study of noni fruit juice. *Pacific Health Dialog*, *15*(2), 21–32.

Whistler, W. A. (1985). Traditional and herbal medicine in the cook islands. In *Journal of Ethnopharmacology* (Vol. 13, Issue 3, pp. 239–280). Elsevier. https://doi.org/10.1016/0378-8741(85)90072-8

Widjastuti, T., Abun, Setiawan, I. & Indrawati. (2019). Effects of addition of Noni fruit juice (Morinda Citrifolia) into drinking water on final body weight, carcass percentage, abdominal fat and meat cholesterol of Sentul Debu Chicken phase growth. *IOP Conference Series: Earth and Environmental Science*, *334*(1). https://doi.org/10.1088/1755-1315/334/1/012032

Widjastuti, Tuti., Setiawan, I., Hasbuna, A. & Asmara, I. Y. (2019). Antibacterial activity of noni fruit juice (Morinda citrifolia L) on performance and haematological indicator on Sentul chicken. *Scientific Papers. Series D. Animal Science*, *LXII*(2), 71–76.

Widjastuti, Tuti, Nurlaeni, L., Hasbuna, A., Setiawan, I., Yudaasmara, I. & Tanwiriah, W. (2023). The microencapsulation of Noni fruit extract (Morinda Citriflia L.) with Maitodextrin and its implementation as Feed additive on carcass quality and histlogy of intestines of Sentul chicken. *International Journal on Advanced Science, Engineering & Information Technology*, *13*(1), 104–109.

Wigati, D., Anwar, K., Sudarsono & Nugroho, A. E. (2017). Hypotensive activity of ethanolic extracts of Morinda citrifolia L. leaves and fruit in dexamethasone-induced hypertensive rat. *Journal of Evidence-Based Complementary and Alternative Medicine*, *22*(1), 107–113. https://doi.org/10.1177/2156587216653660

Williams, K. C. (1992). Some Factors Affecting Albumen Quality with Particular Reference to Haugh Unit Score. *World's Poultry Science Journal*, *48*(1), 5–16. https://doi.org/10.1079/WPS19920002

Windisch, W., Schedle, K., Plitzner, C. & Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science*, *86*(14), E140–E148. https://doi.org/10.2527/jas.2007-0459

Yang, J., Gadi, R., Paulino, R. & Thomson, T. (2010). Total phenolics, ascorbic acid, and antioxidant capacity of noni (Morinda citrifolia L.) juice and powder as affected by illumination during storage. *Food Chemistry*, *122*(3), 627–632. https://doi.org/10.1016/j.foodchem.2010.03.022

Yang, J., Paulino, R., Janke-Stedronsky, S. & Abawi, F. (2007). Free-radical-scavenging activity and total phenols of noni (Morinda citrifolia L.) juice and powder in processing and storage. *Food Chemistry*, *102*(1), 302–308. https://doi.org/10.1016/j.foodchem.2006.05.020

Yeoman, C. J. & White, B. A. (2014). Gastrointestinal tract microbiota and probiotics in production animals. *Annual Review of Animal Biosciences*, *2*, 469–486. https://doi.org/10.1146/annurev-animal-022513-114149

Yilmazer, N., Coskun, C., Gurel-Gurevin, E., Yaylim, I., Eraltan, E. H. & Ikitimur-Armutak, E. I. (2016). Antioxidant and anti-inflammatory activities of a commercial noni juice revealed by carrageenan-induced paw edema. *Journal of Veterinary Sciences*, *19*(3), 589–595. https://doi.org/10.1515/pjvs-2016-0074

Yin, L., Chen, Q., Huang, Q., Wang, X., Zhang, D., Lin, Z., Wang, Y. & Liu, Y. (2020). Physiological role of dietary energy in the sexual maturity : clues of body size , gonad development , and serum biochemical parameters of Chinese indigenous chicken. *Poultry Science*, *102*(12), 103157. https://doi.org/10.1016/j.psj.2023.103157

Yu, Z., Yang, L., Deng, S. & Liang, M. (2020). Daidzein ameliorates LPS-induced hepatocyte injury by inhibiting inflam. *European Journal of Pharmacology*, *885*, 173399. https://doi.org/https://doi.org/10.1016/j.ejphar.2020.173399

Zaboli, G. R., Rahimi, S., Shariatmadari, F., Torshizi, M. A. K., Baghbanzadeh, A. & Mehri, M. (2017). Physiology and reproduction: Thermal manipulation during pre and post-hatch on thermotolerance of male broiler chickens exposed to chronic heat stress. *Poultry Science*, *96*(2), 478–485. https://doi.org/10.3382/ps/pew344

Zaefarian, F., Abdollahi, M. R., Cowieson, A. & Ravindran, V. (2019). Avian liver: The forgotten organ. *Animals*, *9*(2), 1–23. https://doi.org/10.3390/ani9020063

Zhang, B. & Coon, C. N. (1997). The Relationship of Various Tibia Bone Measurements in Hens. *Poultry Science*, *76*(12), 1698–1701. https://doi.org/10.1093/ps/76.12.1698

Zhang, L., Liu, S., Chen, Y., Guo, J., Li, C. & Zhang, J. (2021). Metatranscriptomic approach reveals the functional and enzyme dynamics of core microbes during noni fruit fermentation. *Food Research International*, *141*(November 2020), 109999. https://doi.org/10.1016/j.foodres.2020.109999

Zhao, G. J., Li, D., Zhao, Q., Lian, J., Hu, T. T., Hong, G. L., Yao, Y. M. & Lu, Z. Q. (2016). Prognostic value of plasma tight-junction proteins for sepsis in emergency department: An observational study. *Shock*, *45*(3), 326–332. https://doi.org/10.1097/SHK.0000000000000524

