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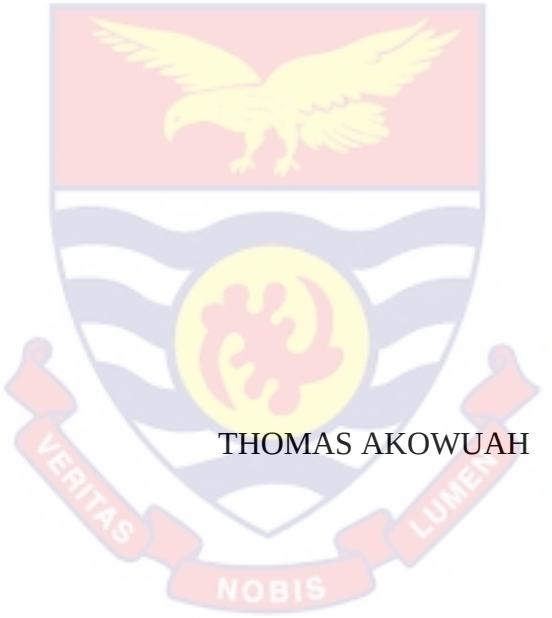
ASSESSMENT OF QUALITY OF TABLE EGGS FROM DIFFERENT LAYER STRAINS AND AGES RAISED IN DIFFERENT HOUSING SYSTEMS USING CONVENTIONAL AND NON-CONVENTIONAL (NIR) METHODS



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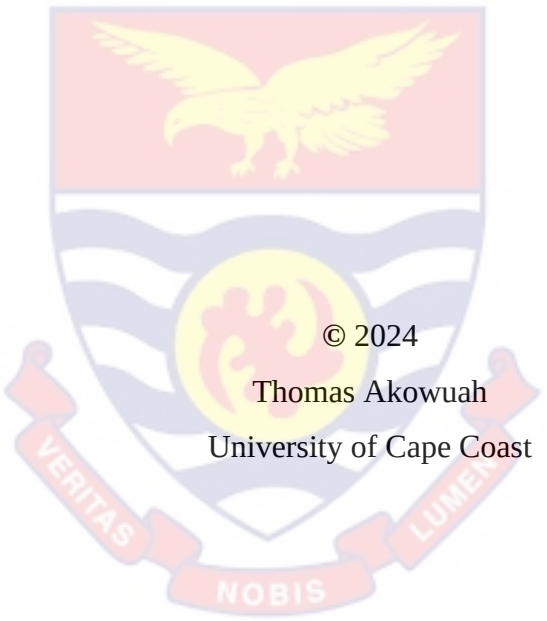

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ASSESSMENT OF QUALITY OF TABLE EGGS FROM DIFFERENT LAYER STRAINS AND AGES RAISED IN DIFFERENT HOUSING SYSTEMS USING CONVENTIONAL AND NON-CONVENTIONAL (NIR) METHODS

BY

THOMAS AKOWUAH

Thesis submitted to the Department of Animal Science of the School of Agriculture, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Animal Science (Management of Livestock Enterprise)

JUNE, 2024

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:..... Date:.....

Name: Thomas Akowuah

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of the thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:..... Date:.....

Name: Professor Julius Kofi Hagan

Co-Supervisor's Signature: ..... Date:.....

Name: Professor Ernest Teye

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ABSTRACT

The objective of this research was to assess the quality of table eggs from two different strains of layers at different ages raised in two distinct housing systems using both conventional (breaking of eggs) and non-conventional (near-infrared spectroscopy) methods. The study was done in two parts, namely: the use of conventional method and the use of non-conventional or the Near Infra-red Spectroscopy (NIR) method for the egg quality assessment. In all five (5) different experiments, namely: physical, nutritional, microbiological, organoleptic egg quality assessment and presence of antibiotic residues in eggs, were carried out. In the first phase of the experiment, a total of 2,490 freshly laid eggs from the two layer strains (Lohmann brown and Lohmann white) aged 24, 39, and 68 weeks, raised in deep litter and battery cage systems, were randomly assessed for the physical, nutritional, microbiological, and organoleptic properties of the eggs as well as the presence of antibiotic residue. The data obtained were subjected to a three-way analysis of variance using the General Analysis of Variance of GenStat (Discovery edition), with strain, housing system, and layer age as fixed factors. The second phase of the study involved the possibility of using the NIR method to predict the physical, nutritional, microbiological and presence of antibiotic residues in the egg. Results obtained from the conventional experiment showed some significant effects of the fixed factors on some of the important physical egg characteristics such as egg weight and egg freshness (Haugh unit); an indication that for improved physical characteristics of eggs, the strain of layers used, the age of the layers when eggs are laid and the type of housing system used for egg production are very important. The portable NIR spectrometer, along with qualitative algorithms, indicated that eggs stored under room temperature had a 95.54% identification rate at five principal components. On the other hand, eggs stored in cold storage had a 100% identification rate at five principal components for determining the lay date. In the independent set, PLS-R produced R = 0.87 and RMSEI = 2.57 for ambient storage, and R = 0.88 and RMSEI = 2.66 for cold storage. Data obtained from the conventional experiments showed significant strain, housing and age effects on the egg internal qualities proximate content and sensory properties of eggs but showed no significant effects on the microbiological properties of eggs. The novel handheld NIR spectroscopy was able to classify (PCA-MSC-LDA gave IR = < 95%) and predict the freshness, storage duration (MSC-PLSR of R = 0.83) and proximate content of eggs. The data from conventional experiments largely correlated with the NIR spectroscopy. The research indicated that portable NIR spectroscopy methods might be employed as a rapid, nondestructive tool for simultaneous egg examination. Moreover, distinct consideration should also be directed to protect eggs against contamination ensuring on-farm biosecurity, reducing cross-contamination, and inspection of eggs and poultry feed.

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LIST OF ABBREVIATIONS

A+Y	Albumen and Yolk
AAS	Atomic Absorption Spectroscopy
AMP	Ampicillin
ANOVA	Analysis Of Variance
AUG	Augmentin
CAM	Campylobacter
CFU	Colony Forming Unit
CIP	Ciprofloxacin
COT	Cotrimoxazole
COX	Cloxacillin
CRD	Completely Randomized Design
CRX	Cefuroxine
CVD	Cardiovascular Disease
DHA	Cardiovascular Disease
EC	Escherichia Coli
EPA	Eicosapentaenoic Acid
ERY	Erythromycin
FAO	Food and Agriculture Organization
FAY	Fayoumi
FFF	Front Face Fluorescence
FIA	Flow Injection Analysis
FIR	Far Infrared Spectroscopy
G	Grams
GEN	Gentamicin

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HDL	Dense Lipoproteins
HU	Haugh Unit
ISO	International Organization for Standardization
K	Quantity of wavelength
KCAL	Kilocalorie
LDA	Linear Discriminant Analysis
LDL	Reduced Density Lipoproteins
LIS	Listeria
LOO-CV	Leave-One-Out Cross-Validation
LSD	Least Significant Difference
M	Mould
MA	Marker
MATLAB	Matrix Laboratory
MC	Mean Centring
MEM	Meropenem
MG	Milligram
MIR	Mid-Infrared
ML	Milliliter
MLS	Multiple Linear Regression
MOFA	Ministry of Food and Agriculture
MRL	Maximum Residual Level
MSC	Multiplicative Scatter Correction
N	Number of Samples
NC	Control
NIR	Near Infrared

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NMR	Nuclear Magnetic Rason
O-H	Hydroxyl
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PH	Potential of Hydrogen
PLS	Partial Least
PLS-R	Partial Least Squares Regression
RH	Relative Humidity
RMR	Risk Management Recommendation
RMSE	Root Mean Square Error
RMSEC	Root Mean Square Error of Calibration
SAL	Salmonella
SAU	Staphylococcus Aureus
SBR	Schmid-Bondzynski-Ratslaff
SEC	Standard Error of Calibration
SEL	Standard Error of The Laboratory
SEM	Standard Error of Means
SEP	Standard Error of Prediction
SIMCA	Soft Independent Modeling of Class Analogies
TET	Tetracycline
TCP	Total Plate Count
UCC	University Of Cape Coast
UG	MicrograM
UK	United Kingdom
USDA	United States Department of Agriculture

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VAN	Vancomycin
VIS-NIR	Visible-Near Infrared
Y	Yeast
YC	Yolk Coefficient
YI	Yolk Index (Yi)

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The most globally preferred and accepted egg amongst the poultry species is the chicken egg. It is eaten by the majority of people worldwide as it stretches beyond people of various socioeconomic class (Chambers, Akhtar, & Abdel-Aal, 2017). Done. The reason for global acceptance were explained as follows: Its widespread acceptance is due to several factors. Chicken eggs are affordable and widely accessible, providing an economical source of high-quality protein and essential nutrients such as vitamins and minerals. They are also highly versatile, used in a wide variety of culinary applications across cultures, from simple preparations like boiling and frying to their inclusion in baked goods and sauces. Additionally, chicken eggs have a relatively long shelf life when stored properly, making them a convenient food item. The cultural and religious acceptance of chicken eggs further contributes to their global popularity. Lastly, chickens are easy to raise and highly efficient in egg production, which supports both small-scale and industrial farming, ensuring a consistent and abundant supply worldwide.

Africa's egg production is expected to be around 2,367,000 tonnes per year, amounting to 3.7 per cent of worldwide egg turnout. There are a lot of variances among countries, with Nigeria producing 533,000 tonnes and countries like the Gambia, Swaziland, Guinea, Central African Republic, Congo, and Comoros producing about 1,000 tonnes. This inadequate result results in an average egg intake of around 36 eggs per individual per annum which is far below the global average of 145 eggs per capita (Tukur, 2011).

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Ghana's egg consumption, on the other hand, has seen a significant increase from 1995 to 2018 where the number of eggs per capita was 12 and 128 respectively (The World Initiative for Soy, 2019).

Table eggs have risen to prominence as a basic diet for humans due to its perfect stability and variety of nutrients, as well as its great digestibility and affordable pricing (Réhault-Godbert, Guyot, & Nys, 2019). Furthermore, eggs offer health-enhancing characteristics; many are naturally preventive, while some are curative potential (Chambers *et al*., 2017). Egg comprises crucial extended-chain fatty acids, including eicosapentaenoic acid (EPA), arachidonic acid, and docosahexaenoic acid (DHA). (Keten, 2019) These are phospholipids elements that ensure cell membrane elasticity and minimise plasma cholesterol levels (Seuss-Baum & Nau, 2011). Inflammation, immunological infections, and mental health, central nervous system, cardiovascular problems are all reduced by EPA and DHA (Fraeye, Bruneel , & Buyse, 2012).

Growing egg production and consumption around the world has resulted from increased public knowledge of the health benefits of egg consumption. However, a quick and precise way to determine egg quality is of paramount importance to stakeholders. This is especially significant since, as a result of growing demand, several producers have flooded the market with various types of eggs such as organic, free-range, pasture-raised, and omega-3 enriched eggs. Moreover, the industry requires correct and trustworthy evidence about the egg so as to grade it exactly and give quality to consumers that conform to their quality and standard criteria (Lourens, Molenaar, van den Brand, & Kemp, 2006).

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Egg quality pertains to the features of an egg thus, egg shape, albumen weight, egg weight, albumen height, albumen percentage, shell strength, thickness of shell, shell colour, weight of yolk, yolk percentage, colour of yolk, eggshell weight, and eggshell percentage that influence customer acceptance and preference (Kowalska, Kucharska-Gaca, Kuźniacka, Lewko, Gornowicz, Biesek & Adamski, 2021). Constituents of egg quality deal with both external and internal quality. The external qualities are contingent on the eggshell quality which is connected to the presence of cracked shells. In contrast, an egg is internal quality comprises three elements: yolk quality, albumen quality, and on the whole quality.

The importance of a particular quality characteristic depends on the stakeholders' handling of the eggs along the supply chain. Producers should be aware of the egg's weight, shell strength, abnormalities, dirt, breaking, and blood stains. Consumers should pay attention to the age, sensory qualities (yolk and shell colour), and nutritional content (fats, vitamin supplements, and essential fats) of the eggs. In the processing industry, quality is measured by the ease with which the shell can be detached, the separation of albumen and yolk, functional qualities, and yolk colour (especially in bakery products) (Giampietro-Ganeco, Borba, Scatolini-Silva, Boiago, Souza & Mello (2015).

Egg quality measurement or assessment relied on visual inspection for an extended period of time. The overall quantity of eggs handled and the rate of sorting (up to 180,000 eggs per hour) have reached a level where this visual assessment is no longer manageable. This pointed to a quest for new non-invasive devices and non-destructive approaches to measuring egg quality

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parameters (Karoui, Kemps, Bamelis, De Ketelaere, & De Baerdemaeker, 2005)

Literature has shown that three types of egg quality sensing devices have been developed and commercialized over the past decades (Karoui *et al*., 2005). The first way, mechanical techniques, measure eggshell parameters like existence of cracks and eggshell strength. Second, spectroscopic equipment which may either be far, mid or near, can be used in determining the albumen properties like pH, Haugh Unit and viscosity based on information obtained from the wavelength. Finally, the computer vision technique relies on external features of the egg such as eggshell color and dirt presence to assess its exterior qualities. Computer vision techniques also enable the quick identification of any cracks that are open problematic for egg grading equipment (Karoui, *et al*., 2005). A scrutiny by (Karoui, *et al.*, 2005) revealed that ultrasonic, magnetic resonance and electronic nose techniques have been developed and used in combination with other measurements to determine other properties of eggs such as eggshell conductance which were not possible in the early years. Development and use of these techniques have contributed to the estimations of both the overall quality of the eggs and the layer flock's condition. which is very beneficial to the consumer because the risk of eating eggs as well as its products has been reduced (Karoui, *et al.*, 2005).

Currently, in Ghana, the destructive method of egg quality assessment is still being used. Even though it is the most basic and extensively used strategy, it is vulnerable to human error. Microbiological, nutritional, organoleptic, physical, and chemical quality can all be evaluated objectively using laboratory procedures. These approaches are time-consuming and

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require the destruction of samples as well as the usage of chemical compounds. Furthermore, they cannot be used online. As a result, accurate, online, and non-destructive procedures are required. According to Hagan & Eichie (2019), the Ghanaian layer industry uses a variety of strains of layers in egg production particularly the Lohmann brown and Lohmann white and the eggs are preserved for a time period at various temperatures before being used and also the layers are normally kept on the farm far beyond the required one-year egg production period. Kusi, Agbeblewu, Anim, & Nyarku (2015) indicated that there is a continuous increase of egg consumption in Ghana regardless of its high price hence commercial layers are used to produce about 80% of the consumed eggs in Ghana. The fact is, there is no observance of standards regarding egg quality, and this makes farmers not willing and ready to follow certain basic management practices that could enable them to produce quality eggs.

1.2 Statement of the Problem

There are several issues associated with the determination of egg quality in egg production. These problems are related to the exterior quality which is concerned with shell cleanliness and thickness, colour, texture, and form, whilst the interior quality is concerned with the egg white (albumen), yolk shape and yolk strength (USDA, 2000). The external egg quality is mainly affected by the housing system, chicken breed and chicken age (Zita, Tůmová , & Štolc, 2009). The internal egg quality is also affected by storage, humidity, diseases (bacteria or fungal), egg handling, temperature and age of the egg (Gerber, 2006). However, molecular genetics also play a critical role in determining both internal and external egg quality. Genetic factors

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influence shell strength, egg size, albumen height, and yolk composition, as well as the hen's resistance to diseases and overall productivity. Advances in genetic research and selective breeding have enabled the development of strains with superior egg quality traits, optimizing both physical characteristics and nutritional content. Understanding the genetic makeup of hens and their eggs can further enhance breeding programs aimed at improving egg quality across various environmental and management conditions.

According to Marelli, Madeddu, Mangiagalli, Cerolini & Zaniboni (2021), there are four egg production systems used by layer farmers; these are "enriched cage and alternative (small outdoor area), litter floor and organic (large outdoor area)". Each of these egg production systems has its own pros and cons. The study by Singh, Cheng, & Silversides (2009) revealed that commercial hens produced more eggs than crossbred hens but crossbred layers produced eggs of better quality than the former Singh *et al*. (2009). Again, it was discovered that eggs from nest boxes on the floor were more probable to be tainted with coliform and E. coli than eggs from cages, and Lohmann brown eggs were more inclined to be contaminated than Lohmann white eggs (Singh *et al*., 2009). The perceived lower quality of eggs from crossbred hens compared to commercial hens in certain aspects could stem from differences in genetic selection and management. Commercial hens are selectively bred for traits that enhance productivity, such as high egg production, consistent shell strength, and resistance to environmental stresses. In contrast, crossbred hens may not be optimized for these specific traits, leading to more variability in egg quality.

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For an extended period, commercial egg farmers have been concerned about poor eggshell consistency because of its economic implications. Consumers are concerned with internal quality because it influences the safety, freshness, nutritional value and sensory characteristics of eggs. Microbial contamination of eggs also has health implications for both the farmer and the consumer. *Bacteria such as Bacillus spp., Salmonella spp., Escherichia coli, Staphylococcus aureus, Listeria monocytogenes and Streptococcus spp., have been identified on eggshells (Mahdavi, Jalali, Safaei, & Shamloo, 2012).* Though *Campylobacter spp* is the most common bacteria linked to food poisoning, *Salmonella spp* is the primary bacterial culprit behind illnesses related to poultry and eggs. (Fardows & Shamsuzzaman, 2015). Aside from *Salmonella enteritidis*, other bacteria linked to chicken and egg contamination include *Listeria monocytogenes*, *Campylobacter jejuni*, and *Escherichia coli*. According to Salihu, Garba, & Isah, (2015), these microbes have the potential to cause decay and contamination in consumers if they enter the food supply chain.

Antibiotics and other veterinary drugs are commonly used to boost productivity, increase feed conversion ratios, and treat diseases in food-producing animals (Beyene, 2016). These residues according to Rana, Lee, Kang , & Hur (2019) can cause serious public health complications such as abnormal intestinal flora, bacterial resistance, mutagenicity, hypersensitivity reactions, cancer, and difficulties reproducing. Current education about the advantages for health offered by eggs intake has increased egg production and consumption across the globe. Nonetheless, one of the primary issues of customers and quality control officers is the difficulty in determining egg

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freshness rapidly and accurately. The significance of this cannot be overstated, since the growing demand for eggs has prompted several manufacturers to flood the market with diverse egg varieties. Specifically, this surplus in supply has resulted in the storage of eggs for lasting durations before they are purchased or sold, a condition that reduces freshness due to the sway of storage days on egg quality Ogunwole, Ojelade, Oyewo, & Essien (2015). Certain producers hoard eggs during periods of excess production and falsely advertise them just like new (eggs that are one day old) during times of high market demand.

The European Union and other statutory institutions have introduced various measures to ensure that quality eggs are sold to consumers. According to Giunchi *et al.* (2008), the specific day the eggshell was deposited as specified according to Regulation 2003/2295/EC by the European Commission, the risk management recommendations (RMRs) and maximum permissible levels of veterinary medication residues permitted in food, and other regulations do not provide sufficient assurance of egg quality. As a result, a combination of factors is required.

Analytical methods for evaluating egg quality, on the other hand, are often require a significant amount of time, involve destructive processes, demand substantial manual effort, and call for intricate laboratory work along with time-consuming sample preparation. Numerous academics have developed effective detection methods in this respect, including an electrical nose-based sensor (Dutta, Hines, Gardner, Udrea, & Boilot, 2003), which fresh eggs possess a limited number of organic volatiles, a factor that contributes to an increase. after storage (Adamiec, Dolezal, Mikova, &

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Davidek, 2002). Viewing eggs under light and finding the air cell is an additional nondestructive procedure. These strategies are ineffective in the first few days after laying (Giunchi , Berardinelli, Ragni, Fabbri , & Silaghi, 2008); Cattaneo, Balzaretti, Quaglia, & di Gian-Camillo, 1997).

Conventional methods in identifying and determining the origin of microbes including bacterium also requires complex cultured procedures. Together with restricted dilution analysis, conventional microbial detection methods are highly sensitive and often result in small forming units per mL (CFU/mL) can be identified. However, quick methods have made use of PCR to detect pathogenic bacteria in microbial DNA samples. (Furutani , Naruishi, Hagihara, & Nagai, 2016) indicated the ability of PCR to amplify specific genes of microbes to millions of copies in about 2 hours. Determination of the DNA of microbes according to the (Chinthapalli 2012) helps prevent outbreaks as the origin of the bacterium is detected in real-time.

In Ghana, there has been no study on the use of handy NIR spectroscopy for synchronous classification and prognostication of egg quality. Furthermore, with the few studies on egg quality, none of them has examined egg quality using both destructive and non-destructive approaches. This research intends to create a unique, instantaneous, and non-destructive method for detecting egg quality utilizing a portable NIR spectrometer paired with multivariate algorithms in contrast to destructive methods.

The international egg industry faces various obstacles. One of them is the necessity to ramp up egg production to converge the demands of a continuously increasing global population. Additionally, they must adapt to more stringent regulations concerning chicken welfare, living area as well as

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building layout. Ensuring food safety and maintaining high-quality standards for egg and egg products also present challenges. Lastly, there is a rising demand for eggs enriched with various essential nutrients. Conversely, the capability to enhance eggs with essential nutrients presents egg farmers with a special opportunity to create high-quality, creative eggs for both international and local markets. Improving egg quality, safety, and production is a shared responsibility that involves various stakeholders within the egg industry. This includes international breeding companies, parent flock and pullet producers, facility and system design companies, feed manufacturers, egg processing equipment manufacturers, and regulators. Effective communication and collaboration among these entities are crucial for advancing the egg industry in the 21st century (Chamber *et al.*, 2017).

1.3 Research Objective

The main goal of the research was to assess the quality of table eggs of different chicken strains of varying ages in layers raised under different housing systems, using destructive and NIR methods.

1.3.1 Specific Objectives

The research was therefore carried out to specifically:

1. assess and predict the physical (internal and external) characteristics of eggs using conventional (destructive) and NIR (non-destructive) means.
2. assess and predict the nutritional characteristics of eggs using conventional (destructive) and NIR (non-destructive) means.
3. assess and predict the presence of microbes on eggs using conventional (destructive) and NIR (non-destructive) means.

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4. assess and predict the organoleptic properties of eggs using both conventional (destructive) and NIR (non-destructive) means.
5. assess and predict the presence of chemical residues in eggs using both conventional (destructive) and NIR (non-destructive) means.

1.4 Justification

The egg quality is extremely important to both consumers and the egg industry because they are a commonly eaten and nutritionally beneficial food source. Table egg quality can vary depending on a number of variables, including layer strains, layer age and housing system. In order to improve production methods and guarantee customer satisfaction, it is necessary to evaluate and understand the elements that affect egg quality.

This research uses destructive and NIR (non-destructive) methods to scrutinize the quality of table eggs from two-layer types at varying ages and reared in diverse housing conditions.

Egg quality is important since it directly affects customer preferences, nutritional value, and safety. For the egg industry to maintain its reputation and satisfy consumers, it is crucial to evaluate and guarantee high-quality eggs.

The evaluation will provide relevant information for key stakeholders (farmers/egg producers, exporters, importers, regulatory bodies, policymakers, consumers, marketers of eggs, egg processing industries, etc.). This study will help in classifying and grading eggs based on different quality assessments, and in doing that helps in pricing, since quality eggs will sell at premium prices just as eggs are currently graded according to size and priced as such.

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Various strains of layers may differ in the physical attributes, nutritional makeup, and organoleptic qualities of their eggs. This research can shed important light on the distinctive characteristics of each strain by examining multiple layer strains.

The housing systems employed in the layer industry for egg production have a big impact, and they may have various effects on egg quality. Comparing eggs from layers raised under different housing systems can assist in determining which of the housing systems best supports the best egg quality.

As egg ages, there's an increase in the air sac which leads to a sharp decline in the eggs' internal qualities. According to Chambers *et al*. (2017), cooling and refrigeration of eggs at an appropriate temperature and humidity should be done to reduce deterioration, and maintain the interior qualities of the egg. (Jones & Anderson, 2013) discovered that the hen strain and housing systems have ramifications on egg microbial levels. The success of the NIR will help in the rapid detection of chicken eggs ages (quality of being fresh), the nutritional, physical, organoleptic and microbial load on eggs. In doing that, it will help farmers to be able to produce to meet special needs, whether for industrial or domestic needs, as each of these will require different egg quality characteristics.

In the egg supply chain, this will support strict quality control and quality assurance monitoring. It is believed that the results of this investigation would shed light on consumer awareness of egg quality in specific areas. The extent of microbial contamination of eggs is also expected to be revealed.

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The traditional techniques of egg quality assessment are destructive, laborious, time-demanding and require extensive preparation of samples (Aboonajmi, Akram, Nishizu, Kondo, Setarehdan, Rajabipour, 2010). Commercially, they cannot be employed since they cannot be used in rapid determination of egg qualities. Among the common spectroscopy techniques in use are: Raman spectroscopy, Near Infrared spectroscopy (NIR), Mid-Infrared spectroscopy (MIR), and Far Infrared spectroscopy (FIR). Several studies have shown that NIR among other approaches is an excellent technique for assessing egg quality and also a non-destructive way of predicting a number of storage days of egg regarding its freshness, and comparing egg Haugh unit (HU) and albumen pH for quality (Wang, Lin, Xu , Bi, & Sun, 2021); Abdel-Nour, , Ngadi, , Prasher , & Karimi, 2011).

1.5 Significance of the Study

This study will provide assistance develop assessment criteria that will make it possible for all stakeholders to assess the quality of table eggs. This will provide a detailed report on table egg quality in Ghana. This will greatly aid in making accurate prediction of table eggs quality utilizing non-destructive (NIR) and destructive means.

The goal of this study, which will be among the first in the country, is to create a unique non-destructive and simultaneous egg quality testing method employing a handy NIR spectrometer and multivariate algorithms. This suggests that our method will significantly reduce egg deceit owing to mislabeling. Across the egg value chain, the study will help to extensively monitor the quality control and assurance of the eggs being produced. The study's originality is the simultaneous identification of egg quality using eggs

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from different layers at different ages and raised under different housing systems.

1.6 Organisation of the Study

This dissertation is organised into six (6) chapters. Chapter 1 features the introduction which discusses the research's historical context, justification for the work, the relevance of the work and finally the objectives of the work. Chapter 2 gives the outline of important works on the research area. Chapters 3 describes the materials and methods whiles chapter 4 presents the research results. The analysis of the research results is outlined in Chapter 5.. The conclusions, recommendations, novelty statement and the list of publications emanating from the study are presented in chapter 6.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The research delved into the quality and safety of chicken eggs by exploring the possibility of developing a model for evaluating and predicting the physical, nutritional, chemical, microbiological and organoleptic properties of chicken egg quality from different breeds and housing management systems. The nutrition and health benefits of eating chicken eggs are reviewed in this chapter's literature and the economic importance of chicken egg production globally and in Ghana. The quality of chicken egg was reviewed being the basis upon which the development of a model will be justifiable. Regarding quality, physical (internal and external), nutritional, microbiological, chemical and organoleptic properties of chicken egg quality were reviewed. The study also examined factors influencing the quality of chicken and eggs. Factors such as housing management systems, chicken age, and breed of chicken were reviewed. Given that housing, age and breed of chicken could account for chicken egg quality, empirical evidence on consumer perception of chicken egg quality was also reviewed. The review also covered methods of assessing chicken egg quality. This includes destructive and non-destructive analytical methods. In a nutshell, the review consisted of chicken egg quality and analytical methods for measuring chicken egg quality.

2.2 Economic Importance of Chicken Eggs Production

Eggs have been a mainstay of nutritive value since the beginning of time, being considered as one of nature's nearly flawless sources of protein

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and additional essential nutrients. Therefore, eggs are used in multiple ways in both the culinary sector and households, with chicken eggs being the utmost popular option. The eggs of birds like geese, ducks, plovers, and quail hold less significance. As a result, when the term "egg" is used without a qualifier, it refers to a chicken egg (Kiosseoglou & Paraskevopoulou, 2014). White eggs come from White Leghorn chickens, brown eggs on the contrary are from Barred Plymouth Rock, New Hampshire, and Rhode Island Red chickens.

Every year, people who raise poultry raise about 50 billion birds, either as layers to produce eggs or as broilers to produce meat, and production is expected to keep going up. Increased demand for animal-based meals is projected as the world population approaches 9.6 billion by 2050 (Mottet & Tempio, 2017). Although pig and beef demand are forecast to rise by up to 43 percent and 66 percent, poultry meat is expected to expand at the breakneck speed of up to 121 percent, while egg demand is likely to go up by 65 percent (Alexandratos & Bruinsma, 2012). Canada transported nearly 39 million hatching eggs to the United States in 2017 for a total of $68 million. To make up for this shortfall, the United States imported about 141,000,000 hatching eggs for broilers ($49,000,000) in 2012 (Young, 2017). Given the importance of chicken products both locally and internationally, it is vital, notably in this age of technological advancements in cognitive computing, that substantial support bolsters egg quality evaluation and freshness.

According to Tukur (2011), Africa's poor egg production translates to a low yearly per capita consumption of 36 eggs, significantly less than the global rate of 145 eggs. Nevertheless, Africa's business has received a makeover successfully in the past years. Statistics from the Nkukwana, (2018)

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indicated that the production of eggs increased by 3.9 percent per year from 1.1 to 3.0 million tonnes, from 2000 to 2012, outpacing the global rate of 2.2 percent. It went on to say that overall egg output in Africa increased from 3.7 percent in 2000 to 4.5 percent in 2012. Africa produced 655,100 tonnes of eggs in 2018, accounting for 21.1 percent of global egg production (Windhorst, 2014).

Egg production in Ghana experienced a 15.5% increase in production to attain 26,000 MT from 1990 to 2008, with Ghana ranked 16th among the top egg-producing African countries (Tukur, 2011). In 2012, Ghana's egg production stood at 40,000 tonnes (Windhorst, 2014). However, this achievement was not sustained, as egg production declined from 47,412 tonnes in 2016 to 46,500 tonnes in 2017 and further to 41,886 tonnes in 2018 (FAOSTAT, 2012).

2.3 Nutritional and Health Benefits of Chicken Egg Consumption

For hundreds of years, eggs have been a homestay in consumers' meals (Forson *et al*., 2011). The nutritional denotation of the chicken egg is presented in Table 1.

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Table 1: Nutritional Constituents of Chicken Egg

Nutrient	Nutrient content per 100g (Large egg)	Nutrient content per 52g (Medium)
Energy (Kcal)	15.0	78
Protein (g)	12.5	6.5
Carbohydrate (g)	Trace	Trace
Fat (g)	11.2	5.8
Cholesterol (mg)	391	225
Retinol equiv. (µg)	190	98
Vitamin D (µg)	1.6	0.9
Riboflavin (mg)	0.47	0.24
Folate (µg)	50	26
Vitamin B12 (µg)	2.5	1.3
Choline (mg)	160	83.2
Biotin (µg)	20	10
Phosphorus (mg)	200	104
Iron (mg)	1.9	0.99
Zinc (mg)	1.3	0.68
Iodine (µg)	53	28
Selenium (µg)	11	5.7

Source: Ruxton, (2010). Recommendations for the use of eggs in the diet. Nursing standard.

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Rich proteins like glycine, cysteine, and tyrosine form the cuticle layer that protects the eggshell from the outside world (Liu, Ren, Yu, Cheng, Guo, Yao, & Xie, 2020). Furthermore, methionine, cysteine, arginine, glutamine, proline, and histidine are abundant in the shell membranes. The chemical compositions of yolk and egg albumen are different in the entire egg. Water, proteins, minerals, carbs, and lipids make egg white (Lv, Huang, Ma, Chen, Batool, Fu & Jin, 2022). The most significant component of egg white is water, which makes up around 87.8% of the total, and the second most important constituent is protein, which makes up 10.6% of the total (Kiosseoglou & Paraskevopoulou, 2014).

The nutritional value of egg protein is relatively high. It is easy to digest and contains a balanced amino acid profile. Albumins and globulins are the most nutritious egg proteins (Kiosseoglou & Paraskevopoulou, 2014). Egg protein is composed of all indispensable amino acids, including methionine, histidine, lysine, phenylalanine, isoleucine, threonine leucine, tryptophan, and valine Rodriguez (2005). As stated by Asghar & Abbas, (2012), people need proteins to develop their bodily tissues, and eggs can provide vital amino acids for healthy growth. Furthermore, according to (Bologa, Pop, & Albu, 2013), eggs contain vital nutrients that strengthen immunity to disorders. To support such a claim, Kiosseoglou & Paraskevopoulou, (2014) noted that eggs might offer significant quantities of nutrients required every day to grow and maintain human tissues.

The importance of amino acids for humans of all ages cannot be overstated because proteins are essential for development and muscle building (Layman & Rodriguez, 2009). According to (Layman & Rodriguez, 2009), for

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example, leucine is a necessary amino acid that can boost a person's body's ability to use energy from muscle contractions and help muscles after workout. According to Asghar & Abbas (2012), the body needs the protein found in eggs to repair and maintain muscles, neurons, blood, and bones. Muscle degeneration and other age-related health issues can also be prevented by using high-quality products (Thalacker-Mercer *et al*., 2007).

Herron and Fernandez (2004) identified substances in eggs that promote optimal brain development. Choline, folate, and selenium are examples of these nutrients. Human health benefits from vitamin B12, vitamin D, *folate, selenium* and choline. According to research conducted by Ruxton & Derbyshire , (2009), vitamin D has the ability to slow down the ageing process of cells and protect against cardiovascular disease (CVD), cancer and. diabetes, According to Wang *et al*. (2007), vitamin B9 can help avert Cardio Vascular Disease (CVD) and lower stroke occurrence. Selenium protects in opposition to lung cancer in the early and late phases of the disease (Zeng & Combs, 2008) and protects against lung cancer (Brinkman *et al*., 2006). .