Zhao, H., Ren, L., Shen, R., Guo, S. & Peng, X. (2022). Identification of the influential odorants for the unpleasant rancid smell of ripe noni fruit (*Morinda citrifolia*). *International Journal of Food Science & Technology*, *57*(4), 2277–2284. https://doi.org/10.1111/ijfs.15578

Ziaei, H., Tonkaboni, A., Shamshiri, A. & Rezaei, N. (2021). "Oral manifestations of patients with inherited defect in phagocyte number or function" a systematic review. *Clinical Immunology*, *229*(May), 108796. https://doi.org/10.1016/j.clim.2021.108796

Zita, Lukas., Ledvinka, Z., Tumova, E. & Klesalova, L. (2012). Technological quality of eggs in relation to the age of laying hens and Japanese quails. *Revista Brasileira de Zootecnia*, *41*(9), 2079–2084. https://doi.org/10.1590/S1516-35982012000900016

Zita, Lukáš., Tůmová, E. & Štolc, L. (2009). Effects of genotype, age and their interaction on egg quality in brown-egg laying hens. *Acta Veterinaria Brno*, *78*(1), 85–91. https://doi.org/10.2754/avb200978010085

11.0 APPENDICES

APPENDIX 1: Effect of noni fruit extract by physiological stage interaction of birds on egg production performance indices

Physiological Stage	Parameters	Treatment (Noni fruit Extract)			SEM	p-value
		T_1 (0 mg/ml)	T_2 (20 mg/ml)	T_3 (40 mg/ml)		
Early-Lay (week 22)	DFI (g)	102.00^{e}	101.16^{f}	99.5^{g}	0.020	0.001
	B.Wt. gain (g)	46.15^{c}	61.95^{b}	64.35^{a}	0.065	0,001
	Egg mass	22.97^{i}	25.75^{h}	29.19^{g}	0.054	0.001
	HDEP %	50.14^{i}	53.29^{h}	55.71^{g}	0.091	0.001
	FCR	4.45^{a}	3.92^{b}	3.41^{c}	0.007	0.001
	NFEI	67.80^{d}	86.89^{b}	94.02^{a}	0.097	0.001
Peak-Lay (week 30)	DFI (g)	110.00^{a}	109.00^{b}	108.00^{c}	0.020	0.001
	B.Wt. gain (g)	14.65^{f}	21.44^{e}	23.61^{d}	0.065	0.001
	Egg mass	40.85^{f}	48.52^{d}	54.45^{b}	0.054	0.001
	HDEP %	80.00^{e}	87.30^{c}	90.95^{a}	0.091	0.001
	FCR	2.51^{e}	2.25^{f}	1.98^{h}	0.007	0.001
	NFEI	53.35^{g}	64.18^{e}	72.28^{c}	0.097	0.001
Late-Lay (week 48)	DFI (g)	110.00^{a}	108.30^{c}	107.00^{d}	0.020	0.001
	B.Wt. gain (g)	3.33^{i}	4.17^{h}	4.28^{g}	0.065	0.001
	Egg mass	43.74^{e}	52.58^{c}	61.00^{a}	0.054	0.001
	HDEP %	72.32^{f}	84.46^{d}	89.29^{b}	0.091	0.001
	FCR	2.70^{d}	2.06^{g}	1.75^{i}	0.007	0.001
	NFEI	40.17^{i}	52.54^{h}	61.01^{f}	0.097	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM. = Standard error of means; DFI = Daily feed intake; BWt.gain = Body Weight Gain; HDEP % = Percent Hen Day Egg Production; FCR = Feed conversion ratio per kg egg mass; NFEI = Net feed efficiency Index;

APPENDIX 2 Effect of noni fruit extract by physiological stage interactions of birds on egg quality indices

Physiological Stage	Parameters	Treatment (Noni fruit Extract)			SEM	p-value
		T_1 (0 mg/ml)	T_2 (20 g/ml)	T_3 (40mg/ml)		
	Albumen height (mm)	4.47	5.22	5.40	0.105	0.647
	Haugh Unit	70.85	75.09	75.39	0.954	0.233
Early-Lay	EST (mm)	0.43	0.47	0.48	0.002	0.138
(week 22)	Yolk index (mm)	0.38	0.43	0.43	0.008	0.280
	Yolk colour	4.20	4.40	4.40	0.276	0.760
	Egg weight (g)	45.03^{g}	48.33^{f}	52.39^{e}	0.991	0.001
	Albumen height (mm)	4.25	5.07	5.27	0.105	0.647
	Haugh Unit	63.97	71.02	71.20	0.954	0.233
Peak-Lay	EST (mm)	0.42	0.47	0.47	0.002	0.138
(week 30)	Yolk index (mm)	0.36	0.39	0.39	0.008	0.280
	Yolk colour	4.20	4.30	4.50	0.276	0.760
	Egg weight (g)	54.95^{d}	55.76^{d}	59.38^{c}	0.991	0.001
	Albumen height (mm)	4.13	5.07	5.26	0.105	0.647
	Haugh Unit	61.80	67.74	67.91	0.954	0.233
Late-Lay	EST (mm)	0.42	0.47	0.47	0.002	0.138
(week 48)	Yolk index (mm)	0.34	0.38	0.39	0.008	0.280
	Yolk colour	4.20	4.40	4.70	0.276	0.760
	Egg weight (g)	56.70^{d}	63.13^{b}	67.98^{a}	0.991	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM = Standard error of means;
EST = Estimated Eggshell Thicknesses

APPENDIX 3. Analysis of variance for interaction effect of noni fruit extract by physiological stage on internal egg quality

Source of Variation	df	Albumin height	Haugh Unit	EST	Yolk index	Yolk colour	Egg weight	p-value
Treatment (Mean Square)	2	8.98	341.71	0.03	0.01	1.21	444.26	0.001
Physiological stage (Mean Square)	2	0.38	485.67	0.00	0.02	0.14^{ns}	1486.45	0.001
Treatment * Physiological stage (MS)	4	0.03^{ns}	6.51^{ns}	0.00^{ns}	0.00^{ns}	0.18^{ns}	33.61	ns
Error	77	0.06	4.55	0.00	0.00	0.38	4.91	

MS = Mean square; df = Degree of freedom; Mean square values with superscript 'ns' are not significant ($p > 0.05$), EST = Estimated eggshell thickness

APPENDIX 4 Effect of Noni fruit extract by physiological stage on haematological parameters of red blood cells and its differentials

Physiological Stage	Parameters	Reference Range	Treatment (Noni fruit Extract)			SEM	p-value
			T_1 (0mg/ml)	T_2 (20mg/ml)	T_3 (40mg/ml)		
Pre-Lay (week 16)	RBC (10^6µL)	2.5 -3.9 [1,2]	2.33^{f}	3.54^{e}	5.54^{c}	0.010	0.001
	Hb (g/dL)	7.0 – 13.0[1,2]	10.14^{de}	10.81^{de}	10.81^{de}	0.447	0,001
	PCV (%)	22.0-35.0[1,2]	33.55^{bc}	34.61^{ab}	34.61^{ab}	1.372	0.019
	MCV (fL)	90.0 – 140.0[1,2]	114.69^{b}	110.75^{d}	110.30^{d}	0.179	0.001
	MCH (pg)	33.0 - 47.0[1,2]	41.15^{a}	33.16^{b}	33.15^{d}	0.164	0.001
	MCHC (g/dL)	26.0 – 35.0[1,2]	30.24^{c}	31.23^{d}	31.22^{d}	0.036	0.001
	Trb (10^9/L)	3.0 - 33.0[1,2]	30.42^{c}	27.85^{f}	20.36^{j}	0.104	0.001
Early-Lay (week 22)	RBC (10^6µL)	2.5 -3.9 [1,2]	3.56^{e}	3.58^{d}	3.58^{d}	0.010	0.001
	Hb (g/dL)	7.0 – 13.0[1,2]	10.20^{de}	11.25^{cd}	11.29^{cd}	0.447	0.001
	PCV (%)	22.0-35.0[1,2]	31.37^{bc}	35.23^{ab}	35.42^{ab}	1.372	0.019
	MCV (fL)	90.0 – 140.0[1,2]	110.80^{d}	110.54^{d}	110.51^{d}	0.179	0.001
	MCH (pg)	33.0 - 47.0[1,2]	39.84^{b}	32.90^{d}	32.87^{d}	0.164	0.001
	MCHC (g/dL)	26.0 – 35.0[1,2]	32.51^{ab}	31.92^{c}	31.87^{c}	0.036	0.001
	Trb (10^9/L)	3.0 - 33.0[1,2]	30.48^{c}	27.31^{hi}	26.93^{i}	0.104	0.001
Peak-Lay (week 30)	RBC (10^6µL)	2.5 -3.9 [1,2]	3.62^{c}	3.73^{b}	3.73^{b}	0.010	0.001
	Hb (g/dL)	7.0 – 13.0[1,2]	9.84^{e}	12.33^{abc}	12.34^{ab}	0.447	0.001
	PCV (%)	22.0-35.0[1,2]	30.17^{c}	37.84^{a}	37.88	1.372	0.019
	MCV (fL)	90.0 – 140.0[1,2]	112.42	112.26	112.14^{a}	0.179	0.001
	MCH (pg)	33.0 – 47.0[1,2]	39.13^{c}	32.87^{d}	32.86^{d}	0.164	0.001
	MCHC (g/dL)	26.0 – 35.0[1,2]	32.65^{a}	32.59^{a}	32.59^{a}	0.036	0.001
	Trb (10^9/L)	3.0 - 33.0[1,2]	30.52^{b}	27.53^{g}	27.24^{h}	0.104	0.001
Late-Lay (week 48)	RBC (10^6µL)	2.5 -3.9 [1,2]	3.65^{c}	3.76^{a}	3.77^{a}	0.010	0.001
	Hb (g/dL)	7.0 – 13.0[1,2]	10.86^{de}	12.54^{a}	12.56^{a}	0.447	0.001
	PCV (%)	22.0-35.0[1,2]	33.31^{bc}	38.47^{a}	38.52^{a}	1.372	0.019
	MCV (fL)	90.0 – 140.0[1,2]	119.15^{a}	118.06^{a}	118.05^{a}	0.179	0.001
	MCH (pg)	33.0 - 47.0[1,2]	39.09^{c}	32.83^{d}	32.84^{d}	0.164	0.001
	MCHC (g/dL)	26.0 – 35.01,2	32.65^{a}	32.59^{a}	32.59^{a}	0.036	0.001
	Trb (109/L)	3.0 - 33.01,2	30.98^{a}	30.18^{d}	29.33^{e}	0.104	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM – Standard error of mean; RBC = Red blood cells; Hb = Haemoglobin; PCV = Packed cell volume; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; Trb = Thrombocytes; [1]Clinical Diagnostic Division (1990); [2]Bounous and Stedman (2000)

APPENDIX 5: Effect of Noni fruit extract by physiological stage on haematological parameters of white blood cells and its differentials in laying birds