Scientists have often criticized eggs as a potential contributor to various ailments due to their high cholesterol content. Studies by Djoussé *et al*., (2009) Barraj *et al*., (2009) and Spence *et al*., (2010), stated that increased egg consumption could be linked to type 2 diabetes, cardiovascular disease (CVD), and heart disease are associated with an increased risk. Conversely, (Schmier *et al*., (2009) discovered that excluding eggs from one's meals increased the likelihood of developing age-related diseases. According to research carried out by Njike, *et al*., (2010) the cholesterol that is consumed in one's diet does not have an impact on one's blood cholesterol levels or their

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risk of developing cardiovascular disease. According to these findings, consuming eggs does not have a negative impact on the function of endothelial cells or the lipid profile of the serum. Drawing on the outcomes of a cumulative statistical examination carried out by Shin *et al*., (2013), consuming eggs does not come with a higher chance of cardiovascular disease or heart attack in the overall population. In addition, Djoussé, *et al*. (2010) discovered that eating eggs was not linked with a higher danger of developing of type 2 diabetes.

2.4 Chicken Egg Formation and Structure

From ovulation until oviposition, egg production takes roughly 25-26 hours. It starts with a fully developed ovum in the uterus and ends with a hard-shelled egg with all of its shielding layers and the nutrients demanded for embryogenesis. It starts with a fully developed ovum in the uterus and ends with the formation of a hard-shelled egg that has all of the protective coverings as well as the nutrients that are necessary for embryogenesis (Perrins 2008). Ovulation, fertilization, creation, and oviposition are the four basic steps of egg development. All of these phases are completed in the ovary and oviduct. The procedure may be improved and more easily understood by using the schematic diagram in Figure 1.

According to Robker, Hennebold, & Russell, (2018), ovulation is how the ovum or egg is liberated from the follicle in the ovary. Following the release of the final egg, ovulation occurs in around 7 to 35 minutes Robker *et al*., (2018). This period came before yolk production, which was made possible by feeding the chickens (hens) proper nutritional diets. Calcium-rich diets are required for practical use later on during shell development. The

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hen's liver transforms the nutrients received into the circulation from the digestive systems of the hens to the yolk (Richards 1997). The yolk is subsequently carried from the hepatic organ to the female reproductive organ. The yolk and some additional essential nutrients are transported to the ovum by the follicular cells around it (Schneider, 2015). Close to the ovary are the immature eggs and the follicular cells that surround them. As the ovum gets more yolk, it gets much bigger and can't fit inside the ovary anymore. So, the nested ovum starts to slowly and steadily push the follicle of the ovary located on the outer periphery

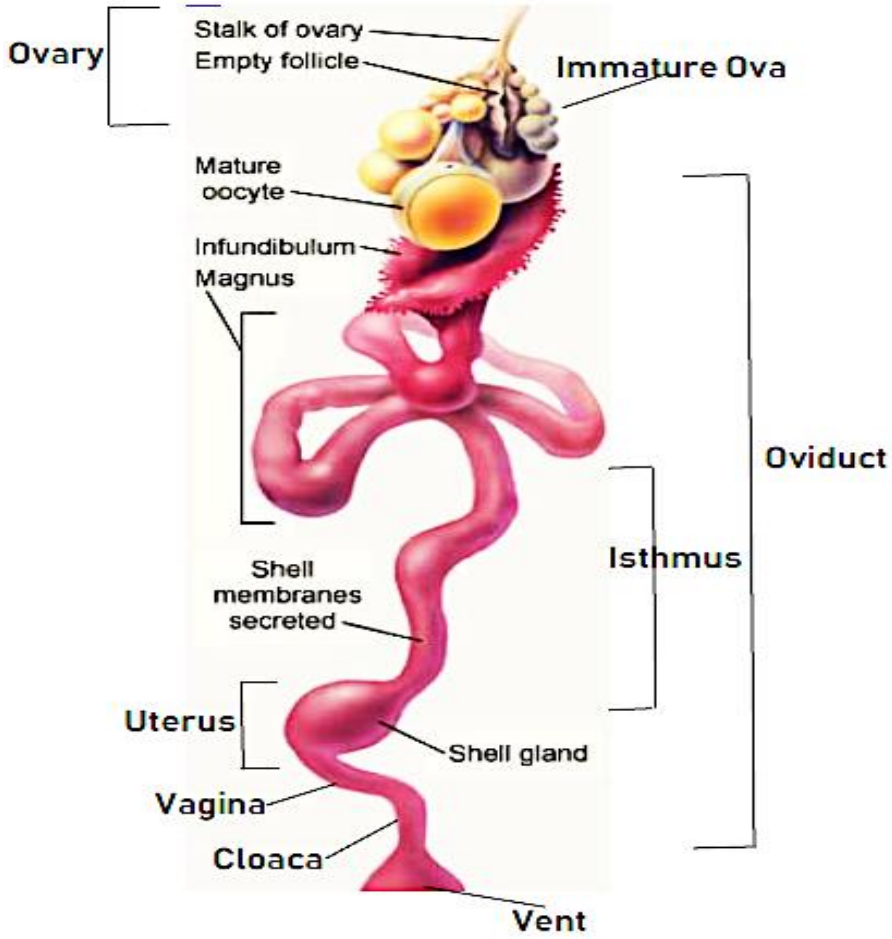

Figure 1: A diagram representing the development of a chicken egg

Source: Adegbenjo, Liu, & Ngadi, (2020). Non-destructive assessment of chicken egg fertility.

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Ovulation is the process by which an egg is released from its follicle once it has accumulated sufficient yolk to sustain the development of a chick. Within a few of minutes, the unattached ovum makes its way into the ovarian pocket, where it is quickly encapsulated by the infundibulum and escorted through the left oviduct's opening. Almost immediately after the ovum is produced by the ovaries, it is transported to the infundibulum, where it is fertilised. Meiosis is a basic cell division process that occurs in the nucleus of the egg during the development of an embryo. An egg can only be detected by the infundibulum if one of the cells generated during meiosis is a developed ovum. Sperm fertilisation occurs in the infundibulum and the resulting zygote divides again through mitosis. As the albumen is generated, the first layer is also created.

The oviduct is comprised of the isthmus, infundibulum, the magnum, the shell gland, the vaginal, and the cloaca. The majority of albumen secretion is carried out by the oviduct segment. Now that the developing ovum including its surrounding layers has reached the magnum, we may refer to the resulting structure as an egg. Albumen layers and chalazae form as a result of such a phenomenon. The shell membranes then are joined to the Isthmus, as well as the shell gland located in the uterus starts the shell development procedure (Bruce & Drysdale, 1994). An ovum will typically remain in each stage of the oviduct for the following amounts of time: Magnum: 2-3 hours; Infundibulum: 15 minutes; Uterus: 21 hours, Isthmus: 1 hour; and a brief period in the vagina or cloaca. This is the typical progression that an ovum makes as it travels down the oviduct. A newly produced entire egg structure has 30-33 per cent

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yolk, roughly 60 per cent albumen, and 9-12 per cent shell, as seen in Figure 2 (Stadelman *et al*., 2017).

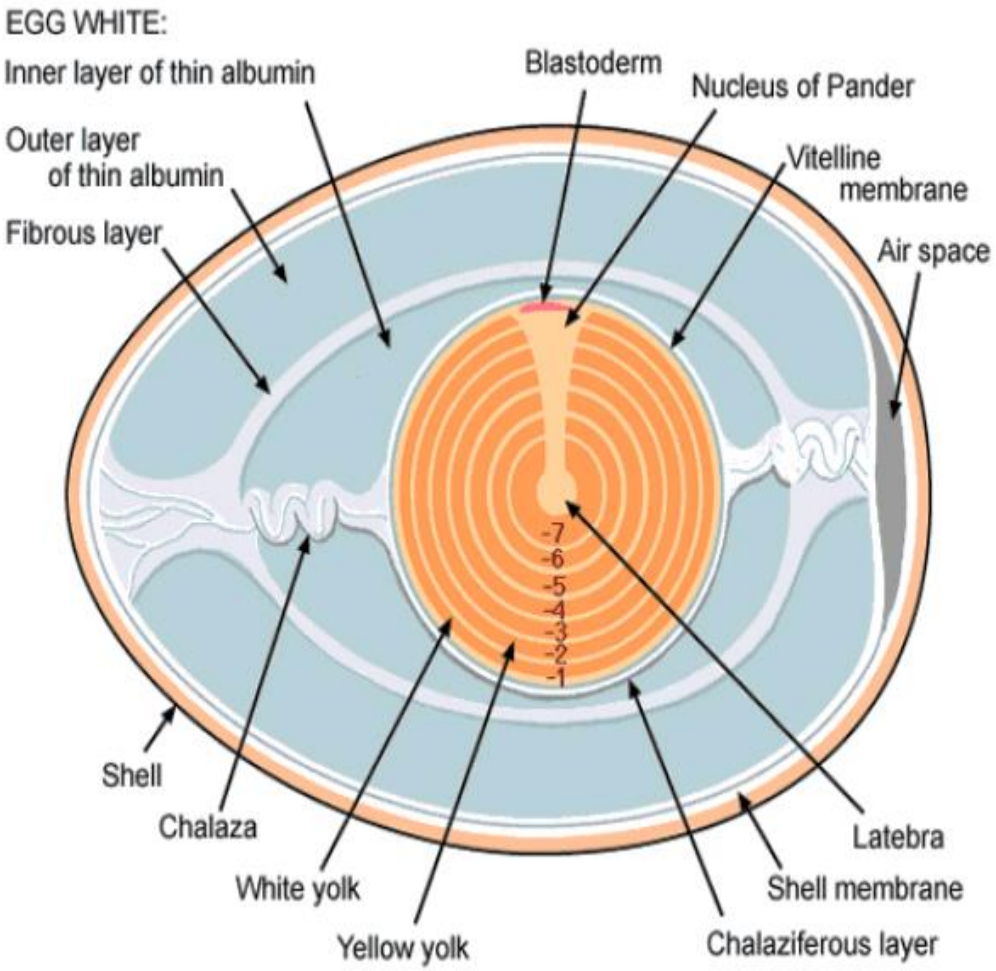

Figure 2: The structure of a chicken egg

Source: USDA Agricultural Handbook (1961)

2.4.1 Eggshell

The eggshell makes around 9 to 12 percent of the overall weight of the egg. Calcium carbonate accounts for 94%, proteins for 4%, magnesium carbonate for 1 per cent, and calcium phosphate for 1% (Stadelman & Cotterill, 1995). A covering of fibrous proteins, primarily keratin, is embedded and seals the carbonates of eggshells. The eggshell contains numerous pores, with 7,000-17,000 pore canals, to facilitate gas exchange. These openings allow carbon dioxide and moisture to leave the egg while allowing microbes to

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enter (Hui & Al-Holy, 2007). The eggshell is made up of a number of different components, including membranes of the external and internal shells. These membranes are made up of semi-permeable protein-polysaccharide fibers. The egg's inside is separated from the shell by membranes called shell membranes, which may be found underneath the eggshell. This allows the albumen and yolk to exist independently from the shell. Keratin and collagen-like proteins make up the inner membrane, which is considerably less thick than the membrane that surrounds it. Additionally, it gives protection against microorganisms (Hui & Al-Holy, 2007). Glycine occurs in low quantity in both membranes, which are composed of diverse proteins with large concentrations of glutamic acid, proline, methionine, histidine, arginine, and cysteine (Stadelman & Cotterill, 1995).

Air cells are also located on the rounded tip of the egg. What is inside the egg compress and increase during refrigeration as moisture and carbon dioxide are lost, resulting in a small initial air cell size (Hui & Al-Holy, 2007). The chalazae, which resemble a rope and are linked to the solid egg white, are still another minute component of the egg. The yolk should always stay in the center of the egg (Hui & Al-Holy, 2007).

2.4.2 Egg Yolk

The chalazae are responsible for holding the yolk, which may be found within the albumen. The egg's yolk accounts for around 36% of its overall mass (Figure 2.2). Huopalahti *et al*., (2007). It is composed of a latebra, an early stage of development disc, as well as two aligned layers of light and dark tissue. The vitelline membrane covers the egg yolk, which comprises all of these components. The vitelline membrane is surrounded by the chalaziferous

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albumen layer and is composed of two layers (Stadelman & Cotterill, 1995). Water, lipids, and proteins form the structure of egg yolk. Lipids account for approximately 65 percent of the dry substance in the yolk (Huopalahti, Anton, López-Fandiño, & Schade, 2007). The main lipid elements of egg yolk are triglycerides (66.2 percent), phospholipids (29.6 percent), and cholesterol (4.2 percent). As per Huopalahti *et al*. (2007) study, fatty acids including; saturated (30-35%), monounsaturated (40-45%), and polyunsaturated fatty acids (20-25%) of various proportions are found in egg yolk. Dense lipoproteins (HDL, nearly 16%), reduced density lipoproteins (LDL, nearly 68%), globular proteins (livetins), phosphoproteins (phosvitin), and smaller proteins are all present in egg yolk.

2.4.3 Egg White

Egg white is the name given to the white part of an egg that comes from a hen's egg (albumen). It is a water-based solution that has a gel-like consistency. Ovomucin is a protein found in egg white that has a fibrous conformational shape. A high concentration of ovomucin is what gives egg white its gel like form. The mucous quality of the egg albumen is mostly because of the presence of this protein (Drakos & Kiosseoglou, 2006). The egg albumen makes up around 63 percent of the egg's total weight and is composed of dual layers: bulky (external covering) and fine white layers (internal layer). The dense external albumen is enclosed by a vitelline membrane, as previously described (yolk membrane). The thick albumen gives rise to double stranded chalazas, which keep the yolk centered within the egg white. (Stadelman & Cotterill, 1995). The fundamental distinction between albumens is stickiness, which is substantially lofty in the bulky

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albumen thanks to the high level of protein called ovomucin (Hui & Al-Holy, 2007). The protein makeup of the two albumen layers is another distinction.

2.5 Chicken Egg Quality

Egg quality is determined by numerous factors, each of which contributes to the overall nutritive value of an egg (Schwaegele, 2001). The shell, membranes, albumen, and yolk are the four major divisions of an egg. By weight, a fresh chicken egg that has been laid a constituent of yolk (32%), albumen (58%), and shell (10%) (Abanikannda *et al.*, 2007). Egg quality refers to characteristics that impact egg consumption as a meal (Schwaegele, 2001). Egg quality is determined by essential features that impact customer acceptance and/or preferences (Hanusova, *et al.*, 2015, (Zaheer, 2015). Therefore, it is necessary to preserve egg quality until they are eventually consumed to ensure the authenticity of eggs and minimise losses. Eggs' physical quality may be classified into several categories Hagan and Eichie, (2019). These categories include chemical quality, morphology, organoleptic quality, physical quality, and microbiological quality. However, some physical (external and internal) egg quality indicators are pertinent to our inquiry.

2.5.1 External Egg Quality Parameters

Various researchers have reported other observable characteristics of an egg's exterior. Examples include cleanliness, freshness, egg weight, and shell weight (Adeogun & Amole, 2004; Dudusola, 2010). According to Bain, (2005), the weight of an egg, its size, its form, the thickness of its shell, the colour of its shell, and its strength are all exterior qualities of an egg. Despite this, the external egg quality parameters that are important to this experiment are given in the next section.

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2.5.1.1 Egg Weight

Egg weight is another crucial phenotypic characteristic determining egg quality and parental reproductive fitness (Islam, *et al*., 2001). As a result, bigger eggs weigh more (Silversides & Scott, 2001). The weight of a globally accepted is expected to be between 58 and 62 grams (Simeonovová, *et al*., 1995) or 60 to 61 grams globally (Arthur & O'Sullivan, 2008). On the other hand, commercial eggs are put into groups based on their weight, namely "AA" (above 62g), "A" (between 58 and 62g), and "B" (between 52 and 57g). Eggs that weigh less than 52g are not good for the market (Jones & Musgrove, 2005). Producers and dealers set prices based on egg weight. It has power over the preferences of customers (Genchev, 2012). Because larger eggs fit better into crates than smaller eggs, there is less egg loss during packaging or shipping (Ebegbulem & Ayuba, 2017). As a result, all egg business actors prefer bigger eggs to smaller ones. However, because of the increased surface area for shell deposition during egg development, a large egg weight (size) may lower shell thickness (Kgwatalala, *et al*., 2016). Alternatively, a decrease in weight of egg would result in decreased price and income. As a result, egg weight has the most significant influence on the farmer. Commercial lines produce bigger, more comprehensive eggs than traditional lines (Hocking, *et al*., 2003). (Hanusova, *et al*., 2015) reported a substantial difference in the mean egg weights from Rhode Island (57.60g) and Oravka (60.96g) chickens when they discovered a genotype (strain) influence on egg weight. Also, Aygün & Narinc, (2016) also found a substantial genotype (strain) influence on the fresh egg weight of the Nick chicken's brown (64.12g) eggs and white (60.39g) Several studies have also shown that the weight of chicken eggs may

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be swayed by the chickens' age (Silversides & Scott, 2001; Silversides & Scott, 2001; Zita *et al*., 2009; Zita *et al*., 2009). Zikic, *et al*., (2017) discovered a good relationship between hen age and egg weight. The Bovans Brown chicken produced a large number of heavy eggs weighing 66.08g in week 59, than week 34 (63.45g), by a significant variance of 2.64g.

2.5.1.2 Egg Length and Egg Width

Producers and consumers generally do not regard egg length and breadth as necessary. They should be thought about, though, when choosing birds to breed. This is due to the fact that they control the size/weight (Silversides & Scott, 2001) and shape of eggs (Cavero *et al*., 2009). For example, it is thought that eggs that weigh more will be longer and wider. On the other hand, eggs with a low ratio of egg width to egg length weigh less (Alsobayel & Albadry, 2011) which may affect other egg quality traits. Though scientists do not pay much attention to the influence of genetic and laying hen age may alter as the variables that affect egg characteristics (Aygun & Yetişir, 2014); Rizzi & Marangon, 2012); Zita , Tůmová, & Štolc, , 2009). A breeding programme aimed at improving the quality of domestic chicken eggs should take into account the aforementioned knowledge regarding each of the external egg quality characteristics, including genotype and age.

2.5.1.3 Egg Size

Because egg size may be very variable, it has been extensively investigated within the framework of avian life-history theory. The breeder layers' production cycle begins with little eggs and progresses to average size and finally to the ideal big-size egg in a couple of weeks. According to the literature, egg size is defined by a diversity of parameters, with the inclusion of breed, genetics, laying age of hen, diet, production procedures, climatic

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circumstances, physiological stress, season, and housing systems (Hanusova, *et al*., 2015; Zemková, *et al*., 2007). In addition, birds' worrying behavioural responses could affect the size of the egg.

Egg size and age inlay were studied by Asuquo & Okon, (1993) for their impact on egg fertilisation and hatchability. They found that larger eggs weighing between 45 and 56 grams hatched more quickly than smaller ones. However, there were no biological explanations given for such a finding. Eggs are marketed in the United States depending on classifications grounded on the weight of consumer (USDA, 2000). Classes of weight of consumer guarantee that the egg size will not change within an egg carton at any point, as well as that consumers will receive an even distribution of egg sizes. Furthermore, it is widely recognized that egg size can be directly influenced by production methods and stress (Cunningham *et al*., 1960; Gardner & Young, 1972); Keshavarz & Nakajima, 1995).

2.5.1.4 Egg Shape Index

The index for egg shape is determined by dividing the width of the egg by its length. (Cavero *et al*., 2009). According to Alsobayel & Albadry, (2011), eggs could either be spherical or oval, with a small egg width-to-egg length ratio yielding an oval shape and the opposite leading to a spherical shape. Even though egg shape is not usually taken into account in the enterprise, it can affect how well an egg is liked by the public or how well it hatches. Round eggs are known for breaking easily (Alsobayel & Albadry, 2011) and thus have a relatively lower hatchability since they do not fit well on setter trays when they are being incubated (Cavero *et al*., 2009). Poultry eggs are typically oval, with minimal variance between species, and this is an

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essential aspect of classifying bird species (Bashir, *et al*., 2015). However, the genetic composition of layers can impact the characteristic (Tůmová *et al*., 2011). According to Alsobayel & Albadry (2011), white-shelled eggs had a much lower egg shape index (74.48 percent) than eggs with brown shells (77.73 percent), while eggs with brown shells with high appearance indexes were circular and more prone to breaking throughout the value chain. According to Hanusova *et al*. (2015), absence of notable statistical variation in the index of egg shape between Rhode Island Red and Oravka eggs. However, it was observed that the egg shape index in Oravka eggs was quantitatively larger, measuring 75.72 percent, compared to Rhode Island Red eggs, which measured 74.76 percent. A previous study and paper looked at the link between the layer age and the index of egg shape. Table eggs from young birds that had been raised for 28 weeks had a much higher egg shape index (76.960%) than eggs from birds that had been raised for up to 80 weeks (75.089%) (Ahmet & Zehra, 2009) . In their study, Zita *et al*. (2009) discovered a notable decrease in the egg shape index for ISA Brown layers, Hisex Brown, and Moravia BSL from week 20–26 to week 36–42 to week 54–60 (Zikic, *et al*., 2017). Zita *et al*. (2012) demonstrated hens age negatively impacts the shape of chicken eggs. They found that the demonstrated the age of the layers decreases steadily from week one (78.06%) to week twenty (76.32%) and week forty (75.85%) during the laying period. Their data, on the other hand, showed that Japanese quail eggs had a snake-like pattern during the first week of laying (77.97 percent) to the twenty-first week (77.37 percent) and then to the forty-first week (78.23 percent).

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2.5.1.5 Egg-Specific Gravity

Eggs with high specific gravity are fresh and of excellent quality. Additionally, the egg index shape is utilized to assess the shell's quality as the hen matures or undergoes periods of stress (Akter *et al*., 2014). In a research by Joubrane *et al*. (2019), there existed no noticeable variance ($p > 0.05$) found between white eggs (1.078 0.008) and brown eggs (1.077 0.008). Nevertheless, when birds are subjected to heat stress, specific gravity decreases, notably during the summer, when egg-specific gravity drops to 1.074 ($p = 0.0021$ 0.05). This was comparable to Mashaly *et al*. (2004), who found that stress and high temperatures can influence specific gravity. Therefore, hen age might limit shell thickness (Samiullah *et al*., 2017).

In addition, storage duration and temperature had an effect on specific gravity (Iqbal *et al*., 2016). At ambient temperature, eggs are more susceptible to quality loss and shell thinning than when stored in the refrigerator (Akter *et al*., 2014). In prior research, it was demonstrated eggs that have shells of a darker color had a greater specific gravity (Ingram *et al*., 2008).

2.5.1.6 Eggshell Colour

Several variables influence eggshell colour, including heredity, sickness or other sources of stress, hen age, and the administration of an antiparasitic medicine (Nicarbazin). Larger eggs are produced as the hen ages, with little or no change in her ability to synthesize pigments. As a result, the eggs become paler. However, thorough investigations of eggshell colour as influenced by these variables are lacking. Nonetheless, eggshell pigmentation is responsible for the wide range of egg colours and patterns seen across the Aves class (Kilner, 2006; Walters , 2006).

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Odabasi *et al*. (2007) demonstrated that the proclivity of hens to lay eggs bearing coloured shells increased with flock age, as seen by an increase in lightness (L*) values over time. According to Joseph *et al*. (1999), the strain had a more substantial impact on shell colour variation than nutrition, and shell colour in broiler breeder eggs was connected with specific gravity and relative shell weight. Some egg quality characteristics change depending on the colour of the eggshell. As a result, the categorization of eggs based on eggshell colour may be financially significant.

2.5.1.7 Eggshell Weight

According to Chepkemoi, *et al*., 2017) shells account for about 30% of eggs from various poultry species; excluding commercial chickens, which only contribute to 10% of an egg's total weight), making shells the second most prevalent ingredient in an egg. Several animals' shells include minerals that are utilized in feed composition. Begli *et al*. (2010), found a genetic effect on eggshell weight when they noticed an average shell weight of 4.45g versus 5.6-7.1g for that recorded in the study by Olawumi and Ogunlade (2008). Abdul-Rehman *et al*. (2016) found that White Leghorn (8.51g) chickens had a somewhat greater shell weight than Fayoumi (FAY) chickens (6.31g). Dudusola (2010), on the other hand, claimed significant disparities in the weight of the shell of 6.31g of guinea fowl and 0.76g of quail. Nonetheless, the apparent variance might be ascribed to species variations rather than the breed effect. The literature also mentioned an age influence on shell weight. According to Rizzi & Chiericato (2005), the eggshell weight for Hyline brown eggs decreased from 6.37 to 6.26g at 40 and 60 weeks. In comparison to older hens (80 weeks), the shell weight of table eggs gathered from younger

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chickens (5.236g) was lower (Ahmet & Zehra, 2009). Padhi *et al*. (2013) ended up finding that the shell weight of Vanaraja male line (PD1) hens increased significantly from 3.99g (at 28 weeks) to 4.48g (at 72 weeks) into laying. As the layers get older, the weight of the shells goes up in a way that is proportional to their age. This is likely because older layers put more minerals into building the shells than younger layers, which use minerals mainly for bone growth.

2.5.1.8 Eggshell Thickness

The thickness of an egg's shell is considered to be an essential characteristic of its quality because of the impact it has on an egg's potential to withstand breaking, hatch, or maintain the quality of its contents (Khan *et al*., 2013). Ledvinka *et al*. (2012) defined quality of eggshell based on its real weight, thickness, and toughness. Eggs with thick shells are less likely to break and are valued by farmers and consumers. Because larger and heavier eggs have a broader surface area for shell deposition, they have a thin shell (Kgwatalala *et al*., 2016). While there is no apparent standard for table eggshell thickness, Yamak *et al*. (2016) classified below 0.30mm is considered thin, 0.30-0.36mm is classified as medium, and 0.36mm is categorized as large. However, the mineralization ability of the layers that lay the eggs can affect the thickness of the eggshell. The eggshell quality can be assessed regarding its weight, proportion of shell, thickness ratio, and durability. The type of layers and their living conditions both impact the quality of the eggshells (Zita *et al*., 2009). Layer genotype has an impact on shell characteristics (strain). White eggs have been shown to have thicker shells than brown eggs (Leyendecker *et al*., 2001), and layers genotype also

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influences shell thickness (Garcês & Casey, 2003). Rizzi and Marangon (2012) found no significant variation in eggshell thickness as hen age increases when comparing Hy-Line brown and Hy-Line white breeds at weeks 30 (376m; 342m) and 42 (364m; 347m). Chung and Lee (2014) found no significant differences in thickness of the shell in Hy-Line brown chickens aged 40 (0.350mm) and 60 (0.347mm). Padhi, *et al*. (2013) found that shell thickness varied significantly but sporadically during week 28 (0.34mm), to week 72 (0.38mm).

2.5.2 Internal Egg Quality Parameters

Unlike outward high quality, the quality of the egg internally starts to degenerate immediately after chickens lay them. Even though hen care and feeding techniques have an impact on the egg's quality from the inside out, handling and storage of eggs have a far more appreciable impact on the overall quality of the eggs marketed, As a consequence of this, the term "egg quality" will have multiple meanings to a diversity of individuals, and the consumer's perceptiveness of quality will probably shift depending on the preferences and the purpose for which the egg was supposed to be used.

2.5.2.1 Albumen Height and Weight

The gap between a thick albumen surface and a flat surface is albumen height. The higher the quality of an egg, the denser the albumen is, with an ideal thickness of 8-10mm (Zeidler, 2002). Monira and colleagues (2003) discovered that the albumen heights of White Leghorn, White Rock, , Barred Plymouth Rock, and Rhode Island Red eggs were 4.33mm, 4.66mm, 4.19mm, and 3.60mm, in that order. Aygün and Narinç (2016) observed a significant positive relationship between the albumen heights of white-shelled Nick

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chicken eggs (5.3 mm) and brown-shelled Nick chicken eggs (3.89 mm). In 2017, they showed that eggs with white shells had a much higher (7.73 mm) albumen height than eggs with brown shells (7.01 mm) (Aygün & Narinç, 2017). A study on how age impacts egg quality revealed that eggs from 35-week-old hens had the highest albumen height (5.836mm) compared to older hens at 40 weeks (5.5mm), 45 weeks (5.2mm), and 50 weeks (4.5mm) (Menezes *et al*., 2012). Silversides and Scott (2001) found that the height of the albumen in eggs from ISA-White hens significantly reduced from 7.8mm to 7.2mm and from 6.5mm to 6.4mm at 25 to 31 and 45 to 59 productive weeks, respectively, while it decreased from 6.81mm to 6.25mm and from 5.58mm to 5.21mm in eggs from ISA Brown. Peri *et al*. (2017) found that the albumen height in Bovans Brown chicken eggs decreased from 7.11 mm at 34 weeks old to 5.7 mm at 59 weeks old.

2.5.2.2 Yolk Colour Quality

The yolk colour is the most important thing about an egg's inside, and it's a good way to measure egg quality in household surveys (Jacob, Miles & Mather, 2000). Because commercial breeds' eggs are watered down and their genes are different, the yolks of eggs from unimproved breeds are darker (Hocking *et al*., 2003) or derived from the xanthophyll in leaves of plants. Alsobayel and Albadry (2011) found that genotype did not affect yolk color in both brown and white-shelled eggs within this specific context. However, their findings revealed that brown eggs had a little lower yolk colour (5.54) than eggs that are white in color priced at 5.55. Aygün and Narinç (2017), on the other hand, found a substantial genotype influence on yolk colour, with brown-shelled eggs having a higher yolk colour (7.88) than white-shelled eggs

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(7.35). Several studies have found that yolk colour has a variable connection with hen age. Zita *et al*. (2012) discovered that the difference in colour of yolk in eggs laid by ISA-Brown breeds varied with production time. The yolk color in the albumen was measured between (6.17-5.37) from the first week to the fortieth week of laying, with the peak color (655) observed on the 32nd week. Previously, Ahmet and Zehra (2009) found that the age of the hen does not significantly affect the colour of the table egg yolk obtained from less old chickens (28 wks. = 10.2) and older hens (80 wks. = 10.206). According to the facts presented above, the interior eggs quality is determined by the strain and age of the layers. Because of this, the genotype and layers can't be ignored in breeding activities that aim for good production of eggs.

2.5.2.3 Haugh Unit

The Haugh unit is a formula that calculates the freshness of eggs using albumen height and egg weight (Haugh, 1937). The United States Department of Agriculture (USDA) categorized eggs into three groups: "AA," "A," and "B," using the respective Haugh unit values of 72 or higher, 60 – 71, and 31 – 59 (USDA, 2000). A higher haugh unit is often thought to indicate superior egg quality. As a result, the albumen height could be linked to the consumer's perception of egg freshness (Arthur & Sullivan, 2008). Haugh unit is often influenced by egg storage conditions, although white hens had a significantly greater Haugh unit than brown hens (Leyendecker *et al*., 2001). Yakubu *et al*. (2008) discovered an important difference in the Haugh unit values of naked neck (73.22) and regular feathered (71.40) chicken breeds. In the same way, Hagan *et al*. (2013) noted a notable difference in the Haugh unit in Bovan Brown (85.2), Lohmann white (77.4), and Lohmann brown (75.9) chickens.

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Zita *et al.,* (2009) observed a decrease in the Haugh unit from 90.34 to 81.34 to 80.02 in ISA Brown hens at 20-26, 36-42, and 54-60 weeks, suggesting that hen age could impact the Haugh unit.

Furthermore, Menezes *et al*. (2012) discovered that the Haugh unit in younger chickens declined significantly from 35 week (83.218) to 40 week (80.667) to 45 week (78.551) to 50 week (74.487). Also, Peri *et al*. (2017) recorded a decrease in the Haugh unit of old Bovans Brown for week 59 (70.35) compared to 81.77 for a 34-week-old group, demonstrating the detrimental effect of hen age on the Haugh unit.

2.5.3 Consumers Perception of Chicken Egg Quality

Defining quality of the egg is a matter of looking at the characteristics that make an egg appealing to customers. Consumers' satisfaction with shelled eggs is significantly impacted by a range of attributes like quality traits cleanliness, freshness, egg weight, and quality of shell (Sonaiya & Swan, 2004). Poultry eggs are highly versatile foods that include several vital elements which aid in sustaining life while the embryo is developing (Abanikannda *et al*., 2007) Additionally, eggs that come from chickens are a popular, healthy, affordable, and simple to-cook food option that provides a variety of essential nutrients for individuals of any age (Matt *et al*., 2009). All these are reasons consumers will gladly want to accept to consume some eggs.

2.5.3.1 Consumer Preferences for Egg Size

According to Chukuwuka *et al*. (2011), there is no standard for grading eggs in several African countries, as egg shells are sold individually. In many developed countries, like the United State, eggs sold are priced according to their weight, as most consumers prefer large egg sizes (USDA, 2000). Large

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and extra-large eggs are in high demand in America, according to Jacob *et al*. (2000). In a study of 273 homes in 23 Japanese areas, Hashimoto *et al*. (2011) found a similar pattern. According to the study, 50.7 percent of those polled preferred big eggs, citing the acceptable pricing and the size of the egg as evidence that egg size does important.

2.5.3.2 Consumer Preferences for Yolk Colour

According to Okeudo *et al*. (2003), the color of the yolk is a significant factor in all customer surveys related to quality of egg. When it comes to a person's taste in yolk colour, there is no universal standard. People's preference for yolk colour varies by geographic region, culture, and customs (Beardsworth, 2007), and those with a preference score of 12 or above, according to the DSM Yolk Color Fan, generally preferred the deepest yellow yolks. However, most European consumers favored yolks with the darkest shades when presented with a range of colors between 8 and 14.