Physiological Stage	Parameters	Reference Range	Treatment (Noni fruit Extract)			SEM	p-value
			T1 (0mg/ml)	T2 (20mg/ml)	T3 (40mg/ml)		
Pre-Lay (week 16)	WBC (10^9/L)	1.9 - 9.5[1]	9.58^{a}	8.97^{b}	8.39^{c}	0.116	0.001
	Heterophil (%)	29.0 - 48.7[1]	49.58^{a}	38.97^{b}	38.48	0.415	0.001
	Basophil (%)	0.0 -6.4[1]	1.01^{a}	0.85^{b}	0.22^{f}	0.017	0.001
	Eosinophil (%)	0.0 -11.5[1,2]	9.61^{a}	9.56^{a}	9.53^{a}	0.026	0.001
	Monocyte (%)	0.0 -6.5[1,2]	3.34^{a}	1.13^{cd}	1.15^{cd}	0.048	0.001
	Lymphocyte (%)	26.9 - 70.6[1,2]	71.01^{a}	61.34^{b}	60.24^{b}	0.920	0.001
Early-Lay (week 22)	WBC (10^9/L)	1.9 - 9.5[1]	8.00^{d}	7.73^{ef}	7.46^{fg}	0.116	0.001
	Heterophil (%)	29.0 - 48.7[1]	31.11^{de}	30.52^{e}	30.40^{e}	0.415	0.001
	Basophil (%)	0.0 -6.4[1]	0.40^{de}	0.35^{e}	0.26^{f}	0.017	0.001
	Eosinophil (%)	0.0 -11.5[1,2]	6.21^{b}	5.53^{c}	5.23^{c}	0.026	0.001
	Monocyte (%)	0.0 -6.5[1,2]	2.52^{b}	1.18^{cd}	1.21^{c}	0.048	0.001
	Lymphocyte (%)	26.9 - 70.6[1,2]	44.01^{c}	38.53^{f}	38.42^{f}	0.920	0.001
Peak-Lay (week 30)	WBC (10^9/L)	1.9 - 9.5[1]	7.81^{de}	7.29^{g}	7.26^{g}	0.116	0.001
	Heterophil (%)	29.0 - 48.7[1]	31.71^{d}	30.29^{e}	30.27^{e}	0.415	0.001
	Basophil (%)	0.0 -6.4[1]	0.61^{c}	0.37^{e}	0.35^{e}	0.017	0.001
	Eosinophil (%)	0.0 -11.5[1,2]	5.15^{f}	3.17^{gh}	3.13^{h}	0.026	0.001
	Monocyte (%)	0.0 -6.5[1,2]	1.06^{d}	1.04^{d}	1.04^{d}	0.048	0.001
	Lymphocyte (%)	26.9 - 70.6[1,2]	46.85^{d}	45.34^{de}	45.24^{de}	0.920	0.001
Late-Lay (week 48)	WBC (10^9/L)	1.9 - 9.5[1]	8.31^{c}	7.52^{fg}	7.49^{fg}	0.116	0.001
	Heterophil (%)	29.0 - 48.7[1]	35.59^{c}	30.54^{e}	30.45^{e}	0.415	0.001
	Basophil (%)	0.0 -6.4[1]	0.60^{c}	0.49d	0.45^{d}	0.017	0.001
	Eosinophil (%)	0.0 -11.5[1,2]	5.39^{ef}	3.23^{g}	3.19^{gh}	0.026	0.001
	Monocyte (%)	0.0 -6.5[1,2]	1.13^{cd}	1.12^{cd}	1.12^{cd}	0.048	0.001
	Lymphocyte (%)	26.9 - 70.6[1,2]	49.05^{c}	43.44^{e}	43.18^{e}	0.920	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM = Standard error of mean; WBC = White blood cells; [1]Clinical Diagnostic Division (1990); [2]Bounous and Stedman (2000)

APPENDIX 6. Analysis of variance for some haematological parameters

SV	df	RBC	Hb	PCV	MCV	MCH	MCHC	Trb	WBC,	Het	Baso	Eos	Mono	Lym	p-value
Treatment (MS)	2	1.14	23.25	211.8	13.75	504.99	0.10	253.35	5.19	219.95	1.14	42.58	8.39	356.46	0.001
Physio stge (MS)	3	7.10	17.80	53.25	382.64	30.05	18.41	35.70	12.87	968.93	1.17	95.30	3.81	2928.52	0.001
Trt*Physio (MS)	6	0.94	3.10	32.73	11.22	37.86	1.10	77.34	0.55	68.04	0.32	11.06	3.44	53.84	0.001
Error	131	0.00	0.80	7.54	0.11	0.11	0.01	0.05	0.06	0.70	0.00	0.00	0.01	3.43	

SV = Source of variation, MS = Mean square df = Degree of freedom; Physio stage = Physiological stage; Trt*Physio = Treatment by Physiological stage interaction; RBC = Red blood cells, Hb = Haemoglobin, PCV = Packed cell volume; Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; Trb = Thrombocytes; WBC = White blood cells; Het = Heterophils; Baso = Basophils, Eos = Eosinophils; Mono = Monocytes; Lym = Lymphocytes.

APPENDIX 7: Effect of Noni fruit extract and physiological stage on serum biochemical parameters (liver function) of laying birds