2.5.3.3 Consumer Preferences for Shell Colour

Another component that determines customer decisions is shell colour. The colour of the eggshell is absent bearing value in terms of nutrition or quality of the egg's interior, nor does it indicate the egg's nutritional value (Flock *et al*., 2007). However, consumers frequently prefer white or brown eggs, which must be considered while selling eggs. According to Odabasi *et al*. (2007), consumers from some European and Asia countries preferred brown eggs to white eggs. In contrast, white eggs are the type of egg that consumers in the United States (Jacob *et al*., 2000; Johnston *et al*., 2011) and Japan are most interested in purchasing (Hashimoto *et al*., 2011). People in New England (Jacob *et al*., 2000), the UK, Australia, and New Zealand

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(Odabasi *et al*., 2007), for example, prefer brown-shelled eggs. This suggests that there is a cultural aspect to consumers' choice of egg shell hue. Uniformity of eggs on the tray was a significant quality attribute, comparable to the USDA system, of grading, where the absence of consistency in both dimensions of the object and color is regarded as a severe egg fault (USDA, 2000). USDA (2000) says that people don't like eggs that are different colours or sizes. Eggs that are categorized and packaged by color ("white" or "brown") are more popular among consumers compared to eggs that are a mixture of colors. On the other hand, Johnston (2011) found that cartooned eggs with different shades of colour were just as good as eggs that were all the same colour. The eggs that customers chose were impacted by their assumptions. They believe that brown eggs contain more nutrients than white eggs. Consumers' perceptions of the nutritional value of eggs must be changed via education, according to the study.

2.5.3.4 Preferences of Consumers Regarding Chicken Egg Breeds

Senbeta, Zekele and Molla (2015) conducted a survey to determine the views and thoughts of consumers in Eastern Ethiopia regarding the quality of chicken eggs. It was found that more than half of the people who took the survey preferred to purchase eggs from local chickens since the quality of the eggs was superior, and the yellow yolk was the most desired colour. Additionally, respondents indicated that local chicken eggs had a more pleasant flavour since the hens fed on natural rather than prepared feed (chemical feed).

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2.5.4 Biochemical Qualities of Chicken Egg

It has been demonstrated that the pH of the albumen is almost entirely impacted by the length of time the egg is stored, making it a reliable indicator of both the quality and freshness of the egg (Lordelo *et al*., 2020). The albumen pH rises as the egg loses water and carbon dioxide. Even though the eggs in this study were of the same age and were kept under the same circumstances, albumen pH was higher in Hybrid eggs and lowered in Preta eggs than in Amarela eggs. This could be an indication that the pH of the albumen is affected by variables other than the amount of time it was stored or the conditions under which it was stored. It has been suggested that the pH of the albumen may drop as the layer ages (Silversides and Scott, 2001). Nevertheless, per the findings of their study, Albumen pH may be profoundly affected by one's genealogy.

2.5.5 Microbial Qualities of Chicken Egg

Egg contamination can occur before oviposition, during oviposition through contact with external surfaces, or via infection of the reproductive tissues. The terms "trans-ovarian," "oviducal," and "trans-shell" refer to these contamination pathways, as discussed by Board *et al*. (1995). The trans-ovarian and oviducal routes are vertical routes of transmission, whereas the trans-shell path is regarded as a transmission path that is horizontal. Vitelline membrane, albumen, yolk, shell membranes, and eggshell can all become immediately contaminated prior to the egg being laid due to trans-ovarian and oviducal infections. Moreover, colonization of the reproductive tissues may occur due to systemic infection (Okamura *et al*., 2001; Gantois *et al*., 2009). When bacteria are devoured, they pass through the intestinal tract, and those

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with the ability to invade intestinal epithelial cells trigger an immune response. Consequently, the bacterial cells are engulfed by macrophages, which are big phagocytic white blood cells, and may even be destroyed when they penetrate the intestinal epithelium.

Nonetheless, certain bacteria have the ability to survive and reproduce inside these immune cells, leading to the widespread dissemination of infected macrophages to various internal tissues, including the reproductive organs, of hens that are kept for egg production (Gantois *et al.*, 2009). Although trans-ovarian transmission might not be a significant concern for many bacterial species, it is particularly alarming for Salmonella spp., especially S. enterica serovar Enteritidis (Gast *et al*., 2004). This is due to its high connection to reproductive tissues and its ability to withstand the defenses of antibacterial agents during the formation of eggs, eventually remaining within the egg (Okamura *et al.,* 2001; Gantois *et al.,* 2009). In addition to this, microorganisms have the potential to penetrate and infect the oviduct through a process known as ascending infection, which begins in the cloaca (Okamura *et al.,* 2001; *Gantois et al.,* 2009).

Subsequent to oviposition, contamination of the trans shell transpires when bacteria penetrate the eggshell, leading to potential contamination. Various factors impacting the level of contamination has been determined (Bruce & Drysdale, 1994). Once the egg is laid, there are multiple opportunities for eggshell exposure to different environmental surfaces, and the degree of infection is impacted by the sanitation conditions in the egg-laying environment (Board *et al.,* 1995). Eggshell contamination can originate from various sources, this includes waste, particles, materials from cages and

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bedding, conveyor belts, nesting areas, fractured eggs, and human hands. The level of contamination across different shell layers is influenced by eggshell porosity and sweating caused by temperature differences. Due to its porous nature, eggshells are susceptible to contamination from external sources. When eggs are laid, their temperature is approximately 42°C, and as they cool down to the temperature of the surroundings changes, the contents of the egg contract, resulting in a reduction in pressure inside the egg. This negative pressure can draw environmental and surface contaminants through the pores of the shell (Bruce & Drysdale, 1994).

Moisture also plays a role in contamination within the outer shell. Water, whether in liquid or vapor state, is crucial for bacteria to infiltrate the eggshell (Board *et al.*, 1995). Moreover, a temperature difference among the egg and the water surrounding it can further facilitate invasion by bacteria (Berrang *et al*., 1999). The level of pollution across different layers within the shell is exacerbated by the exhaustion of the eggshell outer layer (De Reu *et al.*, 2006).

According to Board *et al.* (1995), the levels of aerobic bacteria in eggshells have been analyzed found to vary between 2 and 7 log10 CFU (colony-forming units) per shell, with an average of 5 log10 CFU per unwashed eggshell. In studies involving washing eggs, Knape *et al.* (2002) recorded an average initial pollution rate of 3.9 and 4.6 log10 CFU per shell of an egg. Interestingly, the apparent cleanliness of an eggshell is not a reliable indicator of its contamination level, except for heavily soiled eggs (Smith, Rose, Wells, & Pirgozliev, 2000).

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In their study, Musgrove *et al*. (2006) gathered eggs from three distinct egg processing plants and performed tests to analyze biological molecules and compound are used to distinguish the isolates acquired from the eggshell. *Aeromonas, Chryseomonas, Cedecea, Citrobacter, Erwinia, Enterobacter, Escherichia, Klebsiella, Hafnia, Kluyvera, Listonella, Leclercia, Providencia, Morganella, Pseudomonas,* Prot*eus, Rahnella, Sphingobacterium, Salmonella, Serratia, Vibrio,* and *Xanthomonas* were the genera found in pre-processed eggs. Surface samples of table eggs have revealed the presence of yeasts and molds. Bacteria that are positive for gram staining, due to their ability to tolerate drier conditions, are better adapted for survival on the eggshell. In contrast, Gram-negative bacteria can thrive at experience colder temperatures and require less complex nutritional requirements, making them more prone to survive and thrive within the contents of the egg (De-Reu *et al*., 2008). These bacteria are more prone to causing deterioration of eggs. Aeromonas, Alcaligenes, Citrobacter, Escherichia, Hafnia, Proteus, Pseudomonas, and Serratia are some typical bacterial genera that can cause eggs to spoil.

2.5.6 Factors Affecting Chicken Egg Quality

Genetic and nongenetic factors mostly determine the phenotypic traits of an egg. Following this, the strain and age of the layers should not be overlooked. According to the findings of several different studies (Rizzi & Marangon, 2012; Aygun & Yetişir, 2014; Zita *et al*., 2009), The breed of bird greatly influences the quality of the eggs, whether it be the breed, lineage, or genetic composition. These researchers also found that factors related to the environment like housing, diet, and stress have an effect on egg quality. Concerns about animal welfare and storage, handling, and processing

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techniques for eggs can all impact their quality (Khan *et al*., 2013; Ryu *et al*., 2011). Rizzi and Marangon (2012) also reported the impact of age, which might refer to either the age of the hens who laid the eggs (the hen's age) or the amount of time the eggs were stored . Variations in quality characteristics and relationships of individual eggs as well as the quality of the overall egg can be attributed to these variables. According to the findings of a number of studies, coloured chickens produce eggs that are both heavier and larger than their white counterparts. These findings suggest that the genotype or strain of the chicken plays a significant role in determining egg weight and shell properties (Vits, *et al*. 2005 ; Halaj & Golian, 2011). Different genotypes may differ in egg weight, shell quality, and content (Garces & Casey, 2003). In addition to the genotype of the hen and the age of the hen, other factors, including an approach to management, feeding quality, duration of storage, and storage condition, can have an influence on one or more egg quality characteristics.

2.5.6.1 The Influence of Housing Method on the Quality of Chicken Eggs

The majority of studies on how housing methods affect egg quality have focused on comparing cage systems with free-range systems (Holt *et al*., 2011; Samiullah *et al*., 2017; Sokołowicz *et al*., 2018), but recently, researchers have started to look at how different types of cages affect egg quality. There have been limited studies on whether the housing system of eggs (litter, free-range, organic) affects shell quality, which is significant for both commercial and nutritional reasons.

Tumova *et al*. (2011) discovered that the thickness of eggshells was significantly smaller in cage-produced eggs. Jones *et al*. (2014) looked at eggs

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from hens living using traditional cages, improved colony cages, and free-range aviaries. They found that the significant changes changes were noticed in the weight of egg, the height of albumen, the Haugh unit, and the static compression shell strength. The eggs' weight (g) and shape index (percent) remained the same and were not influenced by the different housing methods. In opposition to the current results, Leyendecker *et al*. (2001) and Tumova *et al*. (2001) recorded heavier eggs deposited in cage housing, whiles Pistekova *et al*. (2006), Tumova and Ebeid (2005), and Hidalgo *et al*. (2008) found eggs with more weight in the conventional housing system. From the review above, it seems that the non-cage housing system negatively impacts the rate of egg laying, quality indicators of eggs, and overall safety of eggs (Matthews & Sumner, 2015). This could result from various bird behaviors and contact between eggs and the floor, with floor-laid eggs being at higher risk of exposure to pathogens.

Furthermore, poor utilization of system resources by the hen's in non-cage systems may result in numerous eggs placed around the nest and droppings on a considerable percentage of the litter and nest (Philippe *et al*., 2020). When in contrast to a littered floor system, Englmaierová *et al*. (2014) observed that cages produced the most eggs and used the least amount of daily. In contrast, Philippe *et al*. (2020) observed that aviaries had lower production of eggs and weight of eggs than cages. Ledvinka *et al*. (2012) found that the cage housing system produced lighter, thinner, and weaker eggs than a littered floor system. Even though the cage housing system makes it hard for the birds to move around, it still reduces the number of broken eggs more than systems without cages (Holt *et al*., 2011; Kontecka *et al*., 2018).

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Van Den Brand *et al*. (2004) looked at the difference in egg quality between hens kept on the range with males and hens kept in individual cages. Consequently, the free-range eggs had a darker yolk color. The authors discovered exterior and interior quality (as measured by the quality of both physical and compositional aspects of the eggs) in free-range eggs. Moreover, as the hen gets older, the variety of free-range egg quality also increases. The authors concluded that according to these findings, additional research was required to discover what factors influence the differences in the standard of eggs sourced from free-range environments.

The most detailed report on how eggs work and how hens live was written by Hidalgo *et al*. (2008). In their research, they looked at the differences between caged, cage-free, organic, and free-range housing. The whipping ability and foam consistency of organic eggs were the best, and their Haugh unit ratings were the lowest (indicating lower quality of eggs). Cage-raised eggs were the freshest because they could be whipped up the least. Hidalgo *et al*. (2008) attempted to create a multivariate method using partial least squares regression to differentiate eggs based on how they were produced. Only eggs that were raised in cages could be told apart from eggs that were not raised in cages in a reliable way. Strength of shell cracking, increase in whip volume, amount of protein, and thickness of shell were the most effective ways to tell them apart. Even though the method could not tell the difference between the different ways of producing eggs, it is hoped that it was able to consistently tell the difference between eggs produced in cages and eggs produced without cages.

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2.5.6.2 The Influence of Breed on the Quality of Chicken Eggs

The breed of chicken directly impacts the quality of eggs. Different species, breeds, strains, and families have different genes that affect the quality of their eggshells (Buss, 1982). For example, the colour of an eggshell is mostly determined by the hen's genes. Chickens that have white feathers produce eggs that are white, while chickens with brown feathers lay eggs that are brown (Jacob *et al*., 2000). Egg weight varies greatly between lines and eggshell thickness. However, eggshell thickness varies slightly amongst related breeds Benavides-Reyes, Folegatti, Dominguez-Gasca, Litta, Sanchez-Rodriguez, Rodriguez-Navarro, & Faruk, 2021). The strain of a breed, hen age, and size of the egg all affect the yolk percentage. (Suk & Park, 2001). The Egg shell quality is defined by the weight and thickness of the shell. and strength. The eggshell quality varies based on climatic circumstances, feed quality, and layer strain (Zita *et al*., 2009). In contrast, Khan *et al*. (2004) discovered that breed did not affect eggshell thickness under semi-scavenging circumstances.

Furthermore, multiple investigations Scott and Silversides, (2001); Silversides and Budgell (2004) found that heights of albumen varied depending on breed. According to Monira *et al*. (2003), the breed substantially impacted Haugh units. Eggs that have a Haugh unit measurement of 70 or higher are classified as high-quality eggs, according to Jayasena *et al*. (2012). Furthermore, Stadelman (2002) revealed that greater Haugh unit values suggest higher albumen quality.

Egg yolk colour resulting from color of eggs is a crucial factor that is taken into account in the egg production sector (Lokaewmanee *et al*., 2011).

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Samiru, Wickramasuriya, Young-Joo, Nu, *et al*. (2015) observed did not notice any significant variation ($p>0.05$) in yolk colour, as egg yolk colour ranged from 7.8 to 10.0, suggesting Korea Native Chicken possess a vibrant golden hue egg yolk. Hagan *et al*. (2013) noted a lack of significant variation in yolk hue across various types of chicken breeds. Likewise, Haunshi *et al*. (2011) found no statistically significant variation among different breeds of chicken.

Singh *et al*. (2009) discovered substantial variations in hen-day egg output, food consumption, and egg mass can be observed between Lohmann Brown H&N White and Lohmann White, and. when they studied hen performance differences amongst commercial hybrids. Additionally, significant differences in eggshell strength were found in tests conducted using brown crosses, including Isa Brown, Moravia BS and Hisex Brown (Ledvinka *et al*., 2012; Tůmová *et al*., 2011). The disparity in the values of strength of the shell of an egg might be attributed to the poor heredity of eggshell strength.

Ali, Campbell, & Siegford, (2020) thoroughly examined the disparities in rearing and quality of strains brown and white and egg. He said that brown chickens laid heavier eggs (61.1g) than white hens (58.3g). The increased egg weight of commercial strains is not surprising, given that such strains have been subjected to significant pressure on breeding for egg weight enhancement (Hocking *et al*., 2003). In addition, egg weights of 43 g 52.5g, 52.1g, and were recorded for Fayoumi, White Leghorn, and Rhode Island Red respectively, in smallholder facilities in northern Ethiopia (Lemlem & Tesfaye, 2010).

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2.5.6.3 Effect of Age on Chicken Egg Quality

According to Johnston and Gous (2007), not only does the housing system and genotype impact egg quality, but the age of the hen has an impact on the outcomes of egg quality assessments. Several studies have found that, except for egg weight, practically all egg quality attributes decrease as chickens age (Altuntaş & Şekeroğlu, 2008; Ledur *et al*., 2002). As the hen grows older, the weight of its egg shells gradually steadily declines, with no discernible pattern (Popova-ralcheva *et al*., 2005; Silversides & Budgell, 2004). Van Den Brand *et al*. (2004) discovered that as hens get older, the weight of their yolks and albumens go up. Danilov (2000) examined the relationships among egg weight, length, and breadth. He revealed that the addition of egg yolk, egg white, and eggshell that makes up an egg's weight keeps increasing as the hen gets older and stays the same when the laying cycle is completed. The weight of egg components, particularly egg albumen and yolk, is influenced by the weight of the egg (Aygun and Yetisir, 2010; Zhang *et al*., 2005). Therefore, weight of egg is crucial in determining the quality of eggs and the productivity of chickens. (Farooq *et al*., 2001; Islam *et al*., 2001). As hens get older, the thickness of their eggshells can either reduce (Benavides-Reyes, *et al*., 2021) or stay the same (van den Brand *et al*., 2004). Alongside temperature, hen age, and quantity of calcium in the hen's diet, all of these factors affect eggshell thickness (Zeidler, 2002). One of the primary concerns is that as the hen ages, its quality of eggshell may decline due to increased egg weight, although no gain in calcium carbonate found in the shells. As a result, the number of broken eggs at the completion of the laying cycle might be as high as 20 percent (Nys, 2001).

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Philippe, Mahmoudi, Cinq Mars, Lefranc Moula, Palacios, Pelletier, and Godbout (2022) found that the age of the chickens affected the quality of the eggs, and that this was true for all housing systems. Increased with time, the proportions of the constituents varied. Because of this, the percentage of egg yolk rose during the laying phase, while the egg white and shell percentages of an egg decreased. This discovery is consistent with previous research findings (Samiullah *et al*., 2017; Yilmaz Dikmen *et al*., 2017). As hen ages, a reduction in shell thickness could indicate a lower level of calcium shell formation cycle (Samiullah *et al*., 2017). This helps to explain the drop in strength of an shell seen in their study.

Aygün and Narinç (2017) showed that discrepancies in quality of eggs characteristics might be evaluated amidst old and not old chickens due to declining egg output. Shell quality degrades in aged hens; whereas egg size grows with age, the weight of the shell can be altered favourably or adversely by age (Roberts, 2004). The height of albumen is associated with the duration of uninterrupted egg-laying by a hen or constantly laid eggs without undergoing a molt. Furthermore, when a hen grows older, the albumen height will decrease (Ledur *et al*., 2002; van den Brand *et al*., 2004). This is because the albumen height of the egg decreases as it matures (Silversides and Budgell, 2004). van den Brand *et al*., 2004) measured height of albumen in addition to shell index and weight. The average height in the youngest hen was 7.27 mm, whereas the mean height in the oldest hen was 7.78 mm.

2.5.7 Chicken Egg Quality Assessment

Eggs are incredibly significant in the diet of human since they are a high-quality proteins source, easily digestible, and an integral ingredient in the

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food industry. However, eggs are considered moderately perishable, as the quality degrades faster when not well preserved or used in the shortest time. This affects physical, chemical, nutritional, and sensory qualities. In addition, the internal component of egg's quality starts to go bad shortly after being laid. This is due to albumen thinning, increased pH, weakened and stretched vitelline membrane, and increased yolk's water content. As a result, fresh production and quality are key to consumer preference for eggs (Karoui *et al*., 2006, Hisasaga *et al*., 2020). Identifying specific criteria to assess the quality and freshness of eggs has posed a significant challenge throughout the century (Stadelman *et al*., 2017). A contrast can be established involving either destructive or non-destructive approaches for assessing egg quality.

2.5.7.1 Destructive Methods of Agricultural Product Quality Evaluation

The most often used parameters for detecting egg quality among destructive processes include strength and thickness of shell, size of internal air cell, ratio of albumen to yolk, viscosity of albumen, height and pH of albumen, color and shape of yolk., and vitelline membrane strength (Eddin *et al*., 2019). Shell strength and thickness of chicken eggs are directly related to their viability as they are transported and stored. Noticeable factors like breed, age, and diet tend to impact egg quality traits significantly. Fragile eggs result in financial losses and increase the chance of microbial infection through cracked eggs (Eddin *et al*., 2019).

An egg's air cell is created as a result of the contraction of the egg's contents that takes place when the egg's temperature decreases after it is laid. This is accomplished by separating the membranes of both the internal and external shells located at the pointed end of the egg. Because water evaporates

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and carbon dioxide escapes via cracks in the eggshell, it continues to grow in size (Stadelman & Cotterill, 1995). Egg weight and storage conditions are also factors that might have an effect on it. The sole numerical measurement for determining egg freshness evaluated by the European Union rule is air cell height (Karoui *et al*., 2006). Albumen thinning is noticeable marker of degradation of quality of internal caused mostly by alterations in the ovomucin-lysozyme complex. Haugh Units (HU) are commonly used to quantify albumen freshness. The micrometre placed on a tripod has been used since 1937 to measure the thickness of the dense egg white 1 cm away from the yolk, while ensuring that chalazae are avoided. The conversion in HU may be done using an appropriate equation that takes the egg weight into account. Due to albumen thinning, HU diminishes with storage duration (Stadelman & Cotterill, 1995).

Another measure of the freshness of eggs is their pH. The pH level of the albumen in newly laid eggs usually falls between 7.6 and 8.5. The level rises as carbon dioxide is released through the shell pores while in storage. (Karoui *et al*., 2006). The yolk undergoes various modifications, including colour change and shape distortion. Colour is an essential component in consumer acceptance, and various countries have distinct colour preferences (Edin *et al*., 2019). The vitelline membrane weakening primarily causes changes in yolk shape, and its measured using the Yolk Index (YI), which is estimated to be the ratio of height of yolk to yolks width (Stadelman & Cotterill, 1995), or the Yolk Coefficient (YC), which is calculated to be the ratio of weight of yolk to the yolks height (Abdanan Mehdizadeh *et al*., 2014). When the membrane of the egg yolk breaks, it can cause the yolk to become

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scattered and can also lead to the formation of foam, particularly during transportation. Human eyes have a hard time detecting such flaws while candling (Zhang *et al*., 2015). Destructive studies provide the advantage of taking measurements directly on top of the egg component of interest, resulting in more dependable results. However, they take a long time, need skilled personnel, and can only be utilised in the laboratory, Ignoring the pressing necessity of fast and dependable techniques for assessing the quality of egg production lines.

2.5.7.2 Non-Destructive Methods of Animal Product Quality

Non-destructive methods for swiftly and accurately detecting the wholesomeness of intact foodstuffs have recently has been examined by various researchers. One of the most researched fields is the food industry. Karoui and De Baerdemaeker (2007) explained how Nuclear Magnetic Resonance , Front Face Fluorescence (FFF), Mid Infrared (MIR) and Near Infrared (NIR) spectroscopy, as well as stable isotope and spectroscopy (NMR), could be applied in the food industry. All of these spectroscopic methods make sure that samples don't need to be prepared much or at all. This cuts down on the time and cost of analysis.

2.5.7.2.1 Visible/Near-Infrared Spectroscopy (NIRS)

Spectroscopy is a non-destructive method that could potentially be utilized online to check the quality of eggs and find problems. Overtones and combinations of C-H, N-H, O-H, and S-H flexing and extending oscillations are seen as absorption bands in the near-infrared range. As a result, NIR technologies apply to all organic substances with numerous C-H bonds, such as; oil-based derivatives, hydroxyl (O-H) bonds such as; carbohydrates,

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moisture and lipids, and N-H bonds, such as those in amino acids and proteins. Furthermore, because all organic materials include several O-H, N-H, and C-H chemical bonds, NIR light impacting a specimen generates a composite spectrum that contains statistical and descriptive data regarding the sample (ElMasry & Sun, 2010).

Dong *et al*. (2017) looked into some interesting ways that transmission Spectroscopy in the Visible-Near Infrared (VIS-NIR) range can be utilised to analyse the freshness and quality of eggs without damaging them. SG, MSC, and SNV were added to the eggs before the spectrums were collected using a fiber optic probe positioned directly on the central portion of the eggs. To craft or produce a predictive model for the shell thickness of eggs, diameter of the air chamber, pH of albumen, and overall pH of egg, the PLSR approach was utilised. Dong *et al*. (2017) conducted an investigation on the thickness of eggshells using a total of 70 entire, eggs with white shells. The training group comprised of 52 samples, whereas the verification group of 18 samples. The best correlation coefficients found in calibration (rc) and prediction (rp) (MSC-treated spectra) were 0.86 and 0.84, respectively, with RMSE in calibration (RMSEC) and prediction (RMSEP) of 0.01 mm, measured using a vernier caliper in the equatorial area. Ninety eggs with brown shells in total were utilized to determine the air chamber diameter, with 68 of those eggs serving as calibration samples and the remaining 22 serving as prediction samples. After shelling, the air chamber's diameter varied from 15.82 to 35.32 mm when measured with a vernier calliper. After applying the MSC treatment to the spectra, we obtained the best PLSR model, which had an rc of 0.87 and

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an RMSEC of 2.13mm, as well as rp and RMSEP, all of which were 0.85 and 2.14mm.

Aboonajmi *et al*. (2016) found similar findings for air chamber height prediction using Visible-Near Infrared spectroscopy on 300 eggs maintained in varying circumstances (temperatures of 25°C and 5°C, with relative humidity (RH) of 40% and 75%, respectively, were maintained for 30 days.). Information gathered from spectroscopic analysis were adjusted for scattering's multiplicative and additive effects, dimensioned using PCA, and elaborated using an ANN approach that was internally confirmed using CV. The eggs held at 5 and 25 ^{o}C had R^2 values of 0.844 and 0.835, respectively. There was no information concerning predicted errors. The HU was also evaluated, with R2 values of 0.767 and 0.745 in CV. Changes in the thickness of the albumen, which could have impacted the outcomes, were linked to the differences seen in the datasets from room temperature and low temperature. So, it was shown that spectroscopic technology could be used for preliminary screening, but more studies are needed regarding the optical fiber utilized. Certainly, integrating data into one model and conducting a validation from outside sources can enhance the reliability of the suggested method.

In fact, Akowuah *et al*. (2020) called for the creation of a forecasting model for HU and the marking of the date of lay, taking into account the different ways eggs are stored, after they found that the HU of eggs kept at low temperatures was indirectly related to how long they were kept. As a result, environmental data is critical in providing consumers with an accurate estimate of how long their items will be stored. One hundred twenty brown-shelled eggs were utilized in the study, placed in either a 4°C or 28°C

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environment for a maximum of 20 days prior to examination using a portable NIR device operating in the 740–1070 nm wavelength range. Following MSC pre-treatment, classification models were created by using LDA, the samples were categorized into four groups based on storage time at the two different temperatures. The prediction accuracy rates were 96 percent for cold storage and 100 percent for ambient storage. The authors subsequently constructed a PLS model to predict storage length of time, with rp scores of 0.89 and 0.91 achieved under ambient and cold storage conditions. Using near-infrared (NIR) reflectance spectroscopy, Zhao *et al.* (2010) created a system that could distinguish between fresh and stale eggs with a 93.3 percent identification rate within the prediction dataset. Later, Lin *et al*. (2011) employed NIR reflectance spectroscopy to predict the HU using a model integrating artificial neural networks with genetic algorithms and discovered a correlation value of 0.879. Furthermore, several papers explored Using VIS-NIR transmission spectroscopy to assess the freshness of eggs. Mehdizadeh *et al*. (2014) discussed the HU, yolk index, and duration of storage using VIS-NIR transmission spectroscopy.

Norris (1996) looked at how storage affected the visual properties of shell eggs within the near-infrared spectrum. Despite the fact that spectral data progressively changed during storage, there was really no significant relation between these variations and inner egg quality indicators, helping to further research and support for new technology. Bamelis *et al*. (2002) used spectrophotometry to find blood in Table eggs and look into the possibility of identifying early embryos in chicken eggs. It was said that embryo development could be seen as early as day 5 (120 hours) of incubation. This

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could be because spectroscopic methods only collect information from a single point, which is bad if the pertinent data isn't in the pixel spot that was measured. This shortcoming is addressed by hyperspectral imaging technology. Near-infrared (NIR) areas have been effectively employed for food high standard and security studies (Abdel-Nour *et al*., 2011; Williams & Norris, 1987Osborne *et al*., 1993) and might also be helpful for chicken egg quality assessment research.

2.5.7.2.2 Raman Spectroscopy

Raman spectroscopy has been utilized sparingly to test the freshness and quality of eggs without damaging them. Liu and colleagues (2020) introduced Raman spectroscopy as an easy, quick, and non-destructive technique for identifying changes in the cuticle related to freshness attributes. 125 Hy-Line Raman spectra (100–3000 cm-1) were used to judge the quality of 59-day-old brown eggs that had been kept in controlled conditions (20 ^{o}C, 40% Relative Humidity). We measured the egg's equatorial and bottom (blunt) areas with a spacing of 6 mm between the probe and the shell. The standard parameters evaluated were HU, YI, egg white pH, and dimensions of the air chamber. When employing Raman spectra, different pre-processing algorithms are used: SG, NL, first and second derivatives, SNV, BL, denoise and MSC. Following that, PLSR models are constructed for each freshness indicator, utilizing 80% of the samples for calibration and 20% for prediction. With rp ranging from 0.807 to 0.895, good results are achieved for Haugh unit (HU), albumen pH, and air chamber size. However, with spectra altered in the second derivative, YI is not successfully predicted, thereby achieving a

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maximum rp of 0.540. This might be owing to a weak correlation between eggshell surface alterations and yolk modifications.

In addition, the optimal acquisition area is examined, demonstrating that the PLSR model constructed from the highest Raman spectrum performed best for the tested criteria for freshness, with rp values increasing from 0.83 to 0.93. This could be attributed to the lack of an air pocket at the egg's apex, resulting in continuous contact between the egg's contents and shell. Other factors, like breed, age of the hen, and how it was raised, must be considered to make the models more flexible and make the best use of Raman spectroscopy.

2.5.7.2.3 Dielectric Spectroscopy

Recently, a few attempts have been made to use dielectric techniques to measure the quality of shell eggs carefully to avoid any damage (Akbarzadeh *et al*., 2019; Soltani & Omid, 2015). Egg quality parameter indices like the air chamber height, thickness of the albumen, HU, the albumen pH, and yolk color were the focus of the research that was conducted by Akbarzadeh *et al*. (2019). The researchers wanted to propose a microwave spectroscopic technique that was utilized on a waveguide and network analyser device in order to do so. Eggs with a white shell (244) were used for the investigation and stored at room temperature for the duration of the research, which was twenty-four days (25 ^{o}C). In the microwave band from 0.9 to 1.7 GHz, mean reflection and transmission spectra were taken right before the destructive assessment of freshness parameters. As a result, a variety of chemometric methods were employed in the development and evaluate regression and classification algorithms. Only albumen pH could not

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be predicted accurately by the return loss reflection spectra, which had a Residual Prediction Deviation (RPD) of greater than two. The RPD for the air chamber height was particularly close to 3. RPD equals the standard deviation divided by RMSEP which serves as a performance metric for constructed models. Higher RPD values indicate effective calibration models as they are produced by decreasing RMSEP and increasing standard deviation. As a result, RPD values of 2 or 3 suggest outstanding calibration. The top predictive models in regression using ANN were discovered by utilizing diverse input spectra. Except for pH albumen (RPD = 1.83), all freshness parameters had RPD values greater than 2. When it came to classification, either SIMCA or the ANN algorithm yielded good results. The return loss reflection spectra showed the highest ability to differentiate between six different freshness classes with a perfect accuracy rate of 100 percent. Even though the outcomes were favorable, the writers emphasized the importance of creating an affordable technique, since the current network analyzer used was costly, and using the method for online activities. Soltani & Omid (2015) investigated the 40 kHz to 20 MHz radio frequency range to create a reliable model for identifying the freshness of eggs without damaging them. Machine learning techniques such as ANN, SVM, BN, and DT were employed to integrate dielectric spectroscopy.

In addition, the CFS algorithm was used on the spectra to reduce the feature vector size from 387 to 24 elements. A total of 150 eggs with white shells were divided into 110 for the calibration set and 40 for the prediction set. These eggs were stored for varying amounts of time (up to 24 days) at 20 ^{o}C and 35% relative humidity. Except for DT, which achieved 87.5 percent

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classification accuracy, all machine learning approaches produced 100 percent classification accuracy. Additionally, air cell height was estimated using regression techniques such as ANN, SVM, and DT. All created models performed well; however, the Meta-Super-Peer DT had the lowest errors (RMSEP = 1.043 mm). To minimize mistakes, Soltani and colleagues (2015) employed a Feedforward Neural Network called Multilayer Perceptron with the Levenberg-Marquardt method. Through the use of HU, YI, yolk/albumen ratio, and yolk weight, we can egg quality, an R2 invalidation of 0.998, 0.998, 0.998, and 0.994 was achieved with 287 white eggs kept at 20 degrees Celsius with a relative humidity of 35% for a maximum of 24 days. The Haugh unit, Yolk index, yolk/albumen ratio, and yolk weight had absolute percent errors of 5.41, 6.84, 8.79, and 4.24 percent, respectively, in prediction mode.

2.6 Chemometrics Algorithms

According to the International Chemometrics Society, chemometrics is defined as "the science of linking measurements taken on a chemical system or process to the system's condition via mathematical or statistical methodologies" (Hibbert *et al*., 2009). Due to the wide absorption bands that overlap in NIR spectra, the analysis and pre-processing are challenging of the data rely on using mathematical transformations through MVA techniques. Utilizing specialized computer programs, mathematical algorithms such as MLR, PCA, or PLS regression are employed to connect chemical data with spectral data (Brereton, 2003). The process of utilizing chemometrics to link chemical and spectral information for developing qualitative and quantitative methods is referred to as spectroscopic calibration (Mark, 2006).

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Various mathematical algorithms may be applied to the primary data at various points during the calibration process. Raw data, like raw materials, can be preprocessed, also known as data pre-treatment, to simplify information extraction from NIR spectral data by reducing spectrum complexity and eliminating interferences (Gemperline, 2006). Unsupervised categorization methods are common in the food industry, but supervised classification methods like correlation and distance-based approaches are preferred. Cluster analysis is one of the observed classification methods, as mentioned by Reich in 2005. Additional supervised methods are linear discriminant analysis (LDA), soft independent modeling of class analogies (SIMCA), and partial least squares discriminant analysis (PLS-DA) as described by Geladi (2003) and Luypaert *et al*. (2007).

2.6.1 Pre-processing Methods

Pre-processing is frequently applied to improve specific spectral characteristics and eliminate noise in the spectra. For instance, methods such as derivatives and multiplicative scatter correction (MSC) are commonly employed to minimize baseline shifts caused by factors like particle size and variations in instrumental settings (Gemperline, 2006).

i. *The second derivative* pre-processing is commonly utilized to enhance spectral clarity, and adjust for spectral shift and incline caused by variations in instrument reaction (Gemperline, 2006). Calculating the first derivative concerning wavelength (w), for example, removes the shifts the component (C) and turns the slope term (a) into a fixed value. As a result, taking the second derivative concerning wavelength is a typical method to reduce both offset and slope (Eq. 2.1):

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$$SEC = \frac{d^2}{dw^2}[f(w) + (a.w + c)] = f'(w) \qquad \text{(Eq. 2.1)}$$

As a result, if each spectrum f(w) has a slightly varied slope (a) in a data set, the first derivative spectra will vary in offset. The second derivative can be calculated as the derivative using a second-order finite-difference method. For this method, the segment length and the spacing between segments must be specified (gap size). Distinguishing three sections at one extremity of the spectrum, each separated by a gap, is the first step in computing the second derivative. The first, second, and third segments' average absorbance values are determined (A, B and C, respectively).

Finally, the value of the second derivative, A-2B+C, is used in the middle of the next section. The entire series consisting of three parts and two intervals is. then relocated to a single data point, as well as the computations are rerun so that every data point in the spectrum has a second derivative value (Gemperline, 2006). Gemperline (2006) mentions additional pre-processing techniques like N-point smooth, SNV, baseline correction, Detrend, S-G, and MSC.

i. N-point smooth is a method of smoothing using a boxcar that has a specific segment size dictating its dimensions in nanometers. The segment's average spectral value is placed in the middle (Gemperline, 2006).

ii. *Standard normal variate (SNV)* is a scatter correction approach for normalizing wavelengths whenever the effective path length differs between samples in a data collection. For example, pathlength variation can arise while analysing the spectra of granular or powdered

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materials owing to non-reproducible sampling or variances in particle sizes among samples (Gemperline, 2006).

iii. Baseline correction helps to minimize spectral shift by deducting either a spectral value at a designated wavelength or a manually given constant value. (Gemperline, 2006).

iv. *Detrending* is a technique for removing a spectrum's baseline displacement, gradient, or curvature. This is performed by subtracting the spectrum from a base function derived from the sample spectrum by performing a least-squares fitting calculation. (Gemperline, 2006).

v. *Savitzky-Golay (S-G)* method of smoothness is well-known, and derivative computation is based on a polynomial's least-squares fit o a section of the spectrum Despite the fact that the S-G and Detrend methods both utilize polynomial functions in a least-squares fit, they have varying scope and impact. Detrend fits the spectrum using a single polynomial function. On the other hand, using coefficients from a segment's data points, one can create a smoothed or derivative spectrum of any order by fitting a polynomial function using the S-G technique. (Gemperline, 2006).

All of the arithmetic pre-treatments that have been discussed up until this point are founded upon and implemented on individual spectra. This means that they function on the data points contained within each spectrum and produce outputs that are determined by the characteristics that are specific to that spectrum. Multiplicative scatter correction (MSC) is a method for scatter correction that relies on a collection of spectra that are related to the sample set. MSC computes the average spectrum from all spectra within a

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specified data set. After that, an analysis is conducted using least-squares linear regression to compare the absorbance values in the sample spectrum with those at corresponding wavelengths in the mean spectrum. This method may be represented as a linear equation with the intercept and slope already determined. Once that stage is finished, the intercept value is subtracted from each data point in the spectrum, resulting in absorbance values that are then divided by the slope value. The same series of procedures are carried out on each analyze the range of frequencies in the dataset using the average spectrum as a reference point (Gemperline, 2006).

Individual spectra are subjected to pre-treatments such as derivatives, SNV, Detrend, and S-G without prior knowledge of how the resultant spectrum should appear. Alternatively, the MSC method calculates an average spectrum by assuming that the spectra in the dataset follow a normal distribution. Therefore, the average spectrum is the most likely spectrum to represent all the spectra in the collection. (Gemperline, 2006). Individual spectra are basically forced to behave as much as possible like the mean spectrum by MSC. The approach is based on a computed average spectrum that nearby resembles the genuine average spectrum based on a huge sample set. In order to create quantitative methods, datasets need to typically include the whole variety of chemical and spectral variations found (Gemperline, 2006). Compared to quantitative models, sample sets for qualitative model building may be rather extensive, with hundreds of goods frequently included. Only a few samples may be included in a specific package. Therefore, MSC may be less effective for qualitative analysis compared to quantitative uses

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that require eliminating pathlength and slope variation with one pre-treatment. (Gemperline, 2006).

2.6.2 Principal Component Analysis (PCA)

It is common to find closely related variables in NIR spectra, showing that absorbance at multiple wavelengths is interconnected. When employing linear regression models, this is known as multi-collinearity, which can pose issues during calibration (Brereton, 2003; Wold, *et al*., 2001). Regressions of observed NIR spectral data against reference data produce calibrations. To conduct chemometrics, these variables must be organised into matrices. Dependent variables and independent variables are the two sorts of variables. The observed absorbances are considered independent variables, whereas the dependent variables must be able to anticipate (for example, levels of concentration). The associated values of absorption for each sample are inserted the absorbance matrix contains X-values arranged as a column vector, while the concentration matrix holds Y-values, specifically as the known concentrations. (Brereton, 2003; Wold, *et al*., 2001). Hence, it is essential to decrease the data in the pre-processed spectra to aid in categorization and adjustment and address interdependence of variables. PCA is frequently used in qualitative NIR analysis to compress data by reducing dimensions and is employed as a tool for identifying patterns without supervision (Brereton, 2003; Wold, *et al*., 2001).

Yet, in the quantitative analysis of NIR data, the PLS regression method is widely used as a linear calibration technique. It decreases the quantity of data and does regression simultaneously (dimension reduction of the NIR spectra matrix) (Folli, de Paulo, Santos, Nascimento, da Cunha,

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Romão, & Filgueiras, (2023).). PCA operates with data that has been pre-processed and does not depend on the outcomes of other analytical techniques. The fundamental objective of principle components analysis (PCA) is to use mathematics to decrease the quantity of originally linked absorption values with fewer principal component factors. Vectors are the primary mathematical components. The typical alterations in the dataset are represented by the most suitable selection of main components. The data's first main component accounts for the majority of the variation. All following principal components are perpendicular to the previous main component and are determined to possess the least amount of leftover variance. This procedure is repeated until all left is noise (Brereton, 2003; Wold, *et al*., 2001).

Over-fitting is characterised as picking an excessive number of basic components, such that one or more of them represent just noise. Nonetheless, reducing the number of principal components too much (under-fitting) can result in the loss of crucial information. Avoiding both over-fitting and under-fitting is essential to maintain the model's robustness and prediction efficiency. For instance, an overfitted model can give great results for samples in the calibration group but struggle to accurately predict sample attributes in an external validation dataset (Brereton, 2003; Wold, *et al*., 2001). Each principal component represents the entire range of data being expressed as a linear mix of the original factors. A score indicates the relationship between each original variable and the main component. Loadings describe the direction of a primary component vector. Score plots are employed for the interpretation of NIR data and detection of outliers. (Brereton, 2003; Wold, Sjöström, & Eriksson, 2001).

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2.6.3 Qualitative Methods

Qualitative analysis involves recognizing patterns chemometric approach that permits the clustering of analytes with similar characteristics in order to build classification strategies for unknown analytes. The qualitative analysis means the classification of analytes based on their NIR spectra information. Generally, classification methods fall into two groups: unsupervised algorithms and supervised algorithms (Reich, 2005). The unsupervised algorithms are pattern recognition methods that do not necessitate any prior information about the analytes to be classified, except the NIR spectra, but instead produce the clustering itself. Whereas the latter category classifies analytes based on prior knowledge, the category membership of analytes is needed. As a result, the classification model is developed using a calibration set of analytes whose categories are already known, and the model's performance is assessed through contrasting the predictions made by the classification model with the actual categories of the validation analytes (Reich, 2005).

2.6.4 Quantitative Methods

Although NIR spectroscopy has the potential to substitute time-consuming and expensive methods, it also comes with its own set of limitations. Hence, assessing the sample type and carrying out a feasibility study are crucial before starting a project involving quantification. The limited molar absorptivity of NIR radiation results in decreased sensitivity but allows for sample analysis. Typically, NIR spectroscopy is unsuitable for measuring components with concentrations below 0.1 % due to the example matrix (Burns & Ciurczak, 2001). Because of the way chemometric approaches work,

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it is essential to avoid having elements that are mutually correlated, which means that they are both rising and decreasing at the same time. This might result in inaccurate calibrations. During the course of In a separate experimental design or within the feasibility study, inter-correlation can be managed by altering component concentrations to make up for their reliance on each other (Reich, 2005).

The calibration set in quantitative method development is the sample spectra that are utilized in creating the calibration model. Similar to qualitative analysis, every sample in the calibration dataset is validated using the laboratory reference method to verify the identity and concentration of the components in order to establish the calibration equation. It is necessary to estimate the standard error of the laboratory (SEL) reference technique in relation to the standard error of calibration (SEC) and standard error of prediction (SEP) of the NIR spectroscopy method (Reich, 2005). In addition, it is essential that the samples be evenly distributed over the scope of concentration, and ensuring the accuracy of the data be centrally balanced, even if, in actuality, the results of analytical tests have a normal distribution the majority of the time, utilizing proper pre-processing techniques like the second derivative and an applicable multivariate approach like PLS can address these concerns. Regression is another term for utilizing multivariate techniques to develop a calibration equation. The multivariate method chosen will vary based on the data type and personal preference of the user (Reich, 2005). MATLAB 9.5.0 from Mathworks Inc., USA provides multilinear regression (MLR) and partial least squares (PLS) regression as multivariate techniques according to Brereton's study in 2003.

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a. Multilinear regression is a basic type of multivariate analysis that depends on the user's expertise in choosing the right wavelengths for calibration with minimal interference from other matrix components. Selecting only one wavelength results in normal linear regression, while choosing two or more wavelengths transforms it into multiple linear regression (MLR). Reich (2005) suggests using MLR for basic matrices with a small number of components, although it is hindered by multi-collinearity.

b. *Partial least squares (PLS*) regression is a method that utilizes PCA and can manage intricate matrices without the need to identify wavelengths that are uncontaminated by interfering substances. Using all wavelengths helps determine the relationship between spectral and chemical characteristics unlike MLR which only uses specific wavelengths (Brereton, 2003; Haaland & Thomas, 1988).

2.6.4.1 Partial Least Squares (PLS) Regression

PLS is a regression approach that enables numerous wavelengths or the complete spectrum to be used while avoiding the MLR problem (Brereton, 2003). In contrast to classic least-squares approaches, PLS doesn't presuppose the accuracy of spectral data or the presence of all-natural variability. Instead, the spectral signal and constituent values are both shown together in steps that address each one sequentially. (Brereton, 2003; Wold, Sjöström, & Eriksson, 2001). Each phase subtracts a portion of the spectrum data (factor) and a matching portion of the component data from the dataset, while excluding residual spectral and constituent data. The remaining data in the calibration dataset shrinks as each factor is determined. The variation predicted for each

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factor is calculated using partial calibrations (loadings represent spectral data, while scores indicate constituent values). They are combined to form a single calibration equation (Brereton, 2003). The calibration models can undergo testing through Validation can be done internally with cross-validation, or externally with a separate validation or test set. The validation data set needs to be a separate sample and is utilized to gather statistical data on how well the calibration model predicts (Reich, 2005). The precision and accuracy need to match that of the reference method. It is important to assess the significance of the slope and bias, while the coefficient of determination (R2) holds equivalent importance to the correlation coefficient (r) in traditional univariate approaches. Evaluation of RMSEC and RMSEP, as well as residuals, is required (Williams *et al*., 2017).

2.6.4.2 Pre-processing Calibration Data

During PLS calibration, the training set's spectral and constituent data undergo different preprocessing compared to operations done on spectra prior to calibration, like the second derivative. The array of individual values is adjusted to have a mean of zero and standardized based on the variance. The ultimate calibration equation considers the average values and scaling factors as stated by Reich in 2005. Weighting vectors (weights) are created prior to spectral loadings in order to evaluate the degree to which data for each wavelength accurately explains remaining concentration levels. The software adjusts the data to give more weight to wavelengths with high absorptivity, based on the multiplication of correlation and variance within the spectral data (Brereton, 2003).

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2.6.4.3 Selecting the Number of Factors

In most cases, the programme makes it possible to compute a greater number of PLS factors than can be successfully utilised in the final calibration. The determination of the optimal number of variables to employ is a crucial component of the PLS calibration process. When there are not enough factors, the model becomes under-fitted, and when there are too many factors, it becomes over-fitted, leading to a model that is unstable and susceptible to prediction errors (Reich, 2005). There are two methods for determining the ideal number of factors: cross-validation and external prediction sets. There are minimal PRESS values in MATLAB version 9.5.0 (Mathworks Inc., USA) and the programme suggests the smallest number of factors for a certain prediction residual error sum of squares. When deciding how many to use, there are numerous of things that can be considered.

2.6.4.4 Cross-validation

Cross-validation can be conducted on the training set rather than requiring external validation samples to determine the residual error sum of squares values. Cross-validation divides the training set into subgroups with multiple or single samples. One subset is excluded from cross-validation while the remaining training samples are used to construct a calibration. The subset's samples are subsequently analysed as unknowns using this calibration. In conclusion, the anticipated values are deducted from the benchmark values, and the differences are squared and totaled. The initial subset is added back to the training set, while the other subsets are analyzed in a similar manner. Therefore, the sum of squares value of the prediction residual error for each component shows the effectiveness of the PLS model. The RMSECV, also

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known as SECV, is a metric calculated in cross-validation to assess the effectiveness of the calibration process (Reich, 2005).

2.7 Calibration and Equations Statistical Analysis

Many different metrics are calculated when calibrating or predicting the validation set, showing the accuracy of the calibration equation and proving its effectiveness in predicting new data points (Williams *et al*., 2017).

2.7.1 Multiple Correlation Coefficient

The correlation coefficient R^2, known as the coefficient of determination, assesses the level of correlation between spectral data and constituent values. When R2 is zero, the absorption spectrum is not influenced by constituent data; when R2 is one, the constituent values perfectly match the spectral data, and all residuals are zero (Reich, 2005).

2.7.2 Standard Error of Calibration

The standard error of calibration (SEC) of the calibration equation shows how accurately it can replicate the component values of the spectra used to develop the calibration. SEC is the measure of the variation in the disparities between the reference and NIR measurements determined in the calibration phase. The SEC is determined from residuals f whenever the calibration equation has been fitted to the training set directly, as illustrated in (Eq. 2.1):

$$SEC = \sqrt{\frac{\sum_1^2 f}{N - K - 1}} \qquad \text{(Eq. 2.1)}$$

The number of samples is N, while the quantity of wavelengths or components is K. The SEC should have a comparable value to the reference method's SEL.

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2.7.3 Standard Error of Prediction

The standard error of prediction (SEP) is determined similarly to the standard error of calibration (SEC), but with the difference that f represents residues that arise from forecasting samples that were not part of the calibration process, specifically those in the external validation set and K is not included in the denominator. Typically, SEP is larger than SEC.

2.7.4 The F-statistic

The F-test statistic (F value) is a valuable indicator of the appropriateness of spectral and constituent data fitting. Another application of PLS is to assess the optimal number of factors for regression and identify which samples should be removed as data points that deviate significantly from the calibration dataset. (Reich, 2005).

2.7.5 Slope and Bias

The gradient and intercept indicate how precise and straight the calibration model is. The mean residual value calculated from a set of predictions constituent values is bias; whereas, if the slope is close to one and the bias is near zero, the deviations are dispersed randomly. A significant bias value (positive or negative) suggests a systemic inaccuracy (Reich, 2005).

2.8 NIR Method Validation Criteria

Specificity and robustness are two of the validation requirements for qualitative techniques, but quantitative methods also include selectivity, proportionality, exactness, precision, range, durability, and outliers among its validation criteria. Since the standard error of the reference measurement is transferred to the NIR spectroscopy technique, this means that the NIR spectroscopy method cannot exceed the reference technique and must instead

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be considered comparable. Due to this, the egg spectra underwent preprocessing with both multiplicative scatter correction and principal component analysis (MSC-PCA). Linear discriminant analysis (LDA) was used to forecast the freshness level, while partial least square regression (PLS-R) was utilized to establish the observed egg-laying date. By utilizing a portable NIR spectrometer operating at wavelengths between 740 to 1070 nanometres and applying chemometrics, we successfully assessed the freshness of eggs kept in cold or room temperature settings

2.9 Chemical Residue Analysis

2.9.1 Effects of Age on Antibiotic Residue in Eggs

The findings from the research papers suggest that the age of eggs can impact the existence of antibiotic residue. Donoghue, *et al*., (1996) found that drug residues were more prevalent in less mature yolks compared to larger preovulatory yolks, implying that eggs released later may have higher residue content even after drug withdrawal. In Tabriz City, Mohammad, Khalilzadeh & Hasseini (2014) discovered that around 30% of chicken eggs contained antibiotic residues, with macrolides being the most common type of contamination. Roudaut and Moretain (1990) observed that drug excretion typically lasted longer in the yolk, with spiramycin being the most heavily eliminated antibiotic in the egg. Additionally, Roudaut (1989) stated that detectable residues in eggs were only found with DHS administered through the intramuscular route, and such residues persisted in the whole egg for up to 8 days.

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2.9.2 Effects of Strain on Antibiotic Residue in Eggs

The papers suggest that antibiotic residues in chicken eggs are a concern for human health. Hakimzadegan, Khalilzadeh and Hasseini (2014) found that 30% of chicken eggs in Tabriz City were contaminated with antibiotic residues, with macrolides being the most common. McReynolds, Caldwell, McElroy, Hargis and Caldwell (2000) found that enrofloxacin treatment in egg-producing chickens resulted in detectable antibiotic residues in eggs. In their 2015 study, Shahbazi, Hashemi, Afshari, & Karami discovered that tetracycline was the most prevalent antibiotic residue in 3.3% of commercial eggs in Kermanshah, Iran. Cornejo, *et al*., (2020) discovered that eggs from backyard poultry production systems in central Chile tested positive for various types of antimicrobials, suggesting a lack of biosecurity measures and posing a potential risk for human consumption. Overall, these papers suggest that antibiotic residues in chicken eggs are a concern and require supervision to ensure human health.

2.9.3 Effects of Housing System on Antibiotic Residues in Eggs

The research papers indicate that the housing system used for chicken production can impact antibiotic residues found in eggs. Álvarez-Fernández Alonso-Calleja & Dominguez-Rodriguez, Capita (2012) discovered that barn, conventional cage and free-range housing systems showed higher resistance to antimicrobials compared to organic and domestic systems. On the other hand, Cornejo, *et al*., (2020) found that eggs from backyard poultry production systems in Chile contained residues of various antimicrobial families, suggesting a lack of biosecurity measures and developing these systems vulnerable to the spread of antimicrobial residues. However, Matt, *et al*.,

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(2009) did not observe a significant difference in the event of pesticide remnants in organic and conventional eggs. Holt, *et al*., (2011) suggests that transitioning from conventional cages to alternative housing systems may have implications for egg safety and quality, including the presence of pathogens or chemicals.

2.9.4 Interactive Effects of Age, Strain and Housing System on Antibiotic Residue in Eggs

The research papers highlight that the combination of age, strain, and housing system can have an impact on antibiotic residue in eggs. Dominguez-Rodriguez, *et al*., (2012) observed that the housing system significantly influenced the frequency of resistance to antibiotics in Escherichia coli strains found in eggs. Vlčková, Tůmová, á & Chodová (2018) discovered that microbial contamination of eggshells and penetration of microorganisms into eggs were mostly influenced by the system for housing and storage time. Jones and Anderson (2013) discovered that the type of hen affected the levels of microbes in eggs within different housing setups emphasizing the importance of considering egg safety when selecting hen strains for each housing system. Lastly, Cornejo, *et al*., (2020) reported eggs produced in small-scale poultry farms in Chile tested positive for at least one antimicrobial residue, indicating a lack of biosecurity measures and the potential for antibiotic-related illnesses and antimicrobial resistance.

2.10 Conclusion

Chicken egg production and quality, near-infrared (NIR) spectroscopy, and chemometrics techniques have been reviewed. Although chicken eggs are mentioned to contribute to several health benefits, the high global demand for

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consumption may increase the risk of food poisoning as both good and bad eggs are allowed to enter the market. This has necessitated the move for more research to strengthen quality control measures. Qualitative and quantitative analysis of commercial eggs is vital after primary grading and sorting of the eggs; however, the traditional method used is laborious, tedious, cumbersome and time-consuming. Many studies have shown that NIR spectroscopy can be used for quality control, but none of them used egg quality validation criteria to demonstrate equivalency. NIR spectroscopy is a quick and affordable method that has the capability to substitute destructive techniques and has been proven, through validation, to meet the standards outlined in food industry guidelines. The literature indicates that combining NIR spectroscopy with chemometric techniques can greatly assess the quality of eggs prior to them being delivered to the market.

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CHAPTER THREE

3.0 METHODOLOGY

3.1 Introduction

This chapter looks at the materials and techniques employed. The study was in five phases, each phase involving the use of conventional (destructive) and non-conventional (non-destructive) approaches to assess the egg quality.

3.1 Phase 1: Physical Egg Quality Analysis

3.1.1 Conventional Method

The goal of this study was to evaluate how different strains of hens, housing systems, and the age of hens impact the physical properties of table eggs, both internally and externally.

Location of the Experiment

The study was carried out at the Teaching and Research Farm, School of Agriculture, University of Cape Coast, Ghana. The temperature in the Teaching and Research Farm at the University of Cape Coast fluctuated between 19°C and 34°C, while the humidity levels ranged from 60% to 80%.

Experimental Design

A total of 360 eggs were collected, with 180 eggs from each strain.of layer chickens called Lohmann Brown and Lohmann White in both the deep litter and battery cage environments assigned in a Completely Randomized Design (CRD) experiment in a 2 X 3 X 2 factorial arrangements with two different layer strains of three age groups under two housing systems, The eggs were collected when the two layer strains were 24, 39, and 68 weeks old.

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The study involved examining egg quality, considering the interactions between the layer strains, housing systems, and age groups.

3.1.1.1 Data Collection

Internal and External Egg Quality Analysis

For each egg assessment, freshly laid eggs were collected from the farm and promptly labeled. Within 24 hours of collection, the eggs were inspected to assess their quality both internally and externally. When the layers were 24 weeks, 30 eggs from each strain from each of the two housing systems (battery cage and deep litter) were collected and within 24 hours, the eggs were analysed for physical characteristics. Same was done when the layers were 39 and 68 weeks old. The ages chosen represented the various stages/phases of egg production. At 24 weeks, the birds were in early lay, at 39 weeks they were at peak lay and at 68 weeks, they were in their late lay, that is, the third phase. These phases are important because they can affect both egg production in terms of quantity and quality.

Farm-fresh eggs were gathered, labeled, and inspected for both internal and external quality checks the following day. After peeling off the shell membrane, three pieces of shell were extracted from each egg: one from the pointed end (the equatorial area), one from the rounded midsection, and one from the wide end (the blunt region). The egg was cracked towards the blunt end, and the albumen was separated using a yolk separator, following the procedure outlined in Nonga *et al*. (2010). The eggshell's equatorial area was cut off from the sharp end using the knife. Following the method described by Ehtesham and Chowdhury (2002), we utilized a digital Vernier caliper for measurement of the thickness of each shell section and then took the mean of

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these three readings. Measurements of the height and diameter of the albumen and yolk were taken using the digital Vernier caliper. As per Parmar *et al*. (2006), the weight of the egg white (albumen) can be obtained by deducting the masses of the yolk and shell from the overall egg weight using the following formula: Albumen weight = total weight - yolk weight - eggshell weight. By employing a knife, the pointed portion of the eggshell was detached from the middle region. The digital Vernier calliper was used to measure the thickness of each shell piece, and the average of the three measurements was determined then calculated based on the method described by Ehtesham and Chowdhury (2002). Moreover, the height and diameter of both the albumen and the yolk were measured using a digital Vernier calliper.

Parmar *et al*. (2006) recommended calculating the albumen weight by deducting the yolk weight and eggshell weight from the total egg weight. Therefore, the albumen's weight was calculated by subtracting the weights of the yolk and eggshell from the overall weight.

The yolk color was evaluated using a Roche yolk color fan, which assigns values that vary from 1 to 14 to represent different shades of yellow, from pale to deep. The pH levels of the albumen and the yolk were determined using a pH meter from Hanna Instruments. (Woonsocket, RI 02895). To determine the Haugh unit, which indicates egg freshness, the albumen height, and egg weight values were used in accordance with the suggested formula by Khaleel (2019).

$$Haugh\ unit = 100log\ (albumen\ height - egg\ weight^{0.37} + 7.57)$$

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3.1.1.2 Data Analysis

The data collected were analyzed using a three-way analysis of variance (ANOVA) which considered strain, housing system, and age of layers as fixed factors. The analysis was completed using the General Analysis of Variance procedure in GenStat (Discovery edition). When substantial differences in the means were noted, the average values were also distinguished using the LSD test at a 5% significance level. The statistical model that follows was utilized:

$Y_{ijk} = \mu + S_i + H_j + A_k + (SH)_{ij} + (SA)_{ik} + (HA)_{jk} + (SHA)_{ijk} + e_{ijk}$

Where:

Y_{ijk} = assessment of the ith strain, jth storage period, and kth storage technique

μ = overall mean

S_i = effect of i_{th} strain of layers

H_j = effect of j_{th} housing system

A_k = effect of k_{th} age of strain of layer

$(SXH)_{ij}$ = the combined impacts of the ith strain and jth housing system

$(SXA)_{ik}$ = the combined impacts of the ith strain and kth age of the strain of a layer

$(HXA)_{jk}$ = changes in layer k's age of strain due to the influence of the jth housing system

$(SXHXA)_{ijk}$ = the combined impacts of the i-th type of layer, j-th type of housing system, and k-th age of layer

e_{ijk} = error term

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3.1.2 Prediction of Physical Egg Quality Characteristics Using NIR (Non-Conventional Method)

The goal of this study was to evaluate the feasibility of using NIR technology to predict physical egg characteristics, especially the egg freshness, which is ascertained by the haugh unit.

3.1.2.1 Experimental Design

Egg Samples- On the Teaching and Research Farm of the School of Agriculture at the University of Cape Coast in the Central region of Ghana, 120 fresh eggs were collected from Lohmann brown layer strains that were 39 and 68 weeks old for this research. The freshly laid eggs were divided into two comprising 60 eggs each with one half stored under refrigeration (4°C), and the other half stored at 28°C, in room temperature conditions, with a 70% relative humidity. Haugh unit suggesting egg freshness was determined. Ten eggs (five each from eggs stored under refrigeration and room temperature) were sampled and analysed for Haugh unit every five days for twenty days.

Sample Spectra Acquisition A portable device called SCIO, which can detect colors in the spectral range of 740 nm to 1070 nm with 1 nm resolution, was used to scan the colors of each egg from the day it was laid until twenty days later in reflectance mode. After rotating each egg by 120 degrees, three scans were taken of its equatorial area. The internal composition variations in this particular section were found to be much greater than in other sections (Chen, Tan, & Lin, 2019). Temperatures of 28.6 1°C and humidity levels of 68% were used for the scanning.

Reference Measurements of Freshness Using Haugh Units - The Haugh unit (Hu), a measure of egg freshness, was determined using the

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specified method and equation (equation 1). Furthermore, the yolk height was gauged utilizing a digital Vernier caliper, as prescribed by Monira, Salahuddin, and Miah (2003). To ascertain the freshness of the eggs, the Haugh unit and yolk height were measured for ten (10) eggs from each storage group over the entire storage period of 0–20 days.

$$HU = 100 \log(h + 7.6 - 1.7w^{0.37}), \quad (1)$$

where HU = Haugh unit, h = albumen height (mm) measured using a digital vernier calliper and w= egg weight (g) by using digital weighing scale (0.001 g).

Software Device **-** The spectral data, along based on their point of origin at the scanning moment, were collected from a cloud-based dataset using a research license of SCIO lab. Afterwards, the information was brought into MATLAB version 9.5.0 (Mathworks Inc., USA) running on Windows 10 Basic, where it was processed for different pre-processing experimental scenarios and multivariate techniques.

Data Partition **-** The raw data were divided into two subsets, a calibration set for developing the model and prediction set for evaluating the predictive ability of the constructed models. In order to guarantee fairness, 75% of the data samples were assigned to the calibration dataset., while the remaining data formed the prediction set. This allocation was conducted in a manner that maintained a balanced 3/1 ratio between the calibration and prediction sets.

Data Pre-processing **-** Since it is fairly uncommon for models constructed with raw spectra data to provide undesirable findings, the raw data-set was pre-processed using multiplicative scatter correction (MSC) on

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the spectra. The MSC tool is a practical method for adjusting for baseline tilt and dispersed light (Geladi, MacDougall & Martens, 1985; Ozaki, McClure & Christy, 2006). Preparation of the spectra data is essential for modeling as it aids in removing background information and noise, concentrating on the important characteristics of the scanned samples. PCA was conducted to identify identifiable cluster patterns. PCA is used for both data description and dimension reduction, mainly applied in spectral data for cluster analysis. (Kong, Zhang, Liu, Nie & He, 2013). Typically conducted prior to any multivariate modeling, PCA helps to identify patterns within the data matrix, revealing visualized trends in dimensional space.

Multivariate Analysis Methods - In this study, the researcher used linear discriminant analysis (LDA) to classify data. The role it serves is to find a linear combination of characteristics that effectively distinguishes between two or more groups of events by increasing the variance between classes relative to the variance within the class, resulting in a linear boundary for decision-making. LDA's effectiveness relies heavily on the number of primary component factors, which is dependent on the count of linear discrimination functions (Chen, Cai, Wan & Zhao, 2011). To construct the identification model, PCA data was fed into a linear discriminant analysis.

In this study, Partial Least Squares Regression (PLS-R) was utilized to predict the marked date of lay. PLS-R is a widely used linear multivariate tool specifically designed for analyzing data containing highly collinear, noisy, and redundant variables. (Wold, Sjöström, & Eriksson (2001).

To evaluate the performance of LDA, the identification rate (%) was used. Alternatively, the PLS model's performance was assessed based on three

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main parameters: RMSECV, RMSEP, and the correlation coefficient. (R), as described by Kalivas, & Palmer, (2014) The calculations for these parameters were conducted using equations (2)-(5):

$$IR = \frac{n1}{n} \times 100, \quad (2)$$

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{n} \left(\hat{y}_{\backslash i} - y_i\right)^2}{n}}, \quad (3)$$

$$RMSEP = \sqrt{\frac{\sum_{i}^{n} \left(y_i - \hat{y}_i\right)^2}{n}}, \quad (4)$$

$$R = \sqrt{1 - \frac{\sum_{i=1}^{n} \left(\hat{y}_i - y_i\right)^2}{\sum_{i=1}^{n} \left(y_i - \bar{y}\right)^2}}, \quad (5)$$

In the equations provided above:

- n1 indicates the quantity of samples that were accurately identified.
- n denotes the overall quantity of samples.
- yi stands for the reference measurement results for sample i.
- ŷ\i signifies the predicted outcome of sample i when the model is constructed with sample i removed.
- ŷi represents the estimated results of the model for sample i.
- ȳ denotes the average of the reference measurement outcomes for every sample.

3.2 Phase 2: Nutritional Quality Analysis

Conventional Method

The objective of this experiment was to evaluate the impact of layer strain, housing systems, and age of layers on the proximate and mineral composition of table eggs.

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3.2.1 Material and Methods

Location of the Experiment

The experiment was conducted at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast, Ghana.

3.2.1.2 Experimental Design

A combined total of 1080 eggs were collected from both Lohmann Brown and Lohmann White layer breeds, divided evenly with 540 eggs from each strain. The eggs were collected from birds aged 24, 39, and 68 weeks, raised in either deep litter or battery cage systems, for nutritional analysis in a factorial experiment. Eggs gathered from the farm were labeled daily during the study, inspected, and blended for proximate and mineral properties within a day of being collected.

3.2.1.3 Proximate and Minerals Analysis

Chemical analyses were carried out at the Nutrition Lab at the A.G. Carson Technology Centre, University of Cape Coast. Pooled content of yolk and albumen mixed was used for the proximate and mineral composition analysis. The dry matter, moisture, crude protein, crude fat/oil, ash, crude fibre, carbohydrate, phosphorus, potassium, sodium and calcium were analysed. The proximate composition was determined following the described method by the Association of Official Analytical Chemistry (AOAC) (Arowora, Oluwabamiwo, Imo, Kukoyi, Ugwuoke, & Eneji, 2020). There were 5 pooled samples per source collected at different time points in the study. The computation method was for the determination of carbohydrate contents. This was done using this formula:

100 % - (protein % + fat % + humidity % + ash %).

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Atomic absorption spectroscopy (5K-TJ-66 AAS flame) was used to estimate sodium and potassium concentrations. Schmid-Bondzynski-Ratslaff (SBR) method was used to quantify the fat content. Flow injection analysis (FIA) spectrofluorometric method was used to evaluate calcium composition while the Kjeldahl method ISO 937:1978 was used to establish nitrogen content. Lastly, phosphorus concentrations in eggs were established through the application of flame spectrophotometric techniques, employing the method prescribed by (Haraguchi & Fuwa, 1976).