Physiological Stage	Parameters	Reference Range	Treatment (Noni fruit Extract)			SEM	p-value
			T_1 (0mg/ml)	T_2 (20mg/ml)	T_3 (40mg/ml)		
Pre-Lay (week 16)	AST (U/L)	9.0 - 49.0[1]	32.42[a]	32.13[b]	24.00[c]	0.027	0.001
	ALT (U/L)	10.0 -109.0[1]	38.13[a]	38.13[a]	36.31[d]	0.020	0.001
	AST/ALT	1.0 – 1.2[1]	0.83[cd]	0.81[cd]	0.66[e]	0.001	0.001
	ALP (U/L)	1.0 – 114.0[1]	40.01	40.02	40.02	0.021	0.741
	GGT (U/L)	3.0 – 19.0[1]	10.26	10.28	10.25	0.059	0.792
Early-Lay (week 22)	AST (U/L)	9.0 - 49.0[1]	32.12[b]	23.62[d]	23.62[d]	0.027	0.001
	ALT (U/L)	10.0 -109.0[1]	38.17[a]	28.14[c]	28.13[c]	0.020	0.001
	AST/ALT	1.0 – 1.2[1]	0.84[bc]	0.83[cd]	0.83[cd]	0.001	0.001
	ALP (U/L)	1.0 – 114.0[1]	40.05	40.03	40.02	0.021	0.741
	GGT (U/L)	3.0 – 19.0[1]	10.34	10.37	10.28	0.059	0.792
Peak-Lay (week 30)	AST (U/L)	9.0 - 49.0[1]	32.13[b]	23.62[d]	23.62[d]	0.027	0.001
	ALT (U/L)	10.0 -109.0[1]	38.14[a]	28.14[c]	28.13[c]	0.020	0.001
	AST/ALT	1.0 – 1.2[1]	0.84[b]	0.83[cd]	0.83[cd]	0.001	0.001
	ALP (U/L)	1.0 – 114.0[1]	40.03	40.01	40.02	0.021	0.741
	GGT (U/L)	3.0 – 19.0[1]	10.27	10.26	10.29	0.059	0.792
Late-Lay (week 48)	AST (U/L)	9.0 - 49.0[1]	32.12[b]	23.62[d]	23.58d	0.027	0.001
	ALT (U/L)	10.0 -109.0[1]	38.14[a]	28.14[c]	28.13c	0.020	0.001
	AST/ALT	1.0 – 1.2[1]	0.84[b]	0.83[cd]	0.83[cd]	0.001	0.001
	ALP (U/L)	1.0 – 114.0[1]	40.03	40.00	40.03	0.021	0.741
	GGT (U/L)	3.0 – 19.0[1]	10.25	10.25	10.29	0.059	0.792

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM. – Standard error of means; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; AST/ALT = Aspartate Aminotransferase/Alanine Aminotransferase ratio; ALP = Alkaline Phosphatase; GGT = Gamma-Glutamyl Transferase; [1]Clinical Diagnostics Division (1990),

APPENDIX 8: Effect of Noni fruit extract and physiological stage on serum biochemical parameters (liver function) of laying birds

Physiological Stage	Parameters	Reference Range	Treatment (Noni fruit Extract)			SEM	p-value
			T_1 (0mg/ml)	T_2 (20mg/ml)	T_3 (40mg/ml)		
Pre-Lay (week 16)	TP (g/l)	54.0 – 75.0[1]	68.35[b]	68.34[b]	56.36[f]	0.019	0.001
	ALB (g/l)	23.0 – 31.0[1]	24.30[e]	28.30[c]	28.37[bc]	0.043	0.001
	GLB (g/l)	0.0 – 45.0[1,2]	44.06[a]	40.05[d]	27.98[f]	0.046	0.001
	ALB/GLB	0.0 – 10.0[1,2]	0.55[f]	0.71[d]	1.01[ab]	0.003	0.001
	TB (µmol/L)	0.0 – 5.13[1]	3.25[a]	3.24[a]	2.83[b]	0.003	0.001
	DB (µmol/L)	1.0 – 2.0[1]	1.33[a]	1.32[a]	1.22[b]	0.008	0.001
Early-Lay (week 22)	TP (g/l)	54.0 – 75.0[1]	68.26[c]	56.36[f]	56.32[f]	0.019	0.001
	ALB (g/l)	23.0 – 31.0[1]	25.42[d]	28.38[bc]	28.42[ba]	0.043	0.001
	GLB (g/l)	0.0 – 45.0[1,2]	43.03[b]	27.98[f]	27.90[f]	0.046	0.001
	ALB/GLB	0.0 – 10.0[1,2]	0.61[e]	1.01[ab]	1.02[a]	0.003	0.001
	TB (µmol/L)	0.0 – 5.13[1]	3.24[a]	2.84[b]	2.84[b]	0.003	0.001
	DB (µmol/L)	1.0 – 2.0[1]	1.32[a]	1.22[b]	1.22[b]	0.008	0.001
Peak-Lay (week 30)	TP (g/l)	54.0 – 75.0[1]	68.49[a]	56.82[e]	56.83[e]	0.019	0.001
	ALB (g/l)	23.0 – 31.0[1]	25.47[d]	28.48[ab]	28.53[a]	0.043	0.001
	GLB (g/l)	0.0 – 45.0[1,2]	43.82[b]	28.40[e]	28.31[e]	0.046	0.001
	ALB/GLB	0.0 – 10.0[1,2]	0.59[e]	1.00[bc]	1.01[ab]	0.003	0.001
	TB (µmol/L)	0.0 – 5.13[1]	3.26[a]	2.86[b]	2.86[b]	0.003	0.001
	DB (µmol/L)	1.0 – 2.0[1]	1.34[a]	1.25[b]	1.25[b]	0.008	0.001
Late-Lay (week 48)	TP (g/l)	54.0 – 75.0[1]	68.22[d]	56.80[e]	56.81[e]	0.019	0.001
	ALB (g/l)	23.0 – 31.0[1]	25.46[ab]	28.54[a]	28.54[a]	0.043	0.001
	GLB (g/l)	0.0 – 45.0[1,2]	42.76[c]	28.26[e]	28.27[e]	0.046	0.001
	ALB/GLB	0.0 – 10.0[1,2]	0.60[e]	0.71[d]	1.01[ab]	0.003	0.001
	TB (µmol/L)	0.0 – 5.13[1]	3.24[a]	2.84[b]	2.84[b]	0.003	0.001
	DB (µmol/L)	1.0 – 2.0[1]	1.32[a]	1.23[b]	1.22[b]	0.008	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM – Standard error of means; TP = Total Protein; ALB = Albumin; GLB = Globulin; ALB/GLB = Albumin/Globulin ratio; TB = Total Bilirubin; TD = Direct Bilirubin; [1]Clinical Diagnostics Division (1990), [2]Harr (2002).

APPENDIX 9: Effect of Noni fruit extract and physiological stage on serum biochemical parameters (kidney function) of laying birds

Physiological Stage	Parameters	Reference Range	Treatment (Noni fruit Extract)			SEM	p-value
			T_1 (0mg/ml)	T_2 (20mg/ml)	T_3 (40mg/ml)		
Pre-Lay (week 16)	CRE (µmol/L)	0.9 - 1.8[1]	1.12[c]	1.13[c]	1.14[bc]	0.001	0.001
	Urea (mg/dL)	2.9 – 10.0[1]	5.43[a]	3.83[d]	3.77[d]	0.124	0.001
	UA (mg/dL)	1.9 – 12.5[1]	3.31[a]	3.31[a]	1.16[b]	0.007	0.001
	Ca^{2+} (mmol/L)	2.2 – 3.0[1]	2.81[b]	2.80[b]	2.70[b]	0.072	0.001
	Na^{+} (mmol/L)	139.0 - 155.0[1]	141.62	141.55	141.70	0.169	0.396
	Cl^{-} (mmol/L)	108.0 – 124.0[1]	121.67	121.64	121.62	0.059	0.451
	K^{+} (mmol/L)	3.5 – 5.2[1]	3.85[e]	5.82[f]	8.62[d]	0.007	0.001
Early-Lay (week 22)	CRE (µmol/L)	0.9 - 1.8[1]	1.12[c]	1.1[ab]	1.15[ab]	0.001	0.001
	Urea (mg/dL)	2.9 – 10.0[1]	4.74[b]	3.87[d]	3.86[d]	0.124	0.001
	UA (mg/dL)	1.9 – 12.5[1]	3.31[a]	1.15[c]	1.14[c]	0.007	0.001
	Ca^{2+} (mmol/L)	2.2 – 3.0[1]	2.86[b]	2.64[b]	2.62[b]	0.072	0.001
	Na^{+} (mmol/L)	139.0 155.0[1]	141.60	141.50	141.34	0.169	0.396
	Cl^{-} (mmol/L)	108.0 – 124.0[1]	121.63	121.66	121.68	0.059	0.451
	K^{+} (mmol/L)	3.5 – 5.2[1]	3.85[e]	9.35[c]	9.37[b]	0.007	0.001
Peak-Lay (week 30)	CRE (µmol/L)	0.9 - 1.8[1]	1.12[c]	1.16[a]	1.16[a]	0.001	0.001
	Urea (mg/dL)	2.9 – 10.0[1]	4.3[c]	3.45[e]	3.44[e]	0.124	0.001
	UA (mg/dL)	1.9 – 12.5[1]	3.30[a]	1.54[c]	1.53[c]	0.007	0.001
	Ca^{2+} (mmol/L)	2.2 – 3.0[1]	2.80[b]	2.41[c]	2.42[c]	0.072	0.001
	Na^{+} (mmol/L)	139.0 155.0[1]	141.26	141.28	141.54	0.169	0.396
	Cl^{-} (mmol/L)	108.0 – 124.0[1]	121.63	121.60	121.49	0.059	0.451
	K^{+} (mmol/L)	3.5 – 5.2[1]	3.84[e]	9.40[a]	9.41[a]	0.007	0.001
Late-Lay (week 48)	CRE (µmol/L)	0.9 - 1.8[1]	1.12[a]	1.16[a]	1.16[a]	0.001	0.001
	Urea (mg/dL)	2.9 – 10.0[1]	4.18[c]	3.24[e]	3.21[e]	0.124	0.001
	UA (mg/dL)	1.9 – 12.5[1]	3.31[a]	1.54[c]	1.53[c]	0.007	0.001
	Ca^{2+} (mmol/L)	2.2 – 3.0[1]	2.73[b]	2.40[c]	2.42[c]	0.072	0.001
	Na^{+} (mmol/L)	139.0 155.0[1]	141.07	141.14	141.17	0.169	0.396
	Cl^{-} (mmol/L)	108.0 – 124.0[1]	121.66	121.61	121.62	0.059	0.451
	K^{+} (mmol/L)	3.5 – 5.2[1]	3.86[d]	9.40[a]	9.41[a]	0.007	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM = Standard error of means; CRE = Creatinine; UA = Uric Acid; Ca^{2+} = Calcium; Na^{+} = Sodium; Cl^{-} = Chloride; K^{+} = Potassium; [1]Clinical Diagnostics Division (1990).

APPENDIX 10: Effect of Noni fruit extract and physiological stage on serum lipid and metabolic profile of laying birds