Table 2: Percentage composition of the layer mash fed to the birds

Ingredient	Percentages
Maize	59
Wheat bran	5
Rice bran	2.5
Soybean meal	15
Palm kernel meal	3
Fish meal	3
Common salt	0.25
Vitamin premix	0.25
Oyster shell	8.5
Dicalcium	2
Mycotoxin binder	0.1
Palm oil	1.4
Total	**100**
Calculated feed analysis	
Me (Kcal/kg)	2,489.05
CP (%)	17.13
CF (%)	4.30

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Ether extract (%)	4.30

Me = Metabolisable energy, CP = crude protein, CF = crude fibre, EE = Ether extract,

3.2.2 Data Analysis

The data collected were analyzed using three-way analysis of variance (ANOVA) using GenStat (Discovery Edition), with strain, housing system, and age of the layers treated as fixed factors. If discrepancies in means were observed, the distinction between the means was determined through the least significant difference (LSD) test, with a significance level of 5%. The following statistical model was employed:

$$Y_{ijk} = \mu + S_i + H_j + A_K + (SH)_{ij} + (SA)_{ik} + (HA)_{jk} + (SHA)_{ijk} + e_{ijk}$$

Where:

Yijk = assessment of the ith strain, jth storage period, and kth storage technique

μ = overall mean

Si = effect of ith strain of layers

Hj = effect of jth housing system

Ak = effect of kth age of strain of layer

(SXH)ij = the combined impacts of the ith strain and jth housing system

(SXA)ik = the combined impacts of the ith strain and kth age of the strain of a layer

(HXA)jk = changes in layer k's age of strain due to the influence of the jth housing system

(SXHXA)ijk = the combined impacts of the i-th type of layer, j-th type of housing system, and k-th age of layer

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eijk = error term

Phase 2: Nutritional Quality Analysis

Non-Conventional Method

The aim of this research was to assess whether NIR can be utilized to forecast the proximate and mineral content of table eggs.

3.2.2 Material and Methods.

3.2.2.1 Egg Samples

In this study, 1080 newly-laid brown eggs from Lohman Brown hens, aged between 36 and 64 weeks, were transferred from the Research Farm School of Agriculture and in Ghana's Central Region to the Animal Nutrition Laboratory of University of Cape Coast's for analysis. In order to have uniform egg samples, the whole egg was homogenised (Su *et al*., 2014).

3.2.3. Data Collection

3.2.3.1 Sample Spectra Acquisition

The fresh egg samples were analyzed using a handheld spectrometer (Tellspec) in reflectance mode to obtain NIR spectra. The measurements were taken between 900 nm and 1700 nm with an interval of 1 nm at 28.6 ± 1°C and 68% humidity. Four scans were captured at the equatorial region of the eggshell by rotating the eggs at 120°C.

Reference Measurements of Eggs Proximate

The samples' moisture, fat, and protein levels were assessed through the AOAC protocols available at http://www.aoac.org. The hot air oven (model FD 115, Binder, Switzerland) was used to determine moisture content at 105^{o}C until reaching a constant weight.

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The reference measurement for the 1080 samples was assessed at the Animal Nutrition Laboratory, University of Cape Coast. Pooled homogenised content of the whole egg was used for the proximate and mineral composition analysis. The moisture, ash, protein, fat/oil, and carbohydrate of pooled homogenised whole egg contents were assessed by the standard AOAC methods (http://www. aoac.org). Hot air oven was utilized for measuring the level of moisture of the eggs at a temperature of 105^{o}C until constant weight. Schmid-Bondzynski-Ratslaff (SBR) method was used to quantify the fat content according to the accepted standard (Abdulhameed *et al*., 2014). The Kjeldahl method was employed to determine the total nitrogen content using digestion (Kjedahtec system) and distillation (Kjedahtec system) unit models after which the total protein content was computed by the formula:

Protein = total nitrogen x 6.25

The computation method was also used in the determination of carbohydrate contents. This was done using this formula:

100% - (protein % + fat % + humidity % + ash %)

3.2.3.1 Software Device

The data related to the spectrum were kept in a dataset Tellspec cloud system alongside their associated reference values were exported for chemometric analysis using MATLAB version 9.5.0 (Mathworks Inc., USA).

3.2.3.1 Preliminary Data Pre-Processing

As shown in Figure 1, the unprocessed spectra pattern of the egg samples was pre-processed prior to analysis to remove background noise and obtain important properties and information contained in the sample. Four spectra pre-processing methods namely mean centering (MC), multiplicative

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scatter correction (MSC), first derivative (1- der.) and second derivative (2-der.) were applied in MATLAB version 7.15. The MC was determined by computing the mean spectrum of the set of data and deducting the average from each. MSC was employed to correct scattered light on the egg samples. Cozzolino *et al*. (2011a) indicated that the derivative techniques are useful in improving additive and multiplicative baseline differences in addition to the superimposed peaks in the spectra. The first derivative pre-treatment was therefore used for improving resolution and adjusting baselines. However, because the spectra data contain noise, the Savitzky-Golay was further used to smoothen random noise in spectra data and also increase the visual appearance of NIR spectra (Vidal *et al*., 2013). The raw and other pre-processed spectra techniques used for all egg samples are shown in figuare 1. Smoothing first derivative (sm-1der) performed better than the other spectral pre-processed methods in this study, hence it was selected.

Further to the selection of the best spectral pre-processed (i.e. sm-1der), various multivariate algorithms were extensively studied and their results compared. In the calibration (R_{cal}) and prediction sets (R_{pre}), the output of each final model was assessed using root-mean-square error of cross-validation, root-mean-square error of prediction, and coefficient of correlation. Equations 1, 2, and 3 were used to measure these parameters, respectively.

where:

n^1 = number of samples correctly identified,

n = the number of samples,

yi = the reference measurement results for sample *i*,

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y\i = the estimated result for sample *i* when the model is constructed with sample *i* removed,

yi = the estimated results of the model for the sample *i*,

y = the mean of the reference measurement results for all samples.

3.2.3.1.1 Different PLS Models' Theories

The leave-one-out cross-validation technique (LOO-CV) was used for RMSECV, as described by Zhang *et al* (2017). This method was chosen because it is the most basic and widely used method for determining predictive ability, and it also allows the optimal application of the training set, ensuring reusability.

3.2.3.1.2 Multivariate Analysis Methods.

In this study, we used LDA, a linear parametric classification method. For its intended purpose, it finds a linear combination of attributes that most effectively distinguishes between two or more groups of events by creating a linear decision boundary between them by maximizing the variance between and within classes (Teye, Huang, Dai & Chen, 2013). LDA's effectiveness relies heavily on the quantity of main constituent elements, which is dependent on the number of linear discrimination functions (Chen, Cai, Wan & Zhao, 2011). In this study, PCA data was use as input data for the LDA to build the identification model. However, proximate and mineral composition predictions were made using partial least squares regression (PLS-R). For further information on PLS-R, see the works of other writers (Wold, Sjöström, & Eriksson, 2001; Teye & Huang, 2015), since this popular linear multivariate method is discussed in more detail by other authors. The effectiveness of LDA was measured in terms of identification rate (percent), while the performance

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of the PLS model was measured consisting of three primary parameters: the correlation coefficient, the root-mean-square error of prediction (RMSEP), and the root-mean-square error of cross-validation (RMSECV) (Guan, Liu, Huang, Kuang & Liu, 2019). Equations 1, 2, and 3 were used to determine these values. The smooth-1der i-PLS model's output for area selection throughout the spectrum is summarized in Table 6.2.

3.3 Phase 3: Microbiological Analysis of Table Egg Quality

Conventional method

The objective of this research was to assess how age of layers and housing systems affect bacterial and fungal loads on eggs of two strains of layers.

3.3.1 Material and methods

3.3.1.1 Location of the Experiment

The enrichment and culturing trial for bacterial and fungal load content was conducted at the Microbiology and Biotechnology Lab, Animal Science Department of University of Ghana, Legon.

3.3.1.2 Experimental Design

Three hundred and sixty (360) fresh eggs, 180 each from the two-layer strains at 24, 39 and 68 weeks old and kept at the two main housing systems were collected and analysed for bacterial and fungal load in a 2 X 2 X 3 factorial experiment (2 layer strains, 2 housing systems and 3 age groups).

3.3.1.3 Data Collection

Inspection was done on the farms in order to understand the structure and sanitation levels on the farm. Fresh eggs collected from the layer strains, kept at the different housing systems and different ages were immediately kept

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in sterile plastic ziplock bags (to avoid contamination) and taken to the Microbiology and Biotechnology Laboratory at the University of Ghana, Legon for the bacterial and fungal load analyses.

3.3.1.3.1 Egg Content Bacterial and Fungi Isolation

Using sterile cotton swabs saturated in 0.1 percent peptone, swabs were obtained from each egg shell. The samples were immersed in a solution of nutrients to allow bacteria to grow before being cultured on the appropriate media. The broth and its contents were plated on different plates: Salmonella Shigella (S-S) agar (Salmonella), Eosin Methylene Blue (E. Coli), Baird parker medium (*Staphylococcus aureus*), Campy CVA agar (Campylobacter), Oxford Listeria selective agar (*Listeria monocytogenes*), oxytetracycline glucose yeast extract agar (yeast and mould) using the streaking method. All media underwent preparation and sterilization through autoclaving at 121EC for 20 minutes as per the manufacturer's guidelines. The agar plates were placed in an incubator at 37 degrees Celsius for 24 hours and monitored for signs of growth.

3.3.1.3.2 Bacterial and Fungi Isolation on Egg Content

The eggshells were cleaned with seventy percent (70%) ethanol before being cracked open to determine what was inside. The eggs were carefully cracked using a clean spatula, and their yolk and albumen were gently poured into separate glass Petri dishes labeled clearly for sterility, and then mixed together. The contents of the eggshell were treated with identical pre-enrichment and subsequent enrichment methods as the eggshell itself.

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3.3.2 Data Analysis

Using GenStat's General Analysis of Variance method, we conducted a three-way analysis of variance (ANOVA) on the gathered data, where the independent variables were the strain, housing system, and age of the layers (Discovery edition). The LSD test, with 5% significance threshold was used to differentiate between means when they differed. The following statistical model was employed:

$Y_{ijk} = \mu + S_i + H_j + A_k + (SH)_{ij} + (SA)_{ik} + (HA)_{jk} + (SHA)_{ijk} + e_{ijk}$

Where:

Yijk = assessment of the ith strain, jth storage period, and kth storage technique

μ = overall mean

Si = effect of ith strain of layers

Hj = effect of jth housing system

Ak = effect of kth age of strain of layer

(SXH)ij = the combined impacts of the ith strain and jth housing system

(SXA)ik = the combined impacts of the ith strain and kth age of the strain of a layer

(HXA)jk = changes in layer k's age of strain due to the influence of the jth housing system

(SXHXA)ijk = the combined impacts of the i-th type of layer, j-th type of housing system, and k-th age of layer

eijk = error term

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3.4 Phase 3: Microbiological Table Egg Quality Analysis using PCR Non- Conventional method

The goal of this research is to assess. the potential of PCR in determining the levels of bacteria and fungi in eggs from two different strains of layers at varying ages and in various housing conditions.

3.4.1 Material and Methods

3.4.1.1 Location of the Experiment

The enrichment and culturing experiment for bacterial and fungal load content was conducted at the Microbiology Lab of the Animal Science Department and the PCR experiment was done at the Biotechnology Laboratory at the University of Ghana, Legon.

3.4.1.2 Experimental Design

Three hundred and sixty (360) fresh eggs, 180 each from the two-layer strains at 24, 39 and 68 weeks old and kept at the two main housing systems were collected and analysed for bacterial and fungal load in a 2 X 2 X 3 factorial experiment (2 layer strains, 2 housing systems and 3 age groups).

3.4.1.3 Data Collection

The eggs were moved to the University of Ghana, Legon's Microbiology and Biotechnology Laboratory for analysis on ice, where they were stored in sterile plastic ziplock bags. DNA from bacteria and fungi was extracted using the method outlined by Soumet *et al*. (1999), albeit with changes.

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3.4.1.3.2 PCR Primers

Two primers (primer 1 = forward and primer 2 = reverse) as listed in Table 3.2 were used for the amplification of the gene.

Table 3: Primers used for the amplification of the gene

Primer	Primer Sequence (5′–3′)	Amplification target	Target size (bp)	Reference
SM1F	ATCGCTGACTTATGCAATCG	Salmonella typhi (genus)	204	Kwang *et al*. (1996)
SM2R	CGGGTTGCGTTATAGGTCTG			
SM3F	TGTGTTTTATCTGATGCAAGAGG	S. enteritidis	304	Jawale and Lee (2013); Aron *et al* (2001)
SM4R	TGAACTACGTTCGTTCTTCTGG			
EC3F	GCGCTGTCGAGTTCTATCGAGC	E. coli	625	Shaltout *et al* (2020)
EC4R	CAACGGCGACTTTATCGCCATTCC			
SA3F	CGATCCATATTTACCATATCA	Staph aureus 2	450	Kareem *et al*. (2020)
SA4R	ATCACGCTCTTCGTCTAGTT	2		
LM1F	CAAATAAAGTTAGAGGTAGAATGT	Campylobacter jejuni	159	
LM2R	CCATAAGCACTAGCTAGCTGAT			
LM3F	TTCCTTAGGTACCGTCAGAA	Campylobacter coli	287	
LM4R	CTGCTTAACACAAGTTGAGTAGG			
QL1F	TGCAAGTCCTAAGACGCCA	Listeria	497	Chen *et al* (2017)
L2R	CACTGCATCTCCGTGGTATACTAA	Listeria		
YS1F	GTTAGATCCCAGGCGTAGAACAG	1 Yeast	300-400	Pereirat al (2010)
YS2R	GCGAGTACTGGACCAAATCTTATG	1Yeast		
YS3	TGGAAAGTCTACGAGAACAAGCC	2 Moulds	700	Pereira *et al* (2010)
YS4	CCTCTATGTGAAGTCCGTATACTG	2 Moulds		

New England BioLabs designed primers using the **NEBuilder Assembly Tool**, which generates primers by analyzing the entered DNA fragment sequences, ensuring compatibility with the selected polymerase and amplification requirements.

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3.4.1.3.3 PCR amplification using Oligonucleotide and species-specific primers

The PCR protocol implemented in the study involved preparing a 20 µL reaction mixture, comprising 16.8 µL of ultra-pure water (Gibco), 0.5 µL of Taq DNA polymerase, 0.2 µL of template DNA, and 0.5 µL each of forward and reverse primers. The thermocycling process started with an initial denaturation at 94°C for 5 minutes to ensure complete strand separation. This was followed by 35 amplification cycles, each consisting of denaturation at 94°C for 30 seconds, annealing at 58°C for 10 seconds, and extension at 72°C for 20 seconds, ensuring precise target amplification. A final extension at 72°C for 7 minutes was performed to complete DNA synthesis. The PCR products were subjected to electrophoresis on a 1.5% agarose gel, run at 80V for 1 hour. Post-electrophoresis, the gel was stained with ethidium bromide, and bands were visualized under UV light to confirm the amplicon size against a 50 bp DNA ladder (Permantas SM1211).

3.5 Phase 4: Sensory Quality Analysis

Conventional Method

The aim of this study was to evaluate how the strain of hens, the type of housing, and the age of the hens impact the sensory traits of table eggs.

3.5.1.1 Location of the Experiment

The research was conducted at the Teaching and Research Farm, University of Cape Coast's.

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3.5.1.2 Experimental Design

Thirty (30) eggs; 15 from each of the two-layer strains/types raised under the two housing systems and at three different categories were sampled and evaluated for sensory analysis. Each sample was replicated three times.

3.5.1.3 Product preparation

The eggs were boiled in water at 100^{o}C. The prepared eggs were peeled, with the yolk and white separated, then cut into four pieces and placed on plastic dishes. Panelists were instructed to refrain from eating any food prior to the testing. In order to prevent partial responses based on eggshell colors, the panelists were not allowed to see or touch the eggshells prior to unwrapping them.

3.5.2 Data Collection

3.5.2.1 Sensory Evaluation

Thirty (30) untrained panelists, all of whom claimed to be frequent users of egg products, were given a 7-point hedonic scale on which to score and rank the samples according to their level of preference (Granato *et al*., 2010a):

1 = Strongly disliked/preferred

2 = Moderately disliked/preferred

3 = Slightly disliked/preferred

4 = Indifferent

5 = Slightly liked/preferred

6 = Moderately liked/preferred

7 = Strongly liked/preferred

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Sensory attributes evaluated were: appearance, aroma/odour, flavour /taste, texture and overall preference (Cardona *et al*., 2023).

3.4.3 Data Analysis

Using GenStat's General Analysis of Variance method, we performed a three-way analysis of variance (ANOVA) on the collected data, where the independent variables were the strain, housing system, and age of the layers (Discovery edition). The least significant difference (LSD) test, with a significance threshold of 5%, was used to differentiate between means when they differed.

The following statistical model was employed:

$Y_{ijk} = \mu + S_i + H_j + A_k + (SH)_{ij} + (SA)_{ik} + (HA)_{jk} + (SHA)_{ijk} + e_{ijk}$

Where:

Y_{ijk} = observation on the i_{th} strain, j_{th} housing system and k_{th} age of birds

μ = overall mean

S_i = effect of i_{th} strain of layers

H_j = effect of j_{th} housing system

A_k = effect of k_{th} age of strain of layer

$(SXH)_{ij}$ = the combined effects of the j_{th} housing system and i_{th} strain

$(AXS)_{ik}$ = the combined effects of the k_{th} age of strain of layer and i_{th} strain

$(AXH)_{jk}$ = the combined effects of the j_{th} the k_{th} age of strain of layer and housing system

$(HXSXA)_{ijk}$ = the combined effects of the j_{th} housing system, i_{th} strain of layer, and k_{th} age of layer

e_{ijk} = random error of interest.

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3.5 Phase 5: Chemical Residue Quality Analysis

The objective of this study was to evaluate the antibiotics residues of two strains of layers of different ages raised under different housing systems using the disk diffusion method.

3.5.1 Location of the Experiment

The antibiotic residue a test was carried out at the Microbiology Lab, Animal Science Department at the University of Ghana, Legon.

3.5.2 Experimental Design

Antibiotic residues were measured in 540 eggs collected from layers which were 24, 39, and 68 weeks old

3.5.3 Data Collection

The inspection was done on the farms in order to understand the structure and sanitation levels on the farm. Five hundred and forty eggs (540) eggs (18 crates) were systematically selected from three farms. These farms include; Akate Frams, Universty of Cape Coast School of Agriculture Farms, and Delawin Farms. These farms were selected based on their homogeneity housing system, the strain and of birds of the birds. A total of eighteen crates of eggs were numbered starting with the first crates. with the egg located in the upper left corner of the crate, the number 1 was given to the egg located at the bottom right corner. A total of 120 eggs were chosen through a basic random sampling technique from all the eggs available. Immediately after sampling, the eggs were sent to the Microbiology Laboratory at University of Ghana, Legon for analysis (Kabir *et al*., 2004).

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3.5.3.1 Procedure

Using the manufacturer's recommendations, nutrient broth and Mueller Hinton agar (MHA) were prepared. A 12 mm diameter blank paper disc was used to place samples and controls onto plates for inoculation (Kabir *et al*., 2004). 100 uL of the controlled strains of *Staphylococcus aureus* and *E. coli* were inoculated by streaking evenly on Mueller Hinton agar medium respectively. The samples, egg yolk and washed egg shells were aseptically dispensed into the wells in the center of the plates. *Staphylococcus aureus* is a gram-positive bacterium that can be found in various environments, including on human skin and in dust particles. It is known to be capable of contaminating food products, including eggs, under certain conditions. Therefore, if the study aims to investigate the antibiotic residues in egg yolk and eggshells during handling or storage, selecting a bacterium like Staphylococcus aureus is relevant, as it is a possible contaminant. Additionally, Escherichia coli (E. coli) is a gram-negative bacterium with a ubiquitous presence in diverse environments, including the human gastrointestinal tract and water sources. This bacterium is known for its capacity to contaminate food items, particularly fresh produce and water supplies, given the right circumstances. In conclusion, the two microbes will be a true representative of the two groups of bacterium thus gram-positive and negative. The cultures were incubated at 35-37 °C overnight. The diameter of each inhibition zone was measured using a ruler, focusing on the well where the yolk or eggshell samples were placed. If an inhibition zone was observed, it indicated the presence of antibiotic residues in the sample; conversely, the absence of a zone suggested no detectable residues. When inhibition zones

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were present, their diameters were measured and compared to standard reference ranges. These standards classify results as "susceptible" (large zone), "intermediate" (moderate zone), or "resistant" (small or no zone), providing an indication of the concentration of antibiotics in the sample.

3.5.4 Data Analysis

Using GenStat's General Analysis of Variance method, we performed a three-way analysis of variance (ANOVA) on the collected data, where the independent variables were the strain, housing system, and age of the layers (Discovery edition). The least significant difference (LSD) test, with a significance threshold of 5%, was used to differentiate between means when they differed. The following statistical model was employed:

$Y_{ijk} = \mu + S_i + H_j + A_k + (SH)_{ij} + (SA)_{ik} + (HA)_{jk} + (SHA)_{ijk} + e_{ijk}$

Where:

Y_{ijk} = observation on the i_{th} strain, j_{th} housing system and k_{th} age of birds

μ = overall mean

S_i = effect of i_{th} strain of layers

H_j = effect of j_{th} housing system

A_k = effect of k_{th} age of strain of layer

(SXH)ij = the combined effects of the jth housing system and ith strain

(AXS)ik = the combined effects of the kth age of strain of layer and ith strain

(AXH)jk = the combined effects of the jth the kth age of strain of layer and housing system

(HXSXA)ijk = the combined effects of the jth housing system, ith strain of layer, and kth age of layer

eijk = random error of interest.

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CHAPTER FOUR

4.0 RESULTS

4.1 Introduction

The results were presented according to how the various phases of the experiments were carried out.

4.2 Results on Physical Characteristics of Eggs

4.2.1 Effects of the Strain of Layers on the External and Internal Egg Quality

The exterior and interior characteristics of the eggs from the two-layer strains are shown in Table 4. Table 4 demonstrates that although the strain did not significantly affect egg weight, it did affect the other quality attributes of the eggs. Lohmann white eggs had better Haugh unit than Lohmann brown eggs. However, shell weight, shell thickness, albumen weight, albumen height, yolk weight, yolk colour, haugh unit and albumen pH were the significant variables.

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Table 4: The effects of the strain of layer on the external and internal egg quality

Characteristics	Lohmann White	Lohmann Brown	SEM	P-value
Egg weight, g	55.3	54.7	0.3	0.20
Shell weight, g	5.8[b]	6.1[a]	0.1	0.01
Shell thickness, mm	0.5[a]	0.4[b]	0.0	<.01
Albumen weight, g	35.5[a]	33.9[b]	0.3	<.01
Albumen height, mm	8.2[a]	7.3[b]	0.2	<.01
Yolk weight, g	13.9[b]	14.7[a]	0.1	<.01
Yolk colour	2.9	3.0	0.2	0.01
Haugh unit, %	90.7[a]	85.8[b]	0.8	<.01
Albumen pH	8.1[b]	8.5[a]	0.0	<.01

abc- means in the same row with different superscripts are statistically different ($p<0.05$); SEM - Standard Error of Means

4.2.2 Effects of age of layer on the external and internal egg quality

Table 5 indicates that the age of the layer affects egg characteristics both inside and outside. There were no notable variations in egg traits such as shell thickness, egg weight, yolk weight, albumen weight, or albumen pH based on the age of the layer strain. The age of the layer strain had a significant impact on the albumen height, shell weight, yolk color, and Haugh unit. There was a significant ($p<0.05$) decline in Haugh unit (a measure of egg freshness) and shell weights as the birds aged. The age of the birds also significantly affected the color of the yolk, as younger birds laid eggs with yolks that were more golden compared to older birds.

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Table 5: Effects of housing system on the external and internal egg quality

Characteristics	24	34	68	SEM	P-value
Egg weight, g	55.7	54.9	54.5	0.5	0.09
Shell weight, g	6.3[a]	5.9[b]	5.8[c]	0.1	<.01
Shell thickness, mm	0.5	0.5	0.4	0.0	0.85
Albumen weight, g	34.8	34.7	34.6	0.3	0.96
Albumen height, mm	8.4[a]	7.67[b]	7.3[c]	0.2	<.01
Yolk weight, g	14.6	14.3	14.1	0.2	0.08
Yolk colour	4.4[a]	2.6[b]	1.7[c]	0.2	<.01
Haugh unit, %	91.2[a]	87.4[b]	86.1[c]	1.0	<.01
Albumen pH	55.7	54.9	54.5	0.4	0.09

abc- statistically significant differences ($p<0.05$) exist among items in the same row with different superscripts; SEM - Standard Error of Means

4.2.3 Effects of housing system on the external and internal egg quality

Larger eggs were produced by birds raised under the deep litter system as compared to those kept under the battery cages (Table 6). Furthermore, compared to birds kept in battery cages, birds raised under the deep litter system produced eggs with high interior quality. Nonetheless, the housing system type did not have a noticeable impact on the egg shell weight or yolk color.

Table 6 displays a comparison of how two housing systems, deep litter and battery cages, impact different egg quality characteristics. The findings indicate that there are notable variations ($p<0.05$) between the two housing

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systems in multiple important areas. Specifically, eggs from the deep litter system exhibited higher egg weight (56.9g vs. 53.1g), lower albumen weight (35.8g vs. 33.6g), higher albumen height (8.4mm vs. 7.1mm), higher yolk weight (15.2g vs. 13.5g), higher Haugh unit (91.5% vs. 5%), and a higher albumen pH (8.5 vs. 8.1) compared to those from battery cages. Moreover, there was a notable difference in shell thickness ($p<0.05$) between the two setups, as deep litter eggs measured 0.4mm thick, whereas battery cage eggs were 0.5mm thick. These results underscore the substantial impact of housing systems on various aspects of egg quality, with statistical significance observed at both the 5% and 1% levels.

Table 6: The effects of housing system on the external and internal egg quality

Characteristics	Housing system		SEM	p-values
	Deep litter	Battery Cage		
Egg weight, g	56.9[a]	53.1[b]	0.3	<.01
Shell weight, g	6.0	6.0	0.1	0.92
Shell thickness, mm	0.4	0.5	0.01	0.02
Albumen weight, g	35.8[a]	33.6[b]	0.2	<.01
Albumen height, mm	8.4[a]	7.1[b]	0.2	<.01
Yolk weight, g	15.2[a]	13.5[b]	0.1	<.01
Yolk colour	3.0	2.8	0.2	0.45
Haugh unit, %	91.5[a]	5[b]	0.8	<.01
Albumen pH	8.5[a]	8.1[b]	0.04	<.01

abc- indicates that items in the same row with varying superscripts have statistical significance ($p<0.05$); SEM - Standard Error of Means

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4.2.4 The interactive effects of strain, age of layer and housing system on the external and internal egg quality

The research found significant interactions between housing system and age on albumen color, albumen height, shell weight, and Haugh unit, as presented in Table 7. Additionally, significant interaction effects were observed between the type of housing and the strain on the weight of eggs, shells, and albumen. Furthermore, the interaction between strain and age had a significant impact on albumen height, yolk weight, and Haugh unit.

Table 7: The interactive effects of strain, age of layer and housing system on the external and internal egg quality

	P-values						
Characteristics	S	A	H	SXA	S X H	A X H	S X A X H
Egg weight, g	0.13	0.04	<.01	0.14	01	0.77	0.2
Shell weight, g	<.01	<.01	0.91	0.02	<.01	0.03	<.01
Shell thickness, mm	<.01	0.84	0.02	0.02	0.07	0.23	0.5
Albumen weight, g	<.01	1.0	<.01	0.37	<.01	0.9	0.5
Albumen height, mm	<.01	<.01	<.01	<.01	0.21	0.04	<.01
Yolk weight, g	<.01	0.03	<.01	0.54	0.08	0.25	0.3
Yolk colour	0.7	<.01	0.34	<.01	0.01	<.01	<.01
Haugh unit, %	<.01	<.01	<.01	<.01	0.02	0.12	<.01
Albumen pH	<.01	<.01	<.01	0.29	<.01	0.21	0.3

Statistically significant at $p<0.05$, where S represents strain, A represents age, and H represents housing. S X A represent interactions between strain and age S X H represents interactions between strain and housing, and A X H, represent interactions between age and housing and all three factors (S X A X H).

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This study revealed significant effects of the housing system, strain, and age on key egg quality traits such as albumen height, Haugh unit, shell weight, yolk weight, and albumen color. These findings are consistent with previous studies indicating that housing conditions and genetic factors are critical in determining egg quality (Tumova *et al*., 2014; Svobodová, 2014). For instance, layers housed in cages exhibited superior albumen height and Haugh unit values at younger ages, aligning with Freitas, Tinôco, Baêta, Barbari, Conti, Teles Júnior, ... & Sousa, (2017)., who reported that controlled housing conditions reduce environmental stress, improving albumen quality.

The significant interaction between the housing system and age on albumen color, albumen height, shell weight, and Haugh unit suggests that the environmental conditions in different housing systems have age-dependent impacts. Layers housed in cage systems showed higher albumen height and Haugh unit values at early stages of production, which declined more sharply with age compared to those in deep litter systems. This is in line with the findings of Shields & Duncan, (2009), who observed that cage systems generally provide better conditions for maintaining egg quality but may not account for the natural behavioral needs of the birds, potentially exacerbating age-related declines.

The interaction between strain and age demonstrated that different strains respond uniquely to aging in terms of albumen height, yolk weight, and Haugh unit. This aligns with the genetic predisposition for heavier yolks (Ledur, Liljedahl, McMillan, Asselstine, & Fairfull, (2002).

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4.3 Results on egg freshness measurements using the NIR

4.3.1 Egg Freshness Measurements

A destructive standard approach was utilized to observe and verify the freshness of eggs in two separate storage conditions. The Haugh unit (HU) rating shows that the eggs freshness diminishes or is diminished with storage time (Table 8).

Table 8: Reference measurement of egg quality under two storage conditions

	Cold storage		Ambient storage	
Days	Haugh unit	Yok height	Haugh unit	Yolk height
0	96.78	9.3	5.06	8.7
1	95.56	18.6	87.12	18.2
5	88.97	18.4	77.20	16.9
10	86.48	18.3	71.89	13.8
15	82.53	15.9	60.01	11.8
20	82.42	18.2	41.36	9.9

Table 8 presents a comprehensive overview of the reference measurements for egg quality, comparing two distinct storage conditions: cold storage and ambient storage, over a span of 20 days. In cold storage, the Haugh unit values started at 96.78 on day 0, gradually declined to 82.42 on day 20, while yolk height experiences an increase from 9.3 mm to 18.2 mm over the same period. Conversely, ambient storage initially exhibits lower Haugh unit values at 5.06 on day 0, followed by a significant rise to 87.12 on day 1, and then a subsequent decline to 41.36 on day 20. Yolk height in ambient storage starts at 8.7 mm on day 0, peaks at 18.2 mm on day 1, and gradually diminishes to 9.9 mm by day 20. .

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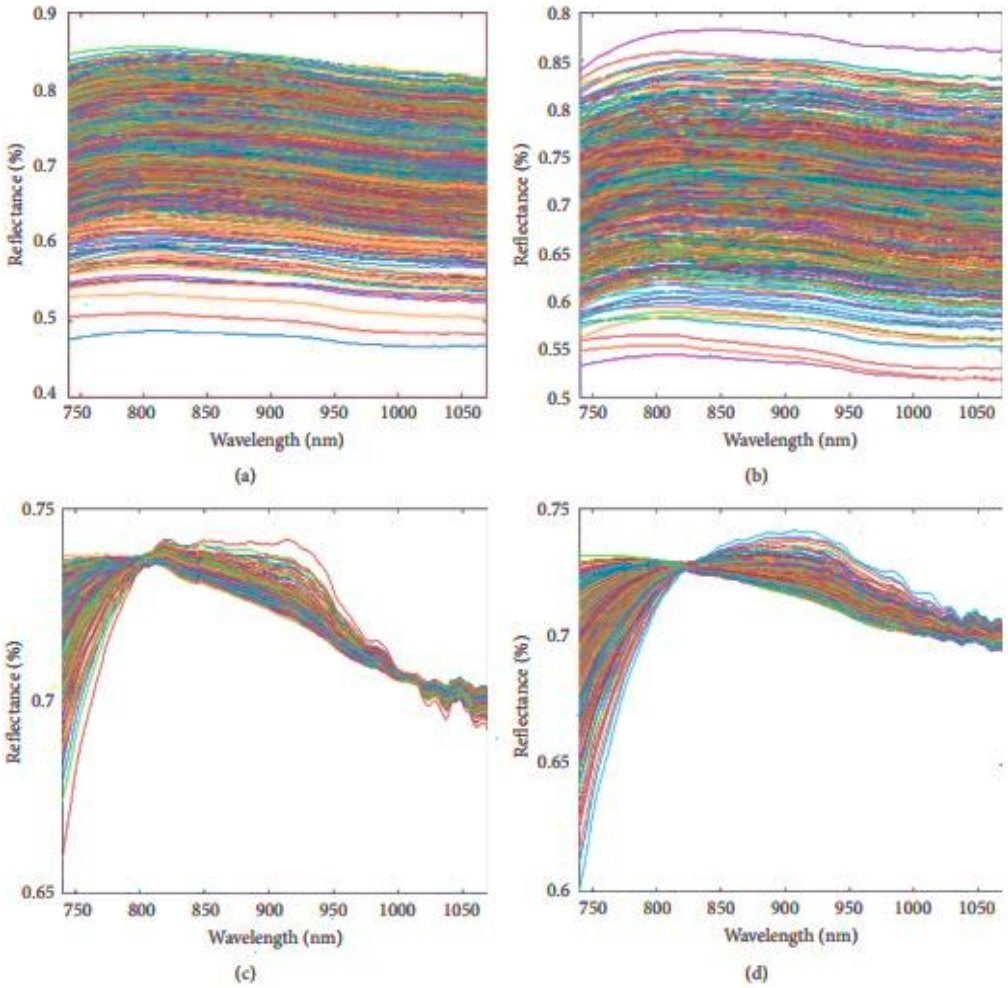

Figure 3: Raw and MSC preprocessed spectra of egg (A1-A2) for ambient storage and (B1-B2) cold storage

4.3.2 *Spectra* Presentation

Figures 3(a) and 3(b) demonstrate the difficulty in distinguishing between the spectral profiles of eggs stored under ambient conditions and cold storage at different time points. Figure 3 showed that the differences between the eggs held for 0 and 20 days were caused by the mean plot. The numbers showed both new and old insights. In addition, there were five distinct categories for the eggs, including a zero-day category. Figure 3 displays the mean plot, which shows a clear spectral profile. Each group of eggs may be identified by its unique fingerprint in the CH 3rd overtone and NH 2nd

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overtone region. Additionally, a PCA was used as an unstructured pattern recognition tool to properly cluster the data.

Principal component analysis was utilized for unsupervised analysis of the spectra data. Figure 4.4 displays the PCA findings at ambient storage and cold storage, illustrating the obvious separations that were detected following pre-processing using MSC. The overall 3 PCs averaged 99.95% for room temperature storage and 99.91% for cold storage. In other words, when MSC was applied to the raw data, the three primary PCs were responsible for the observable cluster trend.

Figure 3 also displays the PCA for the four examined classes, which shows that whereas raw spectra did not provide any separation, MSC-PCA did, as can be seen in Figures 3(a) and 3(b) (d). However, it was shown that data kept in cold storage partitioned more cleanly than data kept in an ambient storage environment. This might be because cold storage provides a more consistent environment than ambient storage.

4.3.3 Classification Models

An LDA-based identification model was created to classify eggs into one of four groups depending on how long they had been in storage. According to Table 4.2, when comparing the classification models for eggs held in ambient and cold temperatures, MSC-LDA achieved the highest performance, achieving a 96% accuracy rate in both the calibration set and prediction set using 5 principal components.

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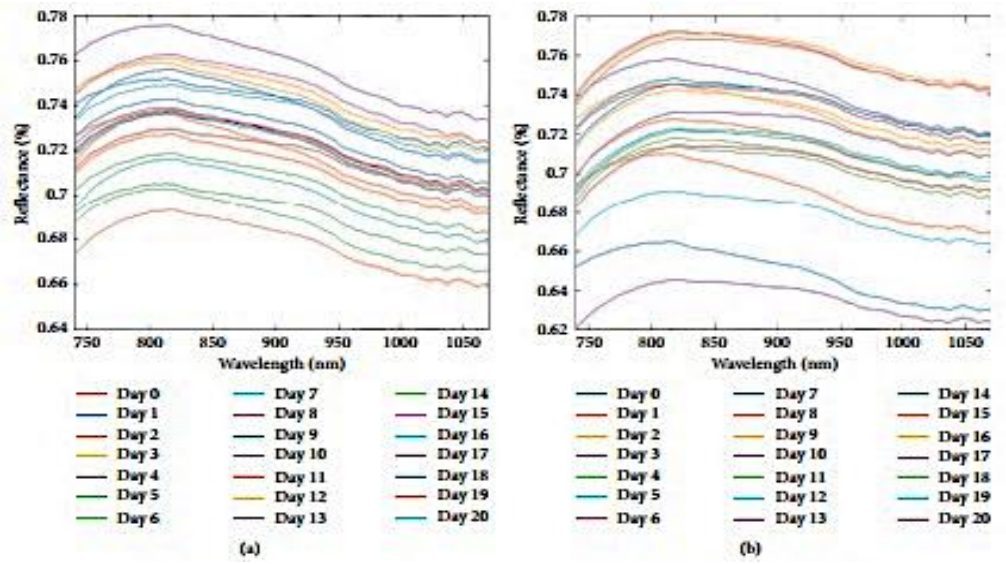

Figure 4: Mean spectral characteristics of eggs stored at room temperature and in the refrigerator for 20 days

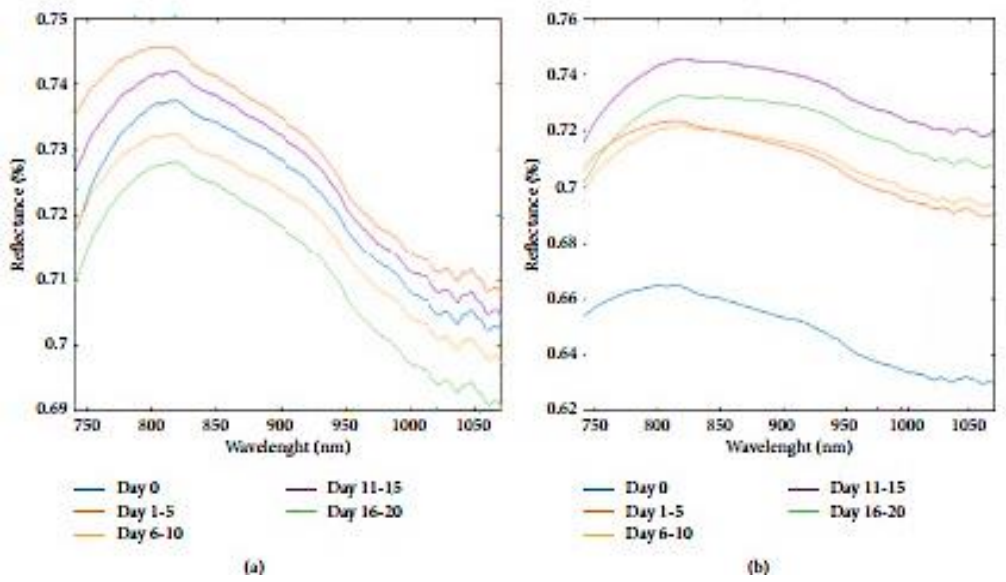

Figure 5: Mean spectra of 5 group of egg freshness for ambient (a) and cold storage. (b) Principal component analysis (PCA)

4.3.4 Quantification Model

During this study, a new model was created at the same time to forecast the exact length of time eggs can be stored in ambient or cold conditions. The PLS-R model was utilized to predict these storage durations in both scenarios. Figures 5(a) and 5(b) demonstrate a significant association between the measured values and the NIR-predicted values for both storage

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conditions. This firm connection was verified by the high R values, exceeding 86%, as presented in Table 9. The measured values showed a linear correlation with the NIR-predicted measurements. Nonetheless, a few outliers were identified, which slightly affected the PLS model. Table 9 shows that the MSC-PLS model had the top performance, with R values greater than 0.85 and RMSEC values under 3.3 days for both the calibration and prediction datasets in both storage conditions.

Table 9: The overall performance of multivariate classification methods

Model		Number of principal components	Correct classification rate (%)	
			Calibration Set	Prediction set
			(472)	(158)
Ambient storage	Raw	9	68.11	65.73
LDA	MSC	5	96.82	95.54
Cold Storage	Raw	9	70.19	71.02
LDA	MSC	5	100.00	100.00

Table 10: The overall performance of PLS regression model

Pre-treatment	Factors	Calibration		Prediction		Independent	
		R	RMSEC (Days)	R	RMSEP (Days)	R	RMSEI (Days)
Ambient storage							
Raw	9	0.61	6.37	0.59	6.47	0.61	6.85
MSC	5	0.83	3.41	0.89	3.12	0.87	2.57
Cold storage							
Raw	9	0.54	5.37	0.49	5.78	0.51	6.05
MSC	7	0.86	3.22	0.91	2.48	0.89	2.66

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4.4 Results of nutritional quality analysis

4.4.1 Effects of the strain of layer on the proximate and mineral composition of eggs

Table 11 presents the proximate and mineral composition of the two-layer strains (Lohmann White and Lohmann Brown). As shown in Table 11 there was no significant ($p<0.05$) strain effect on dry matter, moisture, protein, fibre, carbohydrate and sodium but there were significant ($p<0.05$) strain effects on the other proximate and mineral compositions examined.

Table 11: Effects of strain of layer on the proximate and mineral composition of eggs

Characteristics	Lohmann White	Lohmann Brown	SEM	P-value	Standard values
% DM	25.4	25.9	0.1	0.3	23
Moist (g/100g)	67.4	67.6	1.1	0.9	76.1g
Ash (g/100g)	1.0[a]	1.0[b]	0.0	<.0	1.1g
Protein(g/100)	13.6	13.2	0.2	0.1	12.6g
Fat/Oil(g/100g)	9.7[a]	9.0[b]	0.1	<.01	9.5g
Fibre(g/100g)	0.0	0.0	0.0	0.6	0g
CHO(g/100g)	0.4	0.4	0.0	0.2	0.7g
P (mg/100g)	189.1[a]	184.8[b]	0.3	<.0	198 mg
K (mg/100g)	135.6[a]	135.0[b]	0.2	<.0	138 mg
Na (mg/100g)	141.09	141.4	0.2	0.2	142 mg
Ca (mg/100g)	59.75[b]	62.9[a]	0.9	<.0	56mg

ab - indicates that items in a horizontal line with varying superscripts are significantly different ($p<0.05$); SEM stands for Standard Error of Means. DM= dry matter, MT = moisture, CHO = carbohydrate, P = phosphorus, K = potassium, Na = sodium, Ca = calcium. Source for standard values, https://fdc.nal.usda.gov/fdc-app.html#/food-details/171287/nutrients retrieve 9th February,2021. FDC Published:4/1/2019

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The results in Table 11 indicate that the strain of layers (Lohmann White vs. Lohmann Brown) had varying effects on the proximate and mineral composition of eggs. While the dry matter (% DM) and moisture content were similar across the two strains, no significant differences were observed ($p>0.05$). Notably, significant variations were observed in ash, fat/oil, phosphorus (P), potassium (K), and calcium (Ca) content ($p<0.05$), with Lohmann Brown eggs showing higher fat and calcium levels, whereas Lohmann White eggs had higher phosphorus and potassium content. Protein content showed a slight but non-significant trend, with Lohmann White eggs recording a higher value (13.6%) compared to Lohmann Brown (13.2%). Both strains recorded fiber levels of 0.0g/100g, aligning with the standard values, while carbohydrate (CHO) levels were also low and non-significantly different. The observed mineral levels, although slightly lower than the standard values, highlight strain-specific nutrient profiles, suggesting potential implications for nutritional optimization and consumer preference in egg production systems. These findings emphasize the importance of strain selection in addressing specific nutritional demands and enhancing egg quality.

The findings from Table 11 are supported by studies highlighting the influence of genetic strain on the nutritional composition of eggs. For instance, Basmacioglu, Tokuşoğlu, & Ergül (2004) reported significant differences in proximate and mineral compositions between eggs from different layer strains, attributing these variations to genetic differences that affect nutrient partitioning and egg formation processes. Similarly, Hammershøj and Johansen (2016) observed that brown and white layers often produce eggs

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with distinct nutritional profiles, such as higher calcium content in brown eggs, which is consistent with the increased calcium levels observed in Lohmann Brown eggs in the current study.

4.4.2 Effects of age on proximate and minerals composition of eggs

The impacts of egg layer age on the proximate and mineral composition of eggs are demonstrated in Table 12. The results revealed that there were no significant age-related impacts ($p<0.05$) on dry matter, fiber, sodium, and carbohydrate. Age had a significant impact ($p<0.05$) on moisture, ash, protein, fat/oil, phosphorus, potassium, sodium, and calcium levels. The eggs showed a noticeable decrease in ash content ($p<0.05$) as the birds got older. However, moisture, fat/oil and phosphorus and calcium content fluctuated with age. Moisture, ash and phosphorus contents of the eggs decreased in older age while potassium, sodium along protein increased from mid-lay to late-lay eggs.

Table 12: Effects of age of layer on proximate and mineral composition of eggs

Characteristics	24	34	68	SEM	P-value	Standard values
% DM	25.40	25.40	25.4	0.08	0.82	23
Moist (g/100g)	66.41^{c}	69.44^{a}	66.65^{b}	1.31	0.04	76.15g
Ash (g/100g)	1.00^{a}	0.98^{b}	0.96^{c}	0.05	<.01	1.06g
Protein(g/100g)	13.70^{b}	12.90^{c}	13.70^{a}	0.20	<.01	12.56g
Fat/Oil(g/100g)	9.42^{b}	9.52^{a}	9.20^{c}	0.07	<.01	9.51g
Fibre(g/100g)	0.04	0.04	0.04	0.00	0.77	0g
CHO(g/100g)	0.39	0.39	0.38	0.01	0.65	0.72g
P (mg/100g)	187.30^{b}	188.00^{a}	185.50^{c}	0.41	<.01	198mg

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K (mg/100g)	136.70[a]	133.80[c]	135.30[b]	0.20	<.01	138mg
Na (mg/100g)	140.80[b]	140.80[b]	142.10[a]	0.27	<.01	142mg
Ca (mg/100g)	59.30[c]	62.60[a]	62.10[b]	0.60	<.01	56mg

ab - indicates that items in a horizontal line with varying superscripts are significantly different ($p<0.05$); SEM stands for Standard Error of Means DM= dry matter, MT = moisture, CHO = carbohydrate, P = phosphorus, K = potassium, Na = sodium, Ca = calcium. Source for standard values, https://fdc.nal.usda.gov/fdc-app.html#/food-details/171287/nutrients. Retrieve 9th February, 2021. FDC Published:4/1/2019

4.4.3 Effects of housing system on proximate and mineral composition of eggs

The impact of the housing system on egg moisture, fibre and calcium content were insignificant ($p>0.05$), a notable impact was observed with statistical significance ($p<0.05$) found with the dry matter, ash, protein, fat/oil, carbohydrate, phosphorus, potassium and sodium (Table 13). The results of the research showed that the housing arrangement significantly affects ($p<0.05$) the proximate and mineral content of eggs. Differences in proximate and mineral contents were obvious in dry matter, ash, protein, fats/oil, carbohydrate, potassium, calcium, phosphorus and sodium. Apart from phosphorus and sodium, the content of all the other minerals was significantly ($p<0.05$) higher in deep litter than in battery cage.

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Table 13: The effects of housing system on proximate and mineral composition of eggs

Characteristics	Housing system		SEM	P-value	Standard values
	Battery Cage	Deep litter			
% DM	24.83[b]	25.93[a]	0.07	<.01	23
Moist (g/100g)	68.15	66.86	1.07	0.23	76.15g
Ash (g/100g)	0.93[b]	1.04[a]	0.00	<.01	1.06g
Protein(g/100g)	13.06[b]	13.79[a]	0.17	<.01	12.56g
Fat/Oil(g/100g)	9.00[b]	9.72[a]	0.05	<.01	9.51g
Fibre(g/100g)	0.04	0.04	0.00	0.56	0g
CHO(g/100g)	0.33[b]	0.44[a]	0.04	<.01	0.72g
P (mg/100g)	187.67[a]	186.24[b]	0.34	<.01	198mg
K (mg/100g)	134.36[b]	136.15[a]	0.16	<.01	138mg
Na (mg/100g)	141.81[a]	140.66[b]	0.22	<.01	142mg
Ca (mg/100g)	60.70[b]	61.94[a]	0.49	0.01	56mg

ab - indicates that items in a horizontal line with varying superscripts are significantly different ($p<0.05$); SEM stands for Standard Error of Means DM= dry matter, MT = moisture, CHO = carbohydrate, P = phosphorus, K = potassium, Na = sodium, Ca = calcium. Source for standard values, https://fdc.nal.usda.gov/fdc-app.html#/food-details/171287/nutrients. Retrieve 9th Feburary,2021. FDC Published:4/1/2019

4.4.4 The interactive effects of strain, age of layer and housing system on the external and internal egg quality

Table 14 displayed significant combined effects ($p<0.05$) of age and strain on dry matter, moisture, ash, protein, fat/oil, potassium, and sodium. Age and housing system had significant interaction effects on dry matter, ash, fat/oil, phosphorus, potassium, and sodium levels, with a p-value of less than 0.05. Strain and housing interaction had a significant impact on dry matter, ash, protein, and potassium levels. Interaction of the three factors however

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affected dry matter, protein, fat/oil, potassium and sodium significantly. Heflin *et al*. (2018) indicated the lack of detailed information about the influence of age, strain and environmental effects on egg nutrients even though the nutrient content of eggs is of concern to consumers as stated by Anderson (2011). This research analyzed how strain, age, and living conditions impact the proximate and mineral levels in whole eggs.

Table 14: Interactive effects of strain, age and housing system on proximate and mineral composition

Characteristics	A	S	H	A XS	A X H	S X H	AXBX H
% DM	0.82	0.33	<.01	<.01	<.01	<.01	<.01
Moist (g/100g)	0.04	0.89	0.23	<.01	0.99	0.69	0.99
Ash (g/100g)	<.01	<.01	<.01	0.02	<.01	<.01	0.04
Protein(g/100g)	<.01	0.01	<.01	<.01	0.05	<.01	<.01
Fat/Oil(g/100g)	<.01	<.01	<.01	<.00	<.01	0.62	<.01
Fibre(g/100g)	0.77	0.61	0.56	0.93	0.67	0.99	0.67
CHO(g/100g)	0.65	0.18	<.01	0.55	0.82	0.66	0.55
P (mg/100g)	<.01	<.01	<.01	0.64	<.01	0.33	0.17
K (mg/100g)	<.01	<.01	<.01	<.01	<.01	<.00	<.01
Na (mg/100g)	<.01	0.17	<.01	<.01	<.01	0.04	<.01
Ca (mg/100g)	<.01	<.01	0.01	0.04	<.01	<.01	<.01

ab – different superscripts in the same row indicate statistical significance ($p<0.05$); SEM- Standard Error of Means. DM= dry matter, MT = moisture, CHO = carbohydrate, P = phosphorus, K = potassium, Na = sodium, Ca = calcium.
Source for standard values, https://fdc.nal.usda.gov/fdc-app.html#/food-details/171287/nutrients. retrieve 9th February,2021. FDC Published:4/1/2019

Statistically significant variances ($p < 0.05$) were noted in various characteristics across different groups and combinations in the study. Specifically, dry matter content (% DM) showed significant differences

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between treatments A (0.82%) and H (< 0.01%), A (0.82%) and S (0.33%), A (0.82%) and S x H (< 0.01%), A (0.82%) and A x B x H (< 0.01%), A (0.82%) and S x H (< 0.01%), A (0.82%) and A x S (< 0.01%), and A (0.82%) and H x S (< 0.01%). Moisture content (Moist) exhibited significant differences between A (0.04%) and H (< 0.01%), A (0.04%) and A x H (0.99%), S (0.89%) and S x H (0.69%), H (0.23%) and A x S x H (< 0.01%), A (0.04%) and S x H (0.99%), S (0.89%) and A x S (0.99%), and A x H (0.99%) and S x H (0.69%), among others. These < signs indicate the statistical significance and demonstrate variations in the measured characteristics between the compared groups and combinations.

4.5 Results on prediction of proximate and minerals composition of eggs using NIR

4.5.1 Wet Chemistry data of eggs

Table 15 outlines the statistics parameters of proximate and mineral content in the egg samples. For eggs to be used by processors, it is important to meet proximate and mineral quality limits including dry matter (23%), moisture (76.15g), Ash (1.06g), protein (12.56g) fat/oil (9.51g), fibre (0g), carbohydrate (0.72g). In table 4.12 shows that the range of values for all analyzed proximate and mineral content of eggs were lower than the standard values. The predicted wet chemistry values by the regression model for dry matter (21.65%), moisture (71.84g), Ash (0.50g), protein (8.64g) fat/oil (6.23g), fibre (0g), carbohydrate (0.42g) were narrower than the standard values but very close to the calibrated values in the sampled eggs.

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Table 15: Statistical parameters for proximate compositions of eggs in the calibration and prediction sets

Proximate Composition	Calibration set (1248) / Prediction set (312)	Mean	Minimum	Maximum	Standard Deviation
Dry matter (%)	Calibration set	21.72	15.26	26.92	2.62
	Prediction set	21.65	15.52	26.82	2.59
Moisture (%)	Calibration set	71.88	64.45	76.75	2.63
	Prediction set	71.84	64.45	76.42	2.73
Ash (%)	Calibration set	0.50	-0.09	0.09	0.37
	Prediction set	0.50	-0.06	1.21	0.36
Protein (%)	Calibration set	8.69	0.72	14.71	2.90
	Prediction set	8.64	1.02	14.31	2.90
Fats/Oil (%)	Calibration set	6.33	0.48	10.41	2.14
	Prediction set	6.23	0.06	10.41	2.17
Fibre (%)	Calibration set	0.002	-0.04	0.04	0.02
	Prediction set	0.00	-0.05	0.04	0.02
Carbohydrate (%)	Calibration set	0.42	0.23	1.11	0.21
	Prediction set	0.42	0.24	1.11	0.21

The average NIR spectra of eggs with varying proximate and mineral content from 900-1700nm are shown in Figure 4.5

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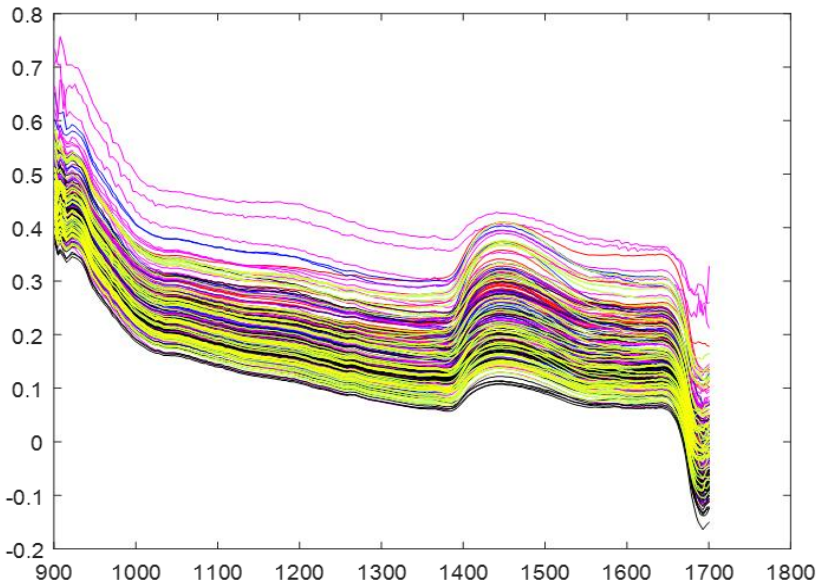

Figure 6: Average Spectra of Eggs

4.5.2 Performance of the different *PLS regression model in* predicting the proximate and mineral content of eggs

Table 16 displays the calibrating and forecasting results of the PLS, i-PLS, and Si-PLS models for assessing dry matter, moisture, ash, protein, fat/oil, fiber, and carbohydrate content. Based on the highest R^2 and the lowest RMSE of calibration set it is known that the PLS model is recognized as being the top model. for predicting dry matter in eggs. The SiPLS model, achieved the best prediction effects (R^2_p = 0.84 for moisture, R^2_p = 0.84 for ash, R^2_p = 0.86 for protein, R^2_p = 0.87 for Fats/Oil, R^2_p = 0.88 for carbohydrate) while the predictive accuracy for PLS was the worst (R^2_p = 0.75 for moisture, R^2_p = 0.74 for ash, R^2_p = 0.79 for protein, R^2_p = 0.80 for fats/oil, R^2_p = 0.79 for carbohydrate.

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Table 16: Comparison of results based on different regression models for proximate content

Parameters	Models	Variables	PCs	Calibration set		Prediction set	
				R^2_{cal}	RMSECV	R^2_{cal}	RMSEP
Dry matter	PLS	256	70	0.69	1.45	0.90	1.45
	i-PLS	42	5	0.62	2.06	0.64	1.99
	Si-PLS	153	10	0.68	1.91	0.66	1.94
Moisture	PLS	6	7	0.75	1.75	0.77	1.75
	-PLS	43	10	0.79	2.05	0.81	2.06
	Si- PLS	153	10	0.84	1.87	0.84	1.94
Ash	PLS	256	6	0.74	0.25	0.71	0.25
	i-PLS	42	10	0.79	0.29	0.73	0.30
	Si- PLS	150	10	0.84	0.26	0.79	0.28
Protein	PLS	256	7	0.79	2.45	0.76	2.39

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	i-PLS	42	10	0.73	1.97	0.81	2.18
	Si- PLS	157	10	0.86	1.28	0.81	1.28
Fat/oil	PLS	256	7	0.80	1.28	0.81	1.28
	i-PLS	42	5	0.81	1.61	0.83	1.58
	Si- PLS	122	10	0.87	1.39	0.87	1.41
CHO	PLS	256	7	0.79	0.13	0.72	0.13
	i-PLS	36	9	0.86	0.14	0.77	0.17
	Si- PLS	156	10	0.88	0.13	0.80	0.16

PCs; principal components, CHO; carbohydrate, Si-PLS model built on the effective selection of the spectral interval by i-PLS

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4.6 Results on Bacterial and Fungal Load Count in Eggs

4.6.1 Prevalence of Bacterial and Fungal Load

Table 17 shows the mean bacterial and fungal count of Lohmann white and brown eggshell and egg content. The percentage of targeted and isolated microbes on the eggshell was higher compared to those found within the egg contents.

Table 17: Prevalence of Bacterial and Fungal Load on Eggs

Microbes		Means	Frequency (n= 540)	Percentage (%)
TPC	Shell	4.48×10^{-4}	388	72%
	Content	1.40×10^{-4}	106	20%
SAL	Shell	1.36×10^{-4}	190	35%
	Content	5.20×10^{-4}	71	13%
E. COLI	Shell	2.06×10^{-4}	183	34%
	Content	8.70×10^{-4}	23	4%
SAU	Shell	9.9×10^{-4}	249	46%
	Content	8.2 0x 10^{-4}	49	9%
CAM	Shell	1.62×10^{-4}	223	41%
	Content	4.80×10^{-5}	32	6%
LIS	Shell	7.3×10^{-5}	260	48%
	Content	1.45×10^{-4}	13	2%
Y and M	Shell	9.8×10^{-4}	168	31%
	Content	8.6×10^{-4}	33	6%

TPC = total plate count, SAL= Salmonella, E. coli = Escherichia coli, SAU = Staphyllococus aureus, CAM = Campylobacter, LIS = Listeria, Y and M = Yeast and Mould

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4.6.4 Effects of Housing System on Bacterial and Fungal Load in Eggs

Eggs gathered from the battery cage system had lower bacterial and fungal loads compared to eggs gathered from the deep litter system. Table 18 demonstrates that the housing type did not have a significant impact on the overall amounts of bacteria and fungus present in eggshells and content from hens in both housing systems.

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Table 18: Effects of Housing System on Microbial Load on Eggs Quality

Microbes		**Battery Cage**	**Deep litter**	**SEM**	**P-value**	**Standard values**
TPC	Shell	4.32×10^{-4}	4.64×10^{-4}	1.50×10^{-4}	1.41×10^{-1}	10,000cfu/g
	Content	1.54×10^{-4}	1.27×10^{-4}	9.79×10^{-5}	7.85×10^{-1}	
Salmonella	Shell	1.91×10^{-4}	8.20×10^{-5}	5.73×10^{-5}	5.80×10^{-2}	1000cfu/g
	Content	6.70×10^{-4}	3.60×10^{-4}	3.04×10^{-4}	3.15×10^{-1}	
E. coli	Shell	1.72×10^{-4}	2.40×10^{-4}	7.49×10^{-4}	3.60×10^{-1}	10cfu/g
	Content	5.80×10^{-4}	1.16×10^{-3}	5.08×10^{-4}	3.17×10^{-1}	
S. aureus	Shell	9.80×10^{-4}	1.01×10^{-3}	5.23×10^{-4}	9.55×10^{-1}	No specific MAL
	Content	8.40×10^{-4}	7.90×10^{-4}	5.62×10^{-4}	9.37×10^{-1}	
Campylobactor	Shell	3.08×10^{-3}	3.45×10^{-3}	8.81×10^{-4}	6.80×10^{-1}	No specific MAL
	Content	6.20×10^{-5}	3.40×10^{-5}	2.85×10^{-5}	3.26×10^{-1}	
Listeria	Shell	4.40×10^{-5}	1.01×10^{-4}	3.27×10^{-5}	8.10×10^{-2}	No specific MAL

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	Content	2.00×10^{-5}	2.69×10^{-4}	1.67×10^{-4}	1.38×10^{-1}
Yeast and Mould	Shell	1.00×10^{-4}	1.86×10^{-3}	1.74×10^{-3}	3.11×10^{-1}
	Content	9.10×10^{-4}	8.00×10^{-4}	5.50×10^{-4}	8.40×10^{-1}

SEM- Standard Error of Means, TPC = total plate count, SAL= Salmonella, E. coli = Escherichia coli, SAU = Staphylococcus aureus, CAM = Campylobacter, LIS = Listeria, YAM = Yeast and Mould.

UK, the Food Standards Agency (FSA),

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There is no specific maximum allowable limit for *Listeria monocytogenes* (*Listeria*) in table eggs set by the European Union or the United Kingdom. However, *Listeria* is a harmful bacteria that can lead to serious sickness, especially in at-risk populations like expecting mothers, people, and older adults with compromised immune systems.

In order to guarantee the safety of food, including the safety of table eggs, the EU and UK implement regulations and guidelines that focus on preventing and controlling the presence of *Listeria* in food production and handling environments.

Under EU and UK food safety regulations, food businesses are required to implement hygiene practices and apply the principles of Hazard Analysis and Critical Control Points (HACCP) to recognize and manage possible dangers, including *Listeria*. These measures are designed to ensure that food, including table eggs, is produced and handled in a manner that minimizes the risk of Listeria contamination.

It's important for egg producers and handlers to follow good hygiene practices, maintain clean production environments, and adhere to proper storage and handling procedures to prevent the growth and contamination of *Listeria*. Regular monitoring and testing of eggs for microbial contamination, including *Listeria*, can also be part of a comprehensive food safety program. While there is no specific limit for *Listeria* in table eggs, the overall aim is to minimize the presence of this pathogen to ensure food safety and protect consumer health. It is advisable to consult the most recent EU and UK regulations and guidelines or consult with local food safety authorities for the

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latest information regarding *Listeria* and any specific limits or guidelines for table eggs.

4.7 Results on PCR Detections of Bacterial and Fungal Load

Figure 7 to 21 show the individual PCR amplification results generated.