Physiological Stage	Parameters	Reference Range	Treatment (Noni fruit extract)			SEM	p-value
			T_1 (0mg/ml)	T_2 (20mg/ml)	T_3 (40mg/ml)		
Pre-Lay (week 16)	TCHOL(mmol/L)	3.34 – 7.7[1,2]	6.85[a]	6.84[a]	4.85[b]	0.005	0.001
	HDL (mg/dL)	0.0 – 10.0[1,2]	1.22[f]	1.31[d]	1.34[bc]	0.012	0.001
	LDL (mmol/L)	0.0 – 10.0[1,2]	2.61[a]	2.62[a]	2.33[c]	0.008	0.001
	VLDL (mmol/L)	0.0 – 10.0[1]	3.00[a]	2.90[c]	1.16[e]	0.014	0.001
	TG (mmol/L)	0.2 – 2.8[1]	2.07[a]	1.54[c]	1.54[c]	0.040	0.001
	GLU (mmol/L)	4.2 - 6.6[1]	4.72[a]	4.62[b]	4.64[b]	0.006	0.001
Early-Lay (week 22)	TCHOL(mmol/L)	3.34 – 7.7[1,2]	6.83[a]	4.83[b]	4.82[b]	0.005	0.001
	HDL (mg/dL)	0.0 – 10.0[1,2]	1.31[d]	1.35[ab]	1.35[ab]	0.012	0.001
	LDL (mmol/L)	0.0 – 10.0[1,2]	2.61[a]	2.22[d]	2.21[d]	0.008	0.001
	VLDL (mmol/L)	0.0 – 10.0[1]	2.90[c]	1.25[d]	1.24[de]	0.014	0.001
	TG (mmol/L)	0.2 – 2.8[1]	1.72[b]	1.48[c]	1.47[c]	0.040	0.001
	GLU (mmol/L)	4.2 - 6.6[1]	4.56[c]	4.42[d]	4.42[d]	0.006	0.001
Peak-Lay (week 30)	TCHOL(mmol/L)	3.34 – 7.7[1,2]	6.82[a]	4.83[b]	4.82[b]	0.005	0.001
	HDL (mg/dL)	0.0 – 10.0[1,2]	1.31[d]	1.35[ab]	1.36[b]	0.012	0.001
	LDL (mmol/L)	0.0 – 10.0[1,2]	2.61[a]	2.31[c]	2.30[c]	0.008	0.001
	VLDL (mmol/L)	0.0 – 10.0[1]	2.90[c]	1.16[f]	1.15[f]	0.014	0.001
	TG (mmol/L)	0.2 – 2.8[1]	1.68[b]	1.45[c]	1.45[c]	0.040	0.001
	GLU (mmol/L)	4.2 - 6.6[1]	4.56[c]	4.42[d]	4.42[d]	0.006	0.001
Late-Lay (week 48)	TCHOL(mmol/L)	3.34 – 7.7[1,2]	6.62[a]	4.83[b]	4.82[b]	0.005	0.001
	HDL (mg/dL)	0.0 – 10.0[1,2]	1.31[d]	1.35[ab]	1.38[a]	0.012	0.001
	LDL (mmol/L)	0.0 – 10.0[1,2]	2.56[b]	2.32[c]	2.31[c]	0.008	0.001
	VLDL (mmol/L)	0.0 – 10.0[1]	2.93[b]	1.16[f]	1.14[f]	0.014	0.001
	TG (mmol/L)	0.2 – 2.8[1]	1.68[b]	1.48[c]	1.48[c]	0.040	0.001
	GLU (mmol/L)	4.2 - 6.6[1]	4.56[c]	4.42[d]	4.42[d]	0.006	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM = Standard error of means; TCHOL = Total Cholesterol; HDL = High-Density Lipoprotein; LDL = Low-Density Lipoprotein; VLDL = Very Low-Density Lipoprotein; TG = Triglycerides; GLU = Glucose (GLU); [1]Clinical Diagnostics Division (1990); [2]Bueno *et al*. (2017).

APPENDIX 11: Analysis of variance for some blood biochemical indices (liver function)

SV	df	AST	ALT	AST/ALT	ALP	GGT	TP	ALB	GLB,	ALB/GLB	p-value
Treatment (MS)	2	6.28^{E+02}	6.38^{E+02}	2.24^{E-02}	0.003^{ns}	0.002^{ns}	1.19^{E+03}	114.51	2025.35	1.67	0.001
Physio stage (MS)	3	1.98^{E+02}	3.64^{E+02}	1.90^{E-02}	0.001^{ns}	0.03^{ns}	3.64^{E+02}	26.86	581.02	0.47	0.001
Trt*Physio (MS)	6	8.10^{E+02}	9.37^{E+01}	2.27^{E-02}	0.00^{ns}	0.01^{ns}	1.30^{E+02}	7.94	208.67	0.16	0.001
Error	131	2.93^{E-03}	2.02^{E+03}	3.57^{E-06}	0.00	0.02	1.59^{E+03}	0.01	0.01	0.00	

Mean square values with superscript 'ns' are not significant ($p > 0.05$); SV = Source of variation, MS = Mean square; df = Degree of freedom; Trt*Physio = Treatment by Physiological stage interaction; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; AST/ALT = Aspartate Aminotransferase/Alanine Aminotransferase ratio; ALP = Alkaline Phosphatase; GGT = Gamma-Glutamyl Transferase, TP = Total Protein; ALB = Albumin; GLB = Globulin; ALB/GLB = Albumin/Globulin ratio; TB = Total Bilirubin; TD = Direct Bilirubin.

APPENDIX 12: Analysis of variance for some blood biochemical indices (kidney function)

SV	df	CRE	Urea	UA	TB	DB	Ca^{2+}	Na^{+}	Cl^{-},	K^{+}	p-value
Treatment (MS)	2	79.40	9.48	2.70^{E+05}	1.42	0.08	0.27	0.03^{ns}	0.05^{ns}	2.52^{E+02}	0.001
Physio stage (MS)	3	1652.08	29.01	8.33^{E+05}	0.43	0.02	1.27	0.29^{ns}	0.06^{ns}	8.60^{E+01}	0.001
Trt*Physio (MS)	6	11.41	1.86	3.62^{E+05}	0.19	0.01	0.45	0.23^{ns}	0.02^{ns}	3.29^{E+01}	0.001
Error	131	0.02	0.06	3.26^{E-02}	0.00	0.00	0.02	0.15	0.02	2.45^{E-04}	

Mean square values with superscript 'ns' are not significant; SV = Source of variation; MS = Mean square; df = Degree of freedom; Physio stage = Physiological stage; Trt*Physio = Treatment by Physiological stage interaction; CRE = Creatinine; UA = Uric Acid; Ca^{2+} = Calcium, Na^{+} = Sodium; Cl^{-} = Chloride; and K^{+} = Potassium.