4.7.1 PCR Amplification of *Salmonella typhi*

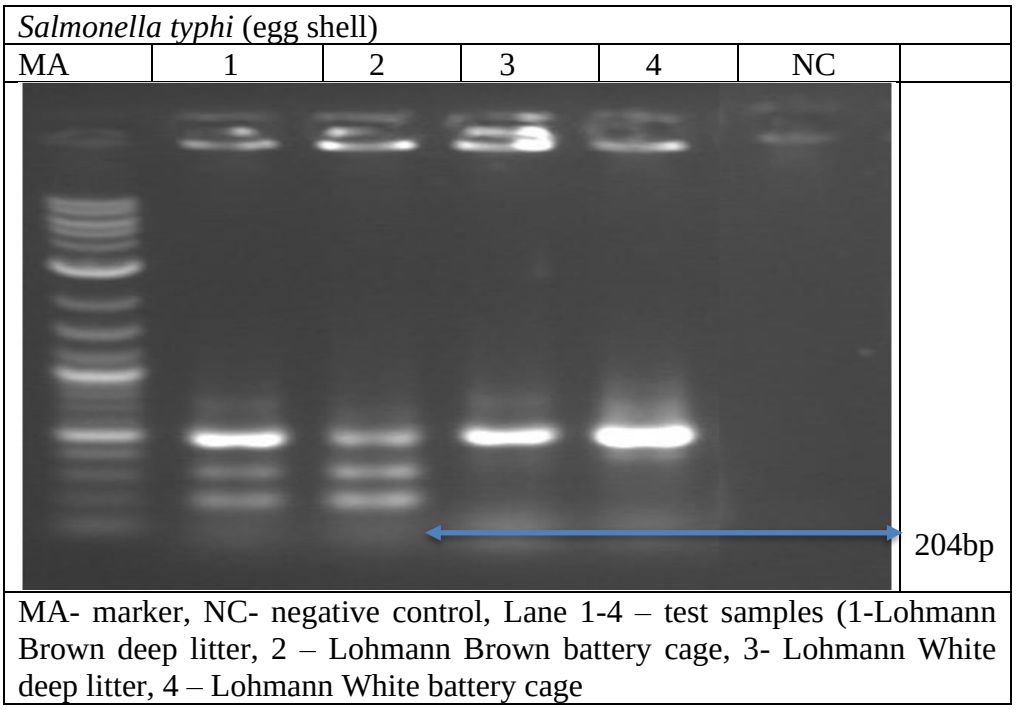
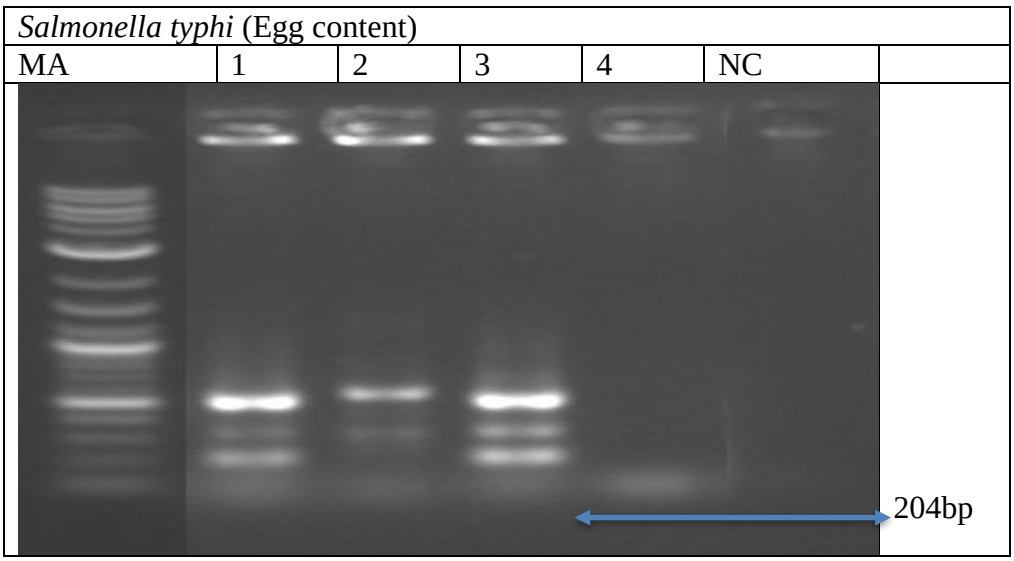


Figure 7: PCR Amplification of Salmonella Typhi (Eggshell)



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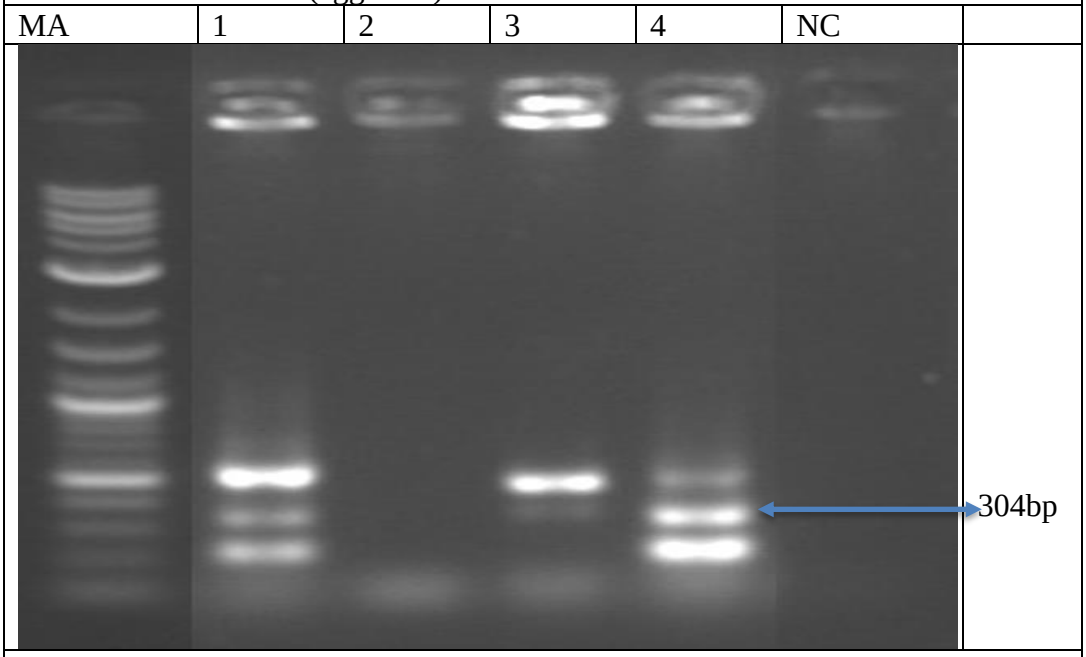
MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 8: PCR Amplification of Salmonella Typhi (Egg Content)

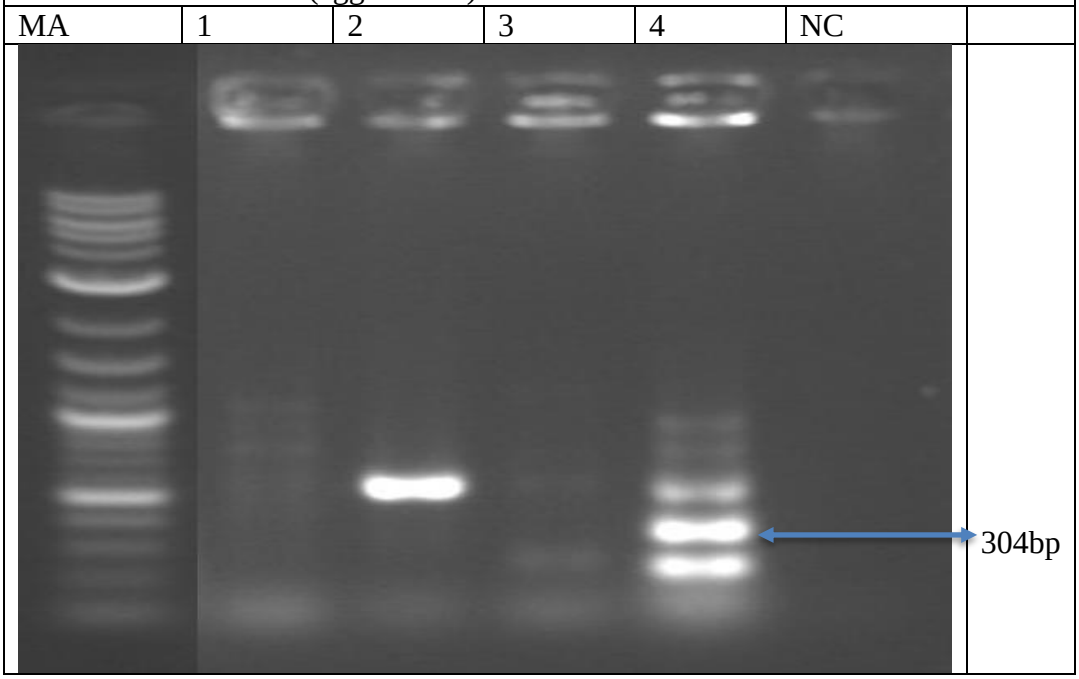
Salmonella enteritidis (egg shell)



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 9: PCR Amplification of Salmonella Enteritidis (Eggshell)

Salmonella enteritidis (egg content)



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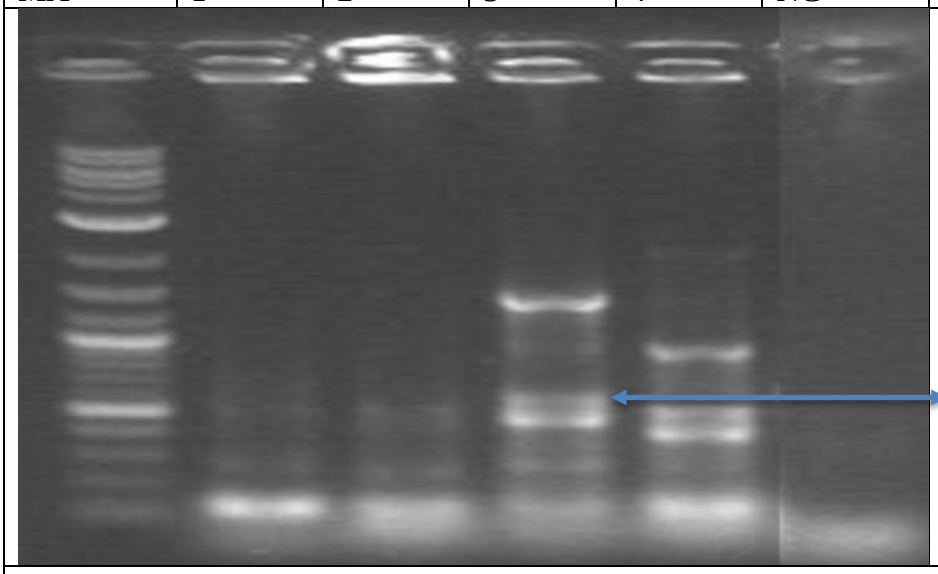
MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 10: PCR Amplification of *salmonella enteritidis* (Egg Content)

4.7.3 PCR Amplification of E. Coli

E. coli (egg shell)

MA	1	2	3	4	NC	



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 11: *E. coli* (Egg shell)

E. coli (egg content)

MA	1	2	3	4	NC	

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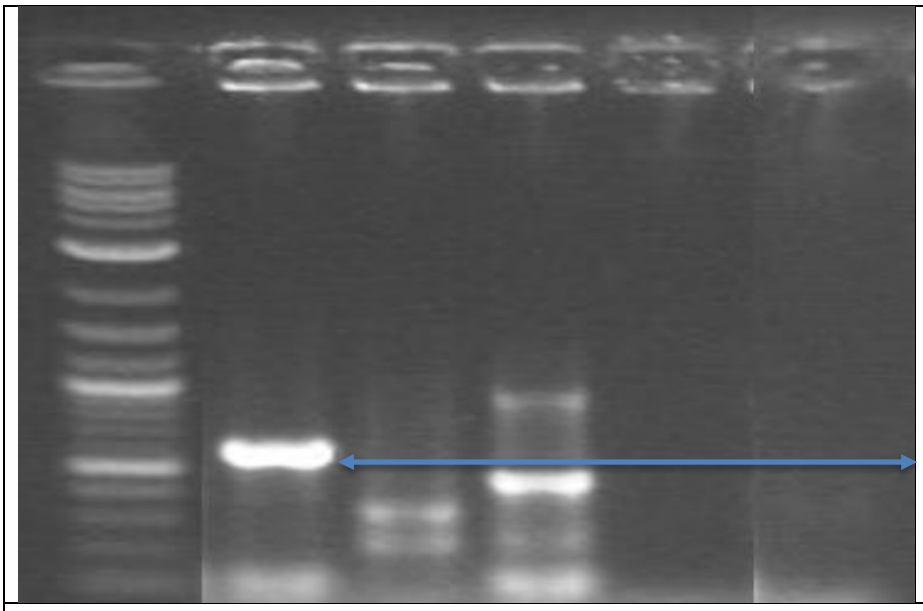


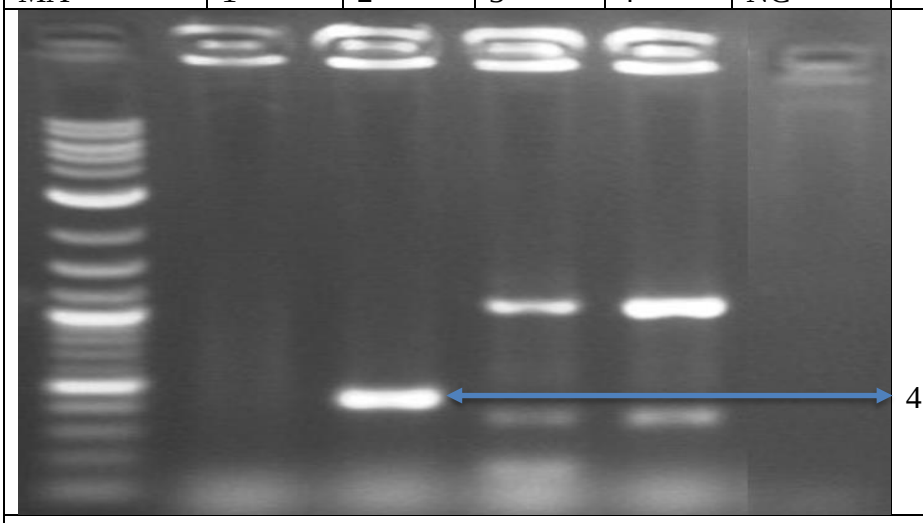
MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 12: *E. coli* (Egg content)

4.7.4 PCR Amplification of Aureus

Staphylococcus aureus (Egg shell)

MA	1	2	3	4	NC	



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 13: Staphylococcus Aureus (Eggshell)

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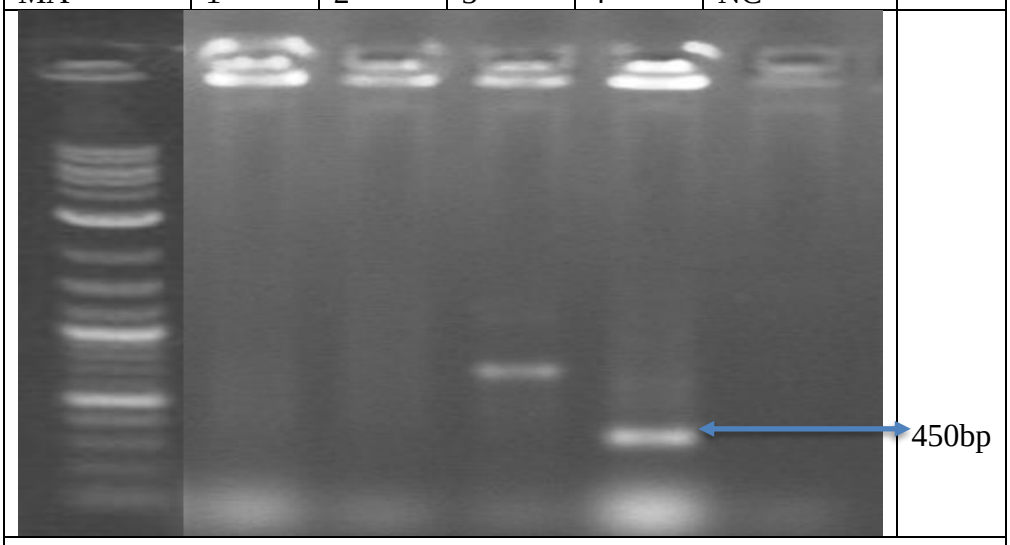

MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 14: *Staphylococcus aureus* (Content)

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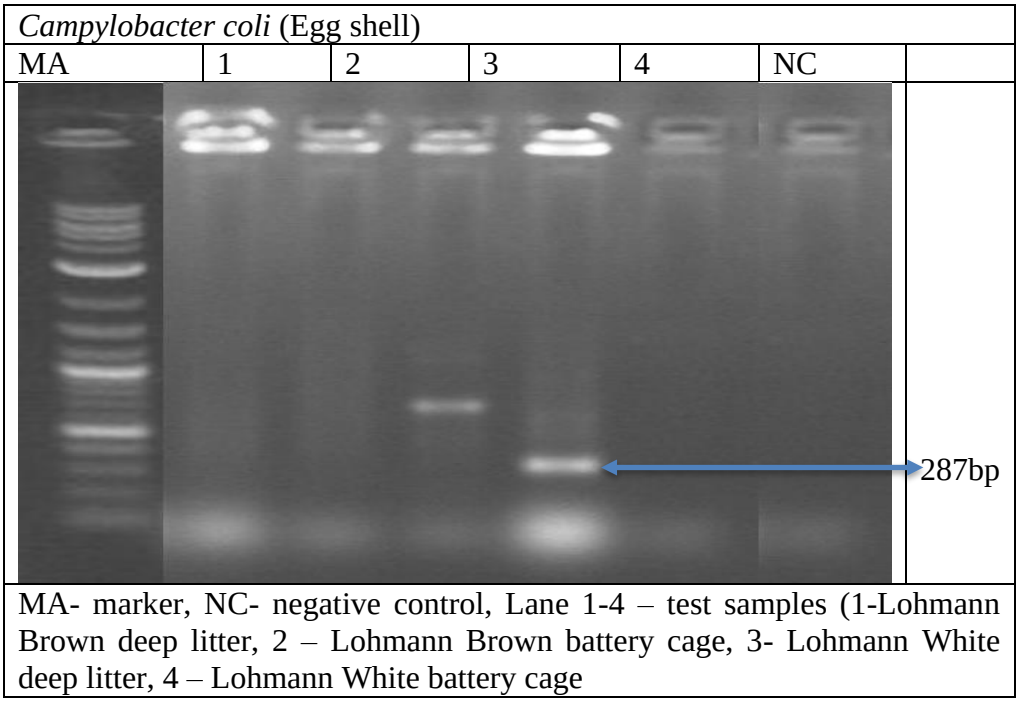
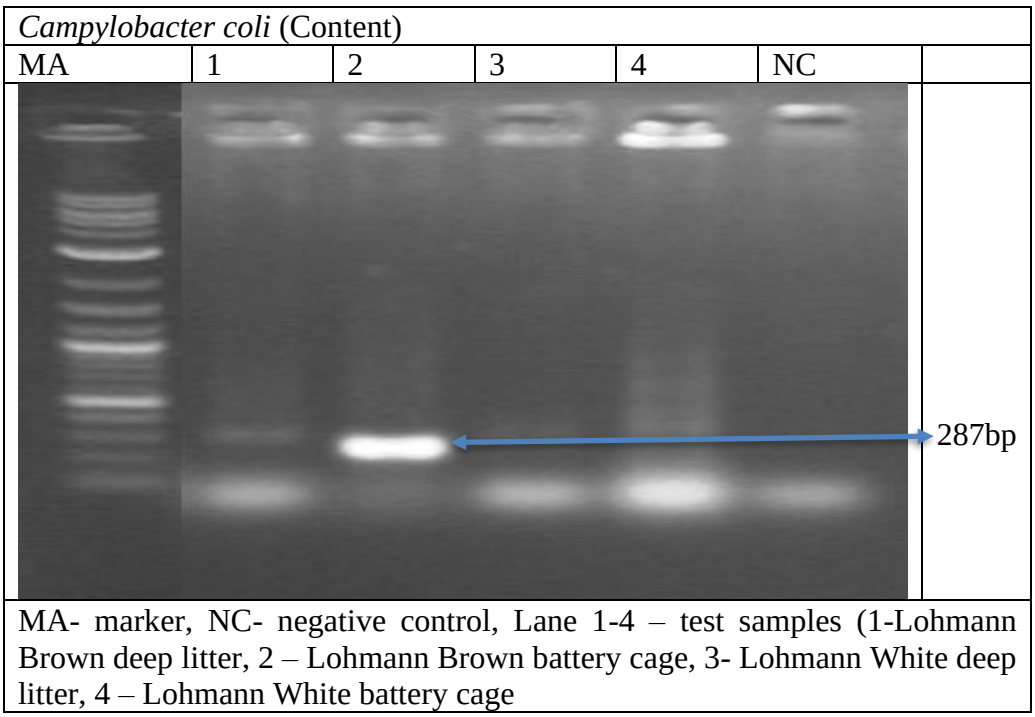
4.7.5 PCR amplification of *Campylobacter coli*



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 14: *Campylobacter coli* (Egg shell)



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 15: *Campylobacter coli* (Content)

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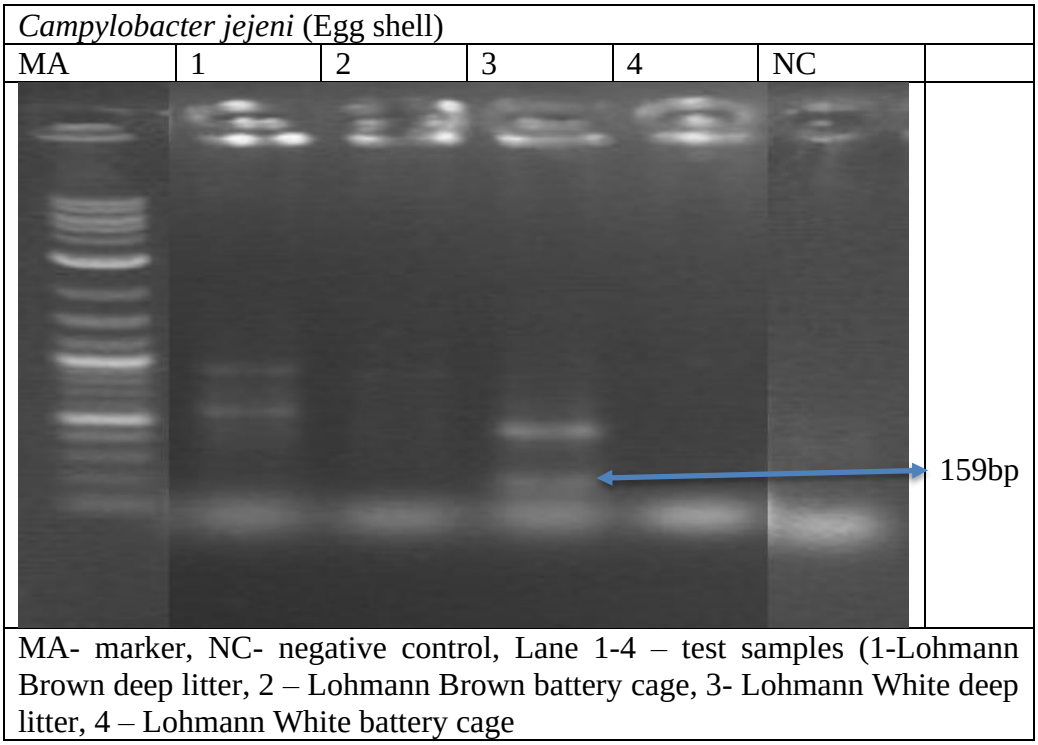
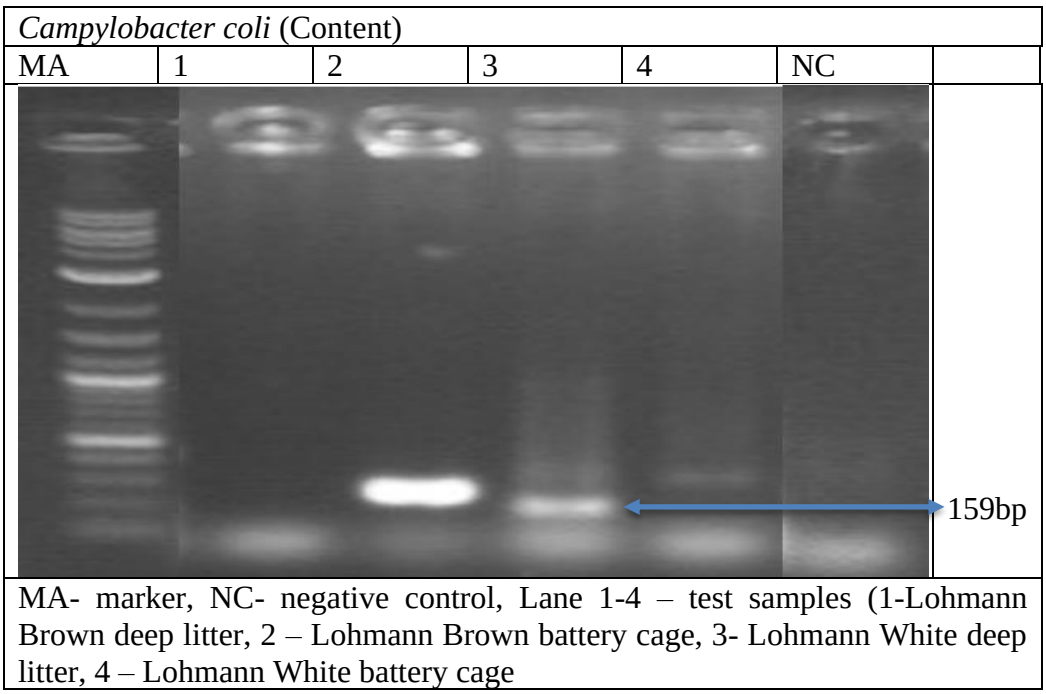
4.7.6 PCR Amplification of *Campylobactor jejeni*



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 16: *Campylobacter jejeni* (Eggshell)



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 17: *Campylobacter coli* (Content)

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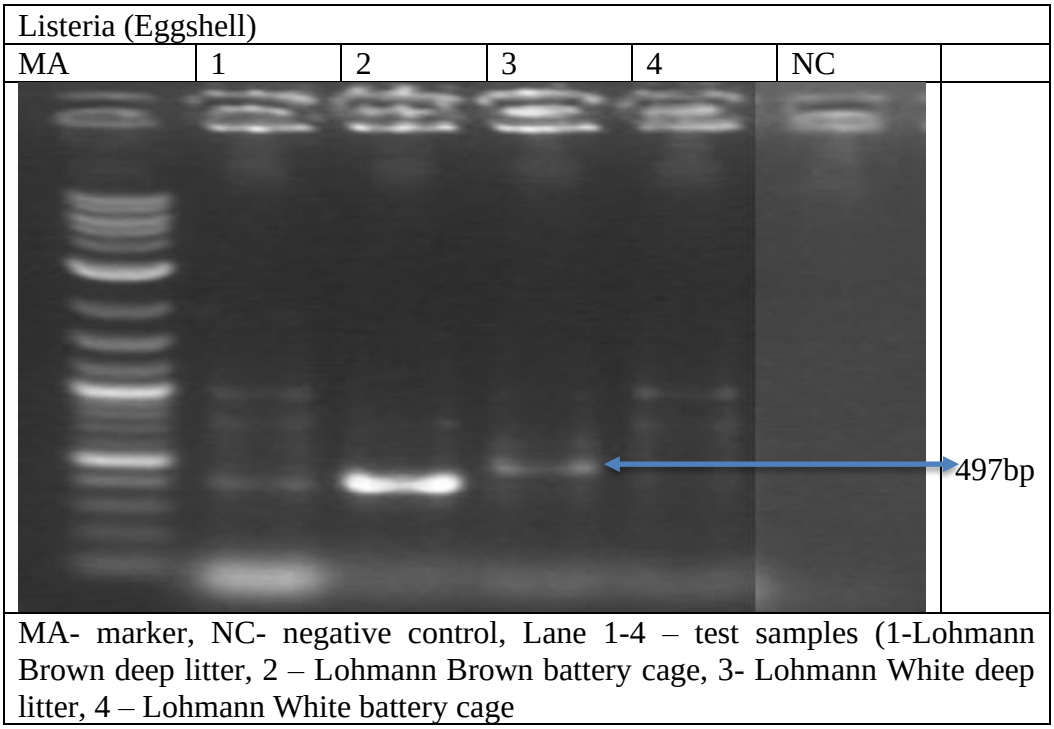
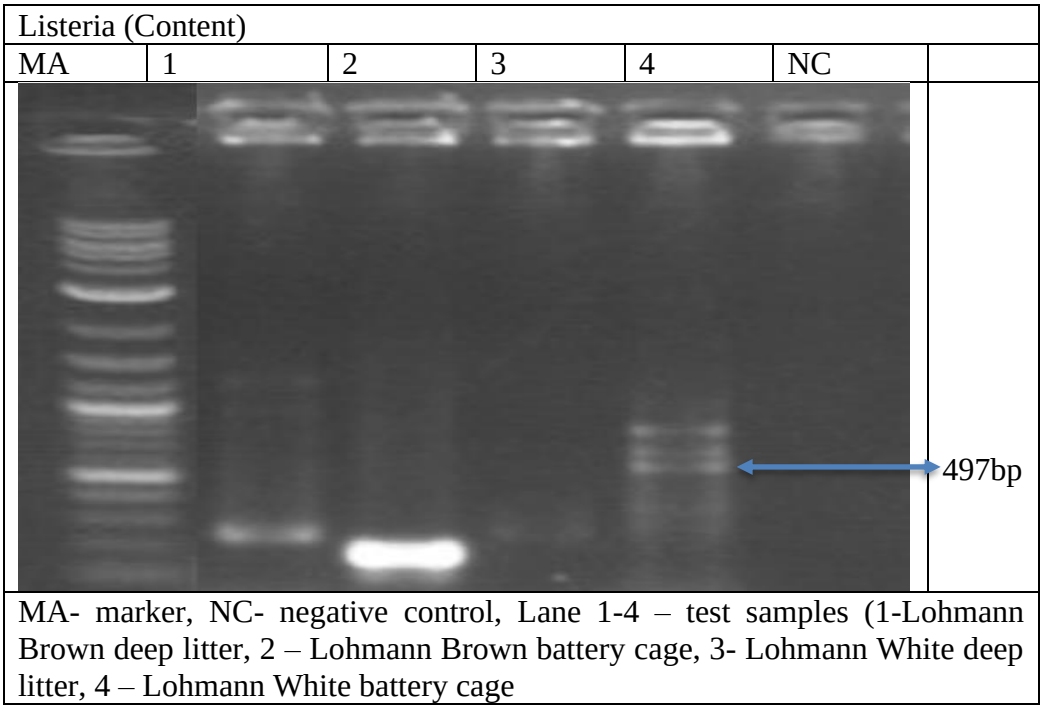
4.7.7 PCR Amplification of Listeria



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 19: Listeria (Egg shell)



MA- marker, NC- negative control, Lane 1-4 – test samples (1-Lohmann Brown deep litter, 2 – Lohmann Brown battery cage, 3- Lohmann White deep litter, 4 – Lohmann White battery cage

Figure 20: Listeria (Content)

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4.7.8 PCR Amplification of Fungal

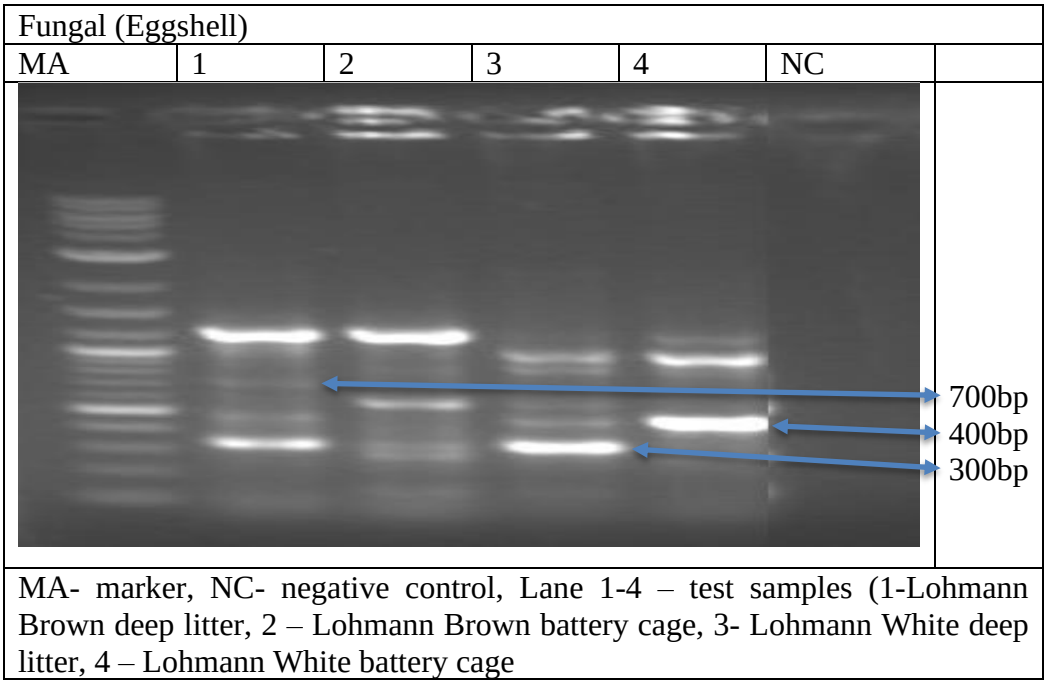

Figure 18: Fungal (Eggshell)

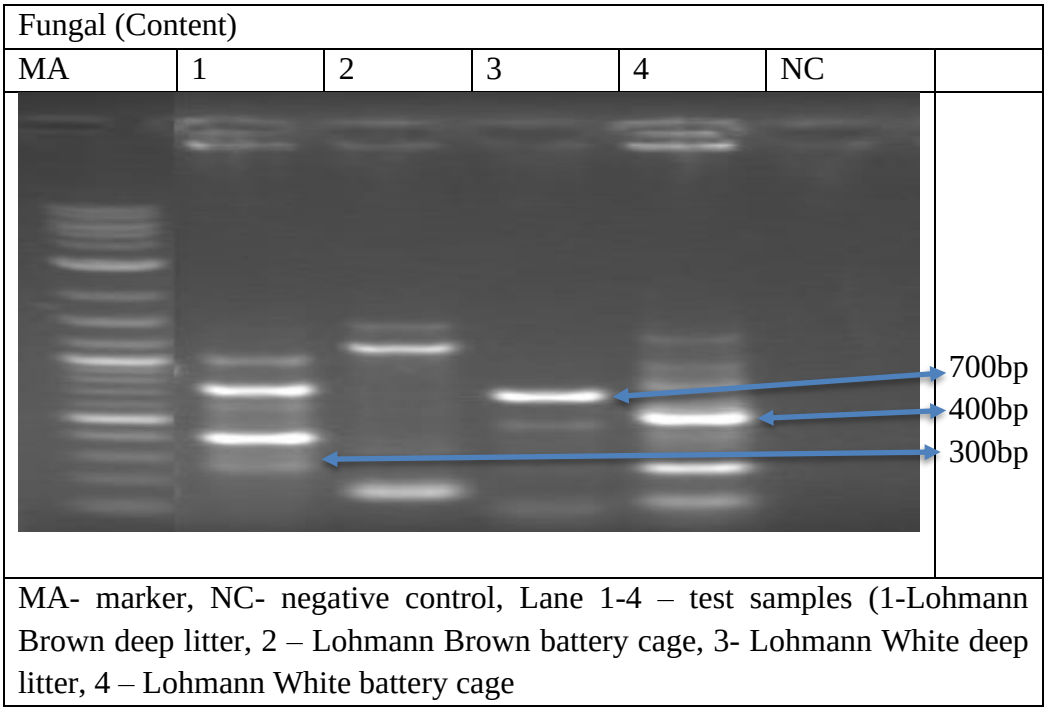

Figure 22: Fungal (Content)

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4.8 Phase 4: Sensory Quality Analysis

4.8.1 Effects of the strain of layer on the sensory quality of eggs

Table 19 shows how the strain of layer impacts the organoleptic attributes of the cooked egg. The variety of the layer had a notable impact ($p < 0.05$) on the visual appeal, smell, flavor, consistency, and overall evaluation of cooked egg whites and yolks. Despite the significant difference, the panel preference shifted toward Lohmann brown in all the sensory characteristics except the texture of the yolk.

Table 19: The effects of the strain of layer on the sensory characteristics of the cooked whole egg

Characteristics	Content	Lohmann White	Lohmann Brown	SEM	P-value
Appearance	Albumen	5.74^{b}	6.54^{a}	0.10	<.01
	Yolk	5.93^{b}	6.32^{a}	0.10	<.01
Aroma/Odour	Albumen	6.26^{b}	6.71^{a}	0.10	<.01
	Yolk	6.28^{b}	6.71^{a}	0.09	<.01
Taste/flavour	Albumen	6.49^{b}	6.71^{a}	0.10	0.06
	Yolk	6.42^{b}	6.80^{a}	0.09	<.01
Texture	Albumen	6.39^{b}	6.69^{a}	0.10	<.05
	Yolk	6.01^{b}	6.67^{a}	0.11	<.01
Preference/Overall rating	Albumen	6.12^{b}	6.56^{a}	0.11	<.01
	Yolk	6.21^{b}	6.50^{a}	0.11	<.01

ab - indicates that averages in the same row but with different superscripts are statistically significant ($p<0.05$); SEM - Standard Error of the Mean.

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4.8.2 Effects of housing system on the sensory characteristics of eggs

Table 20 shows the housing effect on the sensory characteristics of boiled eggs in this study. From Table 20, majority of the sensory characteristics were not affected by the housing system. The taste/flavour of the albumen and yolk and the aroma of the yolk were the two characteristics that were affected significantly by the housing system. The panel liked slightly the aroma for both the albumen and yolk and only the taste of the yolk of eggs from the battery cage system. However, characteristics such as appearance, texture and overall rating were slightly in favour of eggs from the deep litter system.