APPENDIX 13: Analysis of variance for some blood biochemical indices (lipid and metabolic profile)

Source of variation	df	TCHOL	HDL	LDL	VLDL	TG	Glucose	p-value
Treatment (MS)	2	3.47^{E+01}	0.04	0.84	2.68^{E+01}	0.96	0.14	0.001
Physiological stage (MS)	3	1.06^{E+01}	0.02	0.33	7.88^{E+00}	0.74	0.29	0.001
Treatment*Physiological Stage (MS)	6	4.66^{E+00}	0.01	0.12	3.46^{E+00}	0.12	0.02	0.001
Error	131	1.12^{E-04}	0.00	0.00	8.70^{E-04}	0.01	0.00	

SV = Source of variation; MS = Mean square; df = Degree of freedom; TCHOL = Total Cholesterol; HDL = High-Density Lipoprotein; LDL = Low-Density Lipoprotein; VLDL = Very Low-Density Lipoprotein; TG = Triglycerides; GLU = Glucose (GLU).

APPENDIX 14: Interaction effect of Noni fruit extract administered at weeks 16 or 20 and physiological stages (early-lay, peak-lay and late-lay) on internal organ indices

Physiological Stage	Parameters	Treatment (Noni fruit Extract)			SEM	p-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
Early-Lay (week 22)	AF (g)	31.38^{b}	19.30^{e}	24.68^{d}	0.122	0.001
	UWt (g)	37.00^{i}	53.15^{c}	37.74^{h}	0.101	0.001
	Ash (g)	3.48^{a}	2.99^{d}	3.28^{b}	0.037	0.001
	RTL (mm)	111.69^{f}	122.26^{a}	115.59^{d}	0.245	0.001
	RTDD (mm)	5.92^{g}	6.73^{e}	6.58^{f}	0.015	0.001
Peak-Lay (week 30)	AF (g)	33.65^{a}	18.25^{h}	18.63^{g}	0.122	0.001
	UWt (g)	39.86^{g}	55.18^{b}	44.83^{e}	0.101	0.001
	Ash (g)	2.69^{f}	3.42^{b}	3.41^{b}	0.037	0.001
	RTL (mm)	112.53^{e}	122.92^{a}	119.21^{c}	0.245	0.001
	RTDD (mm)	6.73^{e}	8.11^{b}	7.96^{c}	0.015	0.001
Late-Lay (week 48)	AF (g)	33.30^{c}	18.93^{f}	19.03^{f}	0.122	0.001
	UWt (g)	40.82^{f}	56.01^{a}	50.73^{d}	0.101	0.001
	Ash (g)	2.27^{e}	3.32^{c}	3.31^{c}	0.037	0.001
	RTL (mm)	112.52^{e}	122.92^{a}	122.26^{b}	0.245	0.001
	RTDD (mm)	6.94^{d}	8.15^{a}	8.12^{b}	0.015	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$); SEM = Standard error of means; AF = Abdominal Fat; UWt = Weight of uterus; ASH = Mineral content of the right tibia bone; RTL = Right tibia bone length; RTDD = Right tibia bone diaphysis diameter.
* Birds on control received 0 mg/ml Noni fruit extract from week 16 to the end of the experiment.

APPENDIX 15: Interaction effect of Noni fruit extract administered at weeks 16 or 20 and physiological stages (early-lay, peak-lay and late-lay) on egg quality indices

Physiological Stage	Parameters	Treatment (Noni fruit Extract)			SEM.	p-value
		(T_1,control) *(Week 16, 0mg/ml)	(T_2) (Week 16; 40mg/ml)	(T_3) (Week 20; 40mg/ml)		
Early-Lay (week 22)	AH (mm)	3.09^{h}	4.79^{b}	4.51^{c}	0.088	0.001
	HU (mm)	55.72^{d}	69.95^{a}	68.50^{a}	0.769	0.001
	YI (mm)	0.28^{c}	0.37^{a}	0.35^{b}	0.008	0.001
	YC	2.80^{c}	3.20^{b}	3.20^{b}	0.048	0.001
	EST (mm)	0.43^{c}	0.48^{a}	0.45^{b}	0.001	0.001
Peak-Lay (week 30)	AH (mm)	3.88^{f}	4.93^{a}	4.79^{b}	0.088	0.001
	HU (mm)	57.60^{c}	66.74^{b}	66.44^{b}	0.769	0.001
	YI (mm)	0.29^{c}	0.34^{a}	0.33^{b}	0.008	0.001
	YC	2.82^{c}	3.42^{a}	3.40^{a}	0.048	0.001
	EST (mm)	0.42^{c}	0.48^{a}	0.48^{a}	0.001	0.001
Late-Lay (week 48)	AH (mm)	3.46^{g}	4.28^{d}	4.04^{e}	0.088	0.001
	HU (mm)	52.96^{e}	66.28^{b}	66.32^{b}	0.769	0.001
	YI (mm)	0.26^{c}	0.33^{a}	0.32^{b}	0.008	0.001
	YC	2.80^{c}	3.40^{a}	3.40^{a}	0.048	0.001
	EST (mm)	0.40^{d}	0.48^{a}	0.48^{a}	0.001	0.001

Means with different superscripts within a parameter are significantly different ($p < 0.05$);
SEM = Standard error of means; AH = Albumen height; HU= Haugh Unit; YI = Yolk Index;
YC= Yolk Colour; EST= Estimated Eggshell Thickness. * Birds on control received 0mg/ml Noni fruit extract from week 16 to the end of the experiment.