Table 20: The effects of housing system on the sensory characteristics of boiled eggs

		Housing System			
Characteristics	**Content**	**Battery cage**	**Deep litter**	**SEM**	**p-value**
Appearance	Albumen	5.87[b]	6.41[a]	0.10	0.03
	Yolk	5.88[b]	6.38[a]	0.11	<.01
Aroma/Odour	Albumen	6.40[b]	6.71[a]	0.10	0.07
	Yolk	6.42[b]	6.60[a]	0.09	0.10
Taste/flavour	Albumen	6.39[b]	6.77[a]	0.10	<.01
	Yolk	6.42[b]	6.93[a]	0.10	<.01
Texture	Albumen	6.39[b]	6.69[a]	0.10	0.05

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	Yolk	6.25[b]	6.41[a]	0.11	<.24
Preference/Overall rating	Albumen	6.39[b]	6.48[a]	0.11	0.07
	Yolk	6.28[b]	6.55[a]	0.11	0.28

ab - indicates that averages in the same row but with different superscripts are statistically significant ($p<0.05$); SEM - Standard Error of the Mean. 5-Scale; 1=strongly dislike/preferred, 2=moderately dislike/preferred, 3=slightly dislike/preferred, 4=indifferent, 5=moderately liked/preferred, 7=strongly liked/preferred

4.8.3 Effects of age of birds on the sensory characteristics of boiled eggs

Table 21 displays how the age of the hen affects the sensory attributes of boiled eggs. The results obtained indicated significant ($p<0.05$) age effects on appearance, aroma/odour, taste/flavour, texture and overall sensory rating of boiled eggs. Panel preference fluctuated between early lay and mid lay eggs. They were indifferent to the appearance/colour of mid lay eggs but slightly liked the aroma and taste of the albumen and yolk of mid lay eggs than early lay eggs. However, the sensory characteristics of eggs from old lay were the most preferred among the three age groups and all their characteristics were moderately liked by the panelist.

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Table 21: Effects of age of birds on the sensory characteristics of boiled egg

		Age				
Characteristics	**Content**	**24**	**39**	**68**	**SEM**	**p-value**
Appearance	Albumen	5.60^{b}	5.01^{c}	7.81^{a}	0.13	<.01
	Yolk	5.58^{b}	5.05^{c}	7.74^{a}	0.13	<.01
Aroma/Odour	Albumen	5.76^{c}	5.85^{b}	7.84^{a}	0.12	<.01
	Yolk	5.80^{c}	5.85^{b}	7.83^{a}	0.12	<.01
Taste/flavour	Albumen	6.21^{b}	5.83^{c}	7.70^{a}	0.12	<.01
	Yolk	6.49^{b}	5.80^{c}	7.50^{a}	0.12	<.01
Texture	Albumen	6.44^{b}	5.81^{c}	7.36^{a}	0.10	<.05
	Yolk	5.99^{b}	5.74^{c}	7.29^{a}	0.13	<.01
Preference/Overall rating	Albumen	5.64^{c}	5.67^{b}	7.70^{a}	0.11	<.01
	Yolk	5.76^{b}	5.71^{c}	7.58^{a}	0.14	<.01

ab - indicates that averages in the same row but with different superscripts are statistically significant ($p<0.05$); SEM

Table 21 displays how the sensory traits of boiled eggs are impacted by the age of the layer. The results show that age had a significant impact ($p<0.05$) on the visual appeal, scent, flavor, consistency, and overall sensory review of boiled eggs. Panel preference fluctuated between early lay and mid lay eggs. The rating for appearance/colour of mid lay eggs was indifferent while that of the aroma and taste of the albumen and yolk of mid lay eggs was rated slightly preferred than early lay eggs. However, the sensory characteristics of eggs from old lay were the most preferred among the three age groups and all their characteristics were moderately liked by the panelists.

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4.8.4 Interactive effects of age, strain and housing system on the sensory characteristics of boiled eggs.

Table 22 shows interactive impact of age, housing system and strain on the sensory characteristics of boiled eggs. There existed a significant interactive impact of age and strain on the aroma, colour, taste, texture and overall rating of the eggs. There were also interactive impact of housing and age on the colour (albumen and yolk), aroma (albumen and yolk), taste (yolk) and texture (yolk). The aroma (albumen and yolk), texture (yolk) and overall rating of strain and housing system had no significant effects on the sensory characteristic of boiled eggs. The colour, taste, texture of yolk and albumen texture showed no significant impact by the interactive effects of age, strain and housing system.

Table 22: The interactive effects of age of layer, housing system and strain, on the sensory characteristics of boiled eggs

Characteristics		Significance level						
	Content	A	S	H	AXS	AXH	S X H	A X S X H
Appearance	Albumen	<.01	<.01	<.01	<.01	0.04	<.01	<.01
	Yolk	<.01	<.01	<.01	0.01	<.01	0.11	<.01
Aroma/Odour	Albumen	<.01	<.01	0.07	<.01	<.01	0.21	<.01
	Yolk	<.01	<.01	<.10	<.01	<.01	0.40	<.01
Taste/flavour	Albumen	<.01	<.06	<.01	0.02	0.16	0.45	<.01
	Yolk	<.01	<.01	<.01	<.01	<.01	<.01	<.01

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Texture	Albumen	<.01	<.01	0.05	<.01	<.01	0.15	0.03
	Yolk	<.01	<.01	<.24	<.01	<.01	<.01	0.85
Preference/ overall rating	Albumen	<.01	<.01	<.07	<.01	<.01	0.70	0.07
	Yolk	<.01	0.01	0.28	<.01	<.01	0.70	<.01

* Level of significance at p<0.05: strain (S), age (A), housing (H), interaction involving strain and age (S X A), interaction involving strain and housing (S X H), interaction involving housing and age (A X H), and the relationship among strain, age, and housing system (S X A X H) interaction.

4.9 Phase 5: Chemical Residue Analysis

4.9.1 Effects of age on antibiotic residue in eggs

Table 23 gives the effects of the age of birds on some microbes which was observed for eggshells were 7.33,7.50 and 10.00mm for 24-, 39- and 64-weeks old birds for *E. coli* extracts respectively; for *S. aureus* extracts the zone of inhibition for eggshell were 10.92, 7.50 and 7.50mm for 24, 39 and 64weeks old birds. The albumen and yolk samples showed 8.33, 10.00 and 10.00mm for E. coli and 9.75, 8.33 and 10.00mm for *S. aureus* for 24-, 39- and 64-weeks birds.

Table 23: Effects of age on antibiotic residue in eggs

		AGE				
Content	**Microbes**	**24**	**39**	**64**	**SEM**	**P-value**
Shell	EC	7.33	7.50	10.00	0.36	0.05
	SP	10.92	7.50	7.50	0.73	0.32
A+Y	EC	8.33	10.00	10.00	0.73	0.77
	SP	9.75	8.33	10.00	0.63	0.03

A+Y = Albumen and Yolk, EC = Escherichia Coli, SP = Staphylococcus aureus, SEM = standard error of means

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The Table 23 presents data on antibiotic susceptibility of *Escherichia coli* (EC) and *Staphylococcus aureus* (SP) bacteria at different ages and between two groups: "Shell" and "A + Y" (Albumen and Yolk). The data includes various antibiotics, such as AMP (*ampicillin*), CIP (*ciprofloxacin*), CRX (*cefuroxime*), COT (*cotrimoxazole*), MEM (*meropenem*), PEN (*penicillin*), COX (*cloxacillin*), AUG (*augmentin*), TET (*tetracycline*), VAN (*vancomycin*), ERY (*erythromycin*), and GEN (*gentamicin*). Each antibiotic's mean susceptibility value and standard error of the mean (SEM) are stated, along with the corresponding p-values to assess statistical significance.

Several antibiotics show significant differences in susceptibility between the two groups. AMP exhibits significant variations in susceptibility to EC and SP between Shell and A + Y groups ($p = 0.05$ and $p = 0.03$, respectively). Similar patterns are observed with CIP and CRX, where both EC and SP display notable variations in vulnerability among the groups ($p < 0.01$ in both comparisons). For COT, a significant difference in susceptibility is observed only in SP between Shell and A + Y groups ($p = 0.03$). MEM also shows a significant difference in susceptibility to EC ($p = 0.03$). Notably, PEN, COX, and AUG reveal significant differences in SP susceptibility between Shell and A + Y groups ($p = 0.03$, $p < 0.01$, and $p < 0.01$, respectively).

4.9.2 Effects of Strain on Antibiotic Residue in Eggs

The zone of inhibition for Lohmann white as shown in Table 8.2 was 7.33mm for eggshell and 8.72 for albumen and yolk for E. coli extract.

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Lohmann brown samples from egg and albumen and yolk were 7.11 and 10.17mm for S. aureus extract respectively.

Table 24: Effects of strain on Antibiotic Residue in Eggs

		STRAIN			
Content	**Microbes**	**Lohmann white**	**Lohmann brown**	**SEM**	**P-value**
Shell	EC	6.56	10.00	0.37	0.05
	SP	7.11	10.17	0.59	<.01
A+Y	EC	8.72	9.36	0.52	0.05
	SP	10.17	8.89	0.42	0.06

A+Y = Albumen and Yolk, EC = Escherichia Coli, SP = Staphylococcus aureus, Standard error of means (SEM)

Table 24 presents the impact of different bacterial strains (Lohmann White and Lohmann Brown) on antibiotic residue content in eggs. The table includes various antibiotics tested against Escherichia coli (EC) and Staphylococcus aureus (SP) bacteria in two groups, "Shell" and "A + Y" (Albumen and Yolk). Each entry displays the antibiotic content in micrograms (ug) per millilitre (ml) of egg sample, along with the standard error of the mean (SEM) and the corresponding p-value for statistical significance.

For the strain, Lohmann White, the antibiotic residue content for EC and SP in the Shell group is reported for AMP, CIP, COT, CRX, GEN, MEM, TET, VAN, CHL, CTR, CTX, AMK, PEN, ERY, COX, and AUG antibiotics. Significant differences in antibiotic content between EC and SP in the Shell group are observed for AMP, CIP, COT, CRX, MEM, TET, and VAN

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antibiotics ($p < 0.05$). Meanwhile, there was no significant variance observed in the levels of GEN, PEN, ERY, COX, and AUG ($p > 0.05$).

For the Lohmann White strain in the A + Y group, significant differences in antibiotic content between EC and SP are observed for AMP, CIP, CRX, MEM, TET, VAN, CTX, AMK, COX, and AUG antibiotics ($p < 0.05$). However, there is no significant variance observed in the levels of GEN, PEN, ERY, COX, and AUG ($p > 0.05$).in COT, GEN, PEN, and ERY content ($p > 0.05$).

For the strain Lohmann Brown, significant differences in antibiotic content between EC and SP in the Shell group are observed for AMP, CIP, CRX, COT, GEN, MEM, TET, VAN, COX, and AUG antibiotics ($p < 0.05$). There is no significant variance observed in the levels of GEN, PEN, ERY, COX, and AUG ($p > 0.05$) in PEN and ERY content ($p > 0.05$).

For the Lohmann Brown strain in the A + Y group, significant differences in antibiotic content between EC and SP are observed for AMP, CIP, CRX, COT, and AUG antibiotics ($p < 0.05$). Meanwhile, there is no significant variance observed in GEN, MEM, TET, VAN, PEN, and ERY content ($p > 0.05$).

4.9.3 Effects of housing system on antibiotic residues in eggs

The impact of the housing system on the antibiotics residue in eggs as indicated in Table 25 were 9.78 (*E. coli*) and 7.28 (*S. aureus*) for egg shell for birds reared in battery cage housing system; the zone of inhibition for birds raised in deep litter system were 3.33 (E. coli) and 10.00mm (*S. aureus*) for egg shell and 10.00mm (*E. Coli* and *S. aureus*) for albumen and yolk.

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4.9.4 Effects of housing system on antibiotic residues in eggs

Table 25: Effects of housing system on antibiotic residues in eggs

HOUSING SYSTEM					
Content	**Microbes**	**Battery cage**	**Deep litter**	**SEM**	**P-value**
Shell	EC	9.78	3.33	0.37	0.05
	SP	7.28	10.00	0.42	0.06
A+Y	EC	7.44	10.00	0.51	<.01
	SP	8.89	10.00	0.59	<.01

A+Y = Albumen and Yolk, EC = Escherichia Coli, SP = Staphylococcus aureus, Standard error of means (SEM)

Table 25 provides important information on how different housing systems affect the presence of residuals of antibiotics in eggs. The study compares the pair of housing systems: deep litter and battery cage, to evaluate how they influence the content of various antibiotics found in the eggs produced.

The results in the table indicate that the housing system has a considerable impact on antibiotic residues in eggs.

In the Shell group, significant differences in antibiotic content between EC and SP were observed for AMP/EC ($p = 0.04$), AMP/SP ($p < 0.01$), CIP/EC ($p = 0.04$), CRX/EC ($p = 0.01$), MEM/EC ($p = 0.05$), TET/EC ($p = 0.04$), VAN/EC ($p = 0.03$), COX/SP ($p = 0.02$), CTX/EC ($p = 0.10$), and AMK/EC ($p < 0.01$).

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Similarly, in the A + Y group, significant differences in antibiotic content between EC and SP were observed for AMP/EC ($p < 0.01$), AMP/SP ($p < 0.01$), CIP/EC ($p < 0.01$), CIP/SP ($p < 0.01$), COT/EC ($p < 0.01$), COT/SP ($p = 0.21$), CRX/SP ($p < 0.01$), CTX/EC ($p = 0.02$), AMK/EC ($p = 0.06$), COX/SP ($p < 0.01$), and AUG/SP ($p < 0.01$). These significant findings indicate that the housing system may influence the antibiotic residue levels in eggs produced by the hens.

4.9.5 Interactive effects of age, strain and housing system on antibiotic residue in eggs

Table 26 indicates strain and age, age and housing and the combined strain, age and housing interaction had significant effect on the zone of inhibition for egg shell samples for E. coli inoculated extracts. Age and housing system did not impact the zone of inhibition for egg shell, but did impact the albumen and yolk samples for S. aureus extracts. Also, there were no interactive effects on the zone of inhibition of the albumen and yolk samples for E. Coli extracts.

Table 26: Interactive effects of age, strain and housing system on antibiotic residue in eggs

Content	Microbes	S	A	H	S x A	S x H	A x H	SxAxH
Shell	EC	<.01	0.05	0.05	0.03	0.07	<.01	<.01
	SP	<.01	<.01	<.01	<.01	<.01	0.39	<.01
A + Y	EC	<.01	0.32	<.01	0.11	0.11	0.07	0.07
	SP	0.06	<.01	<.01	<.01	<.01	<.01	<.01

A+Y = Albumen and Yolk, EC = Escherichia Coli, SP = Staphylococcus aureus, SEM = standard error of means, S = strain, A = age, H = system of housing

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Table 26 provides valuable insights into the interactive impact of age, housing systems and strain on antibiotic residue content in eggs. The table contains information on various antibiotics, such as Escherichia Coli (EC) and Staphylococcus aureus (SP), and their interactions under different scenarios.

In the Shell group, significant interactions ($p < 0.05$) are observed for several combinations. For example, there are significant interactions between age and strain (S X A) for EC and SP content. Similarly, interactions between strain and housing system (S X H) are significant for EC content. Furthermore, there are significant three-way interactions (S X A X H) for EC content.

In the A + Y group, significant interactions ($p < 0.05$) are found for several combinations. Notably, there are significant interactions between age and strain (S X A) for EC and SP content. Significant interactions between strain and housing system (S X H) are also observed for EC content. Additionally, there are significant interactions between age and housing system (A X H) for EC content. Moreover, there are significant three-way interactions (S X A X H) for AMP/EC, AMP/SP, CIP/EC, COT/EC, CRX/EC, and MEM/EC content.

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CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion on physical egg quality characteristics

5.1.1 Effect of the strain of layers on egg quality

In the same study, a second model was developed to estimate the shelf life of eggs under both room temperature and refrigerated storage conditions. The data in Figures 5(a) and 5(b) indicate a robust relationship between the recorded values and the NIR-predicted values under both storage conditions. Table 9 shows that the correlation between the two variables is high, at 86 percent or higher. There existed a direct correlation between the observed values and the projected values based on NIR. However, certain extreme cases influenced the PLS analysis. In Table 9, it is demonstrated that the MSC-PLS model was the most effective for both storage conditions in both the calibration set and prediction set, achieving an R-value greater than 0.85 and RMSEC below 3.3 days. The thicker the eggshell the better the quality because softer shells tend to break easily. Joubrane *et al*. (2019) also obtained eggshell thickness values of (0.47–0.51mm). Joubrane *et al*. (2019) noted that variations in the thickness of the shells between breeds may result from factors such as the amount of food consumed, the calcium content in the diet, the weight of the eggs, and the date they were collected. These factors may contribute to the observed differences in shell thickness between different breeds of poultry.

Hagan and Eichie (2019) reported that the internal qualities of an egg are important factors in determining the quality of the egg., and in their study,

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Lohmann brown layers had higher albumen weight and height are greater in comparison to Lohmann white layers, resulting in a higher Haugh unit for the former.

Although diet was the primary influence, a significant variation ($p<0.05$) in yolk color was observed between both strains in this research. Nonetheless, the yolk hue identified in this research did not meet the International Markets' minimum standard of 9.0, as reported by Jones *et al*. (2002). Hence, it is necessary to incorporate green vegetables into the feeds of intensively raised layers at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast in Ghana, to enhance the yolk color and adhere to acceptable international standards.

5.1.2 Effect of the housing system on egg quality

As stated by Vlčková *et al*. (2019), egg weight is seen as a key factor in determining egg quality. In the present research, it was observed that eggs produced by layers in the deep litter setup weighed more than eggs from hens in battery cages, agreed with the findings of Zita *et al*. (2018). Nevertheless, Englmaierová *et al*. (2014) as well as Kraus and Zita (2019) observed that eggs were heavier when using the battery cage housing system. The differing outcomes, as noted by Vlčková *et al*. (2019), could be due to varying conditions during the experiments, including environmental variables, differences in strains, or changes in feeding methods.

In this research, the combined impact of age and housing type had no impact on egg size, contrary to results from Vlčková *et al*. (2019) and Yilmaz *et al*. (2017). Additionally, Samiullah *et al*., (2014) discovered that the weight

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of eggs produced by birds in a free-range environment initially rose at the beginning of the laying cycle and then plateaued, whereas eggs from conventional cages continued to increase in weight as the birds aged. Lewko and Gornowicz (2011) stated that the variability in egg weight was a consequence of physical and chemical aspects alterations referred to as egg aging. They mentioned that both water and gases start moving within the egg as well as between the egg's interior and the external surroundings. When eggs are smaller, they lose water quickly, especially when they possess a greater ratio of surface area to volume leading to heavier weight loss in the egg. In Grashorn's (2016) research, showed that as egg weight loss goes up, the quality of both the albumen and yolk changes at a faster rate. Caner and Yüceer (2015) stated that when eggs lose water, it leads to various quality changes such as thinning of albumen, higher pH levels, weakened and stretched vitelline membrane, higher dry matter in albumen, and more water in yolk.

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This study confirmed the results of Kraus and Zita (2019), Ledvinka *et al*. (2012), and Ogunshola *et al*. (2018), indicating that birds raised in deep litter systems yielded eggs of higher interior quality. Benton and Brake (2000) and Roberts (2004) discovered that eggs from birds housed in deep litter were more exposed to ammonia, potentially harming the quality of albumen and the Haugh unit. In the present study, it can be inferred that bird eggs placed under thick litter were exposed to reduced levels of ammonia.

5.1.3 Effects of age of layer on egg quality

In contrast to Kraus and Zita's (2019) previous finding, the current investigation found no significant relationship between age and egg weight. The present study's findings confirmed those of Padhi and Haunshi (2015).

Referencing Padhi and Haunshi (2015), it was noted that the Haugh unit is an important measurement., a finding also supported by Samiullah *et al*. (2017). According to Toussant and Latshaw (1991), the reduction in the quality of albumen with the ageing of layers, leading to lower Haugh unit values, is a result of the decline in protein concentration in the albumen. Wang *et al*. (2019) attributed the strong correlation between ovomucin levels and the Haugh unit to the presence of ovomucin in the albumen fraction. Samiullah *et al*. (2015) and Kraus and Zita (2019) found that the thickness of eggshells was greatly influenced by both age and the type of housing system used. This supports the relationship between age and housing situation, which differs from the findings of the present research.

Krau *et al*. (2019) found that yolk weight tends to increase as eggs age in both types of housing systems, a result that aligns with the current study's

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findings. Moreover, Sokołowicz and colleagues (2018) showed that yolk weight can be influenced by the housing system. Nonetheless, the findings of the present research contrast with those presented by Dikmen *et al*. (2017), who asserted that the correlation among age and housing arrangements had a notable impact on yolk weight. On the contrary, the current research did not find a notable connection between age and housing type when it came to yolk weight.

5.1.4 Interaction effects of age, strain and housing system on egg quality.

There is no notable interaction impact observed in this study among the type of layers, age of layers, and housing system in terms of egg weight. Additionally, no significant correlation between the age of the layer and the housing system in terms of egg weight, in contrast to Vlčková *et al*. (2018) who noticed a noteworthy correlation between strain and housing system regarding the weight of eggs. The research did not discover a noteworthy impact of these variables on egg weight.

5.2 Discussion on egg freshness measurement using NIR

5.2.1 General Discussion

The findings align with those reported by other researchers (Menezes *et al*., 2012), showing that egg freshness decreases with longer storage durations, as indicated by the Haugh unit (HU) rating. According to the HU rating scales, eggs are graded as AA when their HU is 72 and above, A between 60 and 72, and B below 60 (Novita, Putri, Azhari, Rastina, Bakri, Amiruddi & AK, (2021); Hisasaga, Griffin, & Tarrant, 2020). Values below 60 are considered at the consumer resistance point, while below 50 are

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regarded as poor and unacceptable (Zhao *et al*., 2010). However, it was observed that for eggs stored in cold storage conditions, the HU values do not directly correlate with freshness or storage duration.

This data emphasizes the significance of categorizing and forecasting the shelf life of eggs in different storage groups in order to offer useful insights to customers. Additionally, the study confirms the importance of creating a model to forecast egg quality in varying storage environments. Eggs in cold storage conditions retained their quality based on HU values, even after being stored for 20 days.

Analyzing egg samples with a portable spectrometer resulted in the development of a unique spectral profile that showed the freshness composition of the samples. The spectrum between 740 nm and 1070 nm, which was employed for fingerprinting, offered crucial insights into the eggs' chemical and physical characteristics. This information is important for identifying and forecasting the freshness ratings and laying date markings. The spectral profile showed several bands and peaks, believed to be caused by overtones and blends of primary oscillations, indicating the chemical and physical traits of various types of eggs and their respective levels of freshness. The properties identified in the spectral profile can be beneficial for both qualitative and quantitative fingerprinting. When light passes through the material, it undergoes physical and chemical interactions, causing changes in the spectrum. By comparing the altered spectrum with the original one, it becomes possible to link the optical information details of the biological material to its chemical and physical quality. This offers valuable insights for

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various applications and analyses, as highlighted by Aboonajmi & Faridi (2020). Therefore, extensive mathematical models and pre-processing were required to retrieve this important information. The necessity for pre-treatment of the spectral data set was satisfied by the MSC pre-processing approach. Based on the acquired data, it was evident that MSC increased the evaluation of both the categorization and measurement models in this investigation. It indicates that MSC approaches successfully corrected scattered light and baseline fluctuation as indicated by previous studies (Geladi *et al*., 1985; Ozaki *et al*., 2006). It further reinforces the idea that preprocessing spectral data is crucial for modeling to remove extraneous data and interference from the important characteristics of scanned samples. (Coronel-Reyes *et al*., 2018; Blanco & Villarroya, 2002). In addition, the optimal classification findings demonstrated a direct correlation found between the NIR spectrum and the different levels of egg freshness examined. PLS-R was used for the estimation of the indicated date of lay (for the determination of storage duration). The observed values (using destructive approaches) corresponded linearly with NIR projected readings according to the results obtained. However, only some anomalies have affected the PLS model. In Figure 22(b), the progression of the PLS-R model's sophistication in forecasting freshness based on storage duration is illustrated. It also details how the model components analyzed the fingerprint and how to understand the significance of each model component (Suhandy & Yulia, 2017). The main peaks that impacted its performance were observed at 840–855 nm, 875 nm, and 1000–1033 nm, as shown in the PLS-R weight plot for the first two PCs. These frequencies correspond to the second

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harmonics of OH, NH, and CH, which are associated with pH, proteins, and carbohydrates in biological substances.The NH, CH, and OH overtones in the spectra are highly important as they indicate aromatic amino acids, vital organic compounds found in eggs because of the existence of amine (NH2) and carboxylic acid (-COOH) functional groups (Khan, Siddiqi & Salahuddin, 2017). These operational groups are crucial parts of proteins, like proteins are composed of numerous smaller units known as amino acids (Khan *et al*., 2017). Furthermore, studies have demonstrated that the characteristics of both chemical and physical nature of eggs change by considering the length of time in storage, the temperature, and the level of humidity (Nour *et al*., 2011; Yao, Zhou, Wang, Liu & Yu, 2014). These changes have also played a role in identifying and measuring egg freshness through portable NIR spectroscopy. Specifically, the pH level of the egg white in newly laid eggs is between 7.6 and 8.5, but increases to 9.5 within the first few days, resulting in the creation of a thick, gel-like texture in the albumen. (Yao *et al.*, 2014; Omana, Liang, Kav & Wu, 2011). Hence, the identification of the CH third overtone around 875 nm in eggs may be linked to the pH level of the eggs. Furthermore, the changes observed in the overtones of the eggs could be attributed to the decomposition process taking place in eggs (Akter, 2014). These results are consistent with findings from previous studies by Coronel-Reyes *et al*. (2018). The brief OH overtone can be linked to the moisture initially found on the eggshell surface soon after the egg is laid. As time goes by, the cuticle on the egg dries out, causing moisture loss and a gradual rise in the OH band intensity. At the same time, the cuticle thins out, allowing more of the eggshell

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carbonate mineral to be exposed on the surface (Coronel-Reyes *et al*., 2018). In general, the results are consistent with findings from previous research. In particular, the correlation coefficients of 0.87 and 0.88 achieved for autonomous set for ambient and refrigerated storage matched previous studies by Coronel-Reyes and colleagues (Abdel-Nour *et al*., 2011; Coronel-Reyes *et al*., 2018; Sun, Yuan, Cai, Lin, & Zhao, 2015); nevertheless, the root mean square error of calibration values of 2.57 and 2.66 differed from those reported by the aforementioned authors. As an example, Sun and colleagues (Sun *et al*., 2015) accomplished an R value of 0.8653 and RMSECV of 3.745 when utilizing artificial vision and dynamic weighing to evaluate egg freshness. Additionally, some achieved an R-value of 0.89 alongside RM. Overall, the results of this study align with prior research. In particular, the R values of 0.87 and 0.88 acquired for the autonomous group in regular and chilly storing circumstances correspond with the findings disclosed by Coronel-Reyes and peers (Abdel-Nour *et al*., 2011; Coronel-Reyes *et al*., 2018; Sun, Yuan, Cai, Lin, & Zhao, 2015). Nevertheless, the RMSEC values of 2.57 and 2.66 showed minor discrepancies in comparison to the values reported by these authors.

In the study by Sun *et al*. (2015), they obtained an R value of 0.8653 and RMSECV of 3.745 by employing artificial vision and dynamic weighing to evaluate egg freshness. Similarly, a different study also achieved an R value of 0.89 and an RMSECV of 1.65 by utilizing a high-quality VIS/NIR spectroradiometer in the laboratory (Abdel-Nour *et al*., 2011). Additionally, in a different investigation conducted by Aboonajmi and Najafabadi (2014),

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utilizing VIS/NIR spectral readings ranging from 300-1100 nm, a determination coefficient (R^2) of 0.79 was discovered for forecasting Haugh unit. The differences in the results may be due to variations in the predictive models and the introduction of a new experimental configuration (Aboonajmi *et al*., 2016).

5.3 Discussion on proximate and mineral content in eggs

5.3.1 Effects of the strain of layer on the proximate and mineral content in eggs

The findings of the current research regarding protein content were in line with Dawolor's (2017) results but contradicted the studies of Franco *et al*. (2020); Krawczyk *et al*. (2011), and Minieri *et al*. (2016).

According to Krawczyk *et al*. (2011), variations in protein content may be attributed to additional protein sources, such as invertebrates and plants, which hens, particularly those in free-range systems, consume as part of their diet.. There is therefore the need to add plants and other sources of protein in feeding intensively raised hens with diets that boost the protein levels. The results of this study were similar to those of Dawolor (2017) and Chepkemoi *et al*. (2017) indicating that moisture content was not different from Lohmann Brown and Lohmann White eggs but differed from the data from Franco *et al*. (2020) who indicated that strain significantly affected the moisture content. The higher moisture content values according to Rose (1997) may be possible due to the higher proportion of albumen in their eggs.

In conformity with the result, Minieri *et al*. (2016) showed that strain significantly affected the ash content when they compared eggs from

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Mugellese breed to White Leghorn ones. On the other hand, this contradicted the findings of Abdul-Rehman *et al*. (2016), who reported that the ash content was numerically higher in FAY eggs compared to White Leghorn eggs; however, the difference was not statistically significant. The findings also indicated that the fat content in Lohmann white eggs was significantly greater ($p<0.05$) than in Lohmann brown eggs, aligning with Abdul-Rehman *et al*. (2016) but contradicting Chepkemoi *et al*. (2017) who found no notable difference in fat levels among various poultry species' eggs. This may be as a result of higher yolk weight and higher yolk: albumen ratio in Lohmann White eggs than in Lohmann Brown eggs (Bell *et al*., 2002). Chepkemoi and colleagues (2017) discovered that out of five poultry species, domestic guinea fowl eggs had the most calcium at 194.30mg/100g in their research. This is similar to our findings where Lohmann brown eggs were higher in calcium compared to Lohmann white but the values were lower compared to other studies. Sidhu *et al*. (2004) who argued that the decreased mineral content may be linked to many commercial feeds being made up of grains containing anti-nutrients that hinder mineral absorption.

5.3.2 Effects of age of layer on proximate and mineral composition of eggs

Moisture, ash and phosphorus decreased in older age while potassium, sodium along protein increased from mid-lay to late-lay eggs. According to Stanišic *et al*. (2015), the hens' age was an important factor in determining the proximate of the whole egg which is consistent with this study. Also, the finding from this study agrees with the data from Ghane *et al*. (2015) as carbohydrates, fibre, dry matter and sodium were moderately constant. In

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addition, the significant increase of protein, fats and calcium with age also confirms the outcomes of Ghane *et al*. (2015). According to Ghane *et al*. (2015), the reduction in moisture in eggs from older hens is partly caused by a greater amount of nutrients in eggs laid later in life compared to those laid earlier. The high concentration of nutrients in older eggs according to Ahn *et al*. (1997) can be explained by the high yolk: albumen ratio in older eggs since studies have shown that a considerable amount of minerals and nutrients are in egg yolk.

5.3.3 The effects of housing system on proximate and mineral composition of eggs

The findings of this study indicated that the housing arrangement has a substantial impact ($p<0.05$) on the proximate and mineral content of eggs. Differences in proximate and mineral contents were obvious in dry matter, ash, protein, fats/oil, carbohydrate, potassium, calcium, phosphorus and sodium. Apart from phosphorus and sodium, the content of all the other minerals showed a notable increase in deep litter than in battery cage. The protein content discrepancy did not align with the results of Ogunwole *et al*. (2015) and Radu-Rusu *et al*. (2014) but did align based on the results of Krawczyk (2009). The results of this study were alike to Radu-Rusu *et al*. (2014) findings, showing that eggs from organic farming and free-range systems had more fat than eggs from conventional cage systems. Kiczorowska *et al*. (2015) and Sokołowicz *et al*. (2018) found no significant discrepancies in dry matter, total protein, and crude ash levels in egg white and egg yolk, which contrasts based on the results of the current research. Radu-Rusu *et al*.

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(2014) found that decreased movement in caged hens and increased movement and thermoregulation in free-range hens affect the absorption of energy and nutrients from feed and their metabolism, impacting the transfer of excess nutrients like lipids from the blood to eggs. Eggs from cage-raised hens, whether they are kept in conventional cages or furnished cages, will have a higher energy content and therefore be less nutritious than eggs from free-range hens. Attia *et al* (2014) suggested that variations in the nutrient composition of eggs may be credited to the absolute weight and chemical makeup of the eggs.

5.3.4 The interactive effects of strain, age of layer and housing system on the on proximate and mineral composition of eggs

The result from this study demonstrated that the proximate and mineral content of the whole egg is impacted by the interaction of strain, age and housing system which confirms the data of Heflin *et al.* (2018). Aside from the influence of feed, researchers have attributed the difference in egg proximate and minerals to stress (Heflin *et al.*, 2018) and egg size (Anderson, 2011), which affects the ratio of yolk to albumen.

On the other hand, the levels of potassium, sodium, and calcium were discovered to be significantly influenced by the combined effects of strain, age, and housing system ($p<0.05$). This contrasted with the findings of a previous study by Heflin *et al*. in 2018, demonstrating that eggs from hens raised conventionally did not exhibit elevated levels of potassium and manganese in comparison to eggs from hens in alternative systems.

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Dry matter, ash, protein and potassium Significant impacts were observed due to the combination of strain and housing. Interaction of the three factors however affected dry matter, protein, fat/oil, potassium and sodium significantly. Heflin *et al.* (2018) indicated the lack of detailed information about the influence of age, strain and environmental effect on egg nutrients even though the nutrient content of eggs is of concern to consumers as stated by Anderson (2011). This study examined the impacts of age, strain and housing system on the proximate and mineral content within whole eggs.

The result from this study demonstrated that the proximate and mineral content of the whole egg is impacted by the interaction of strain, age and housing system which confirms the data of Heflin *et al.* (2018). Aside from the influence of feed, researchers have attributed the difference in egg proximate and minerals to stress (Heflin *et al.,* 2018) and egg size (Anderson, 2011), which affects the ratio of yolk-to-albumen.

On the other hand, the levels of potassium, sodium, and calcium were notably affected by the combination of strain, housing system and age, with statistically significant results ($p<0.05$). This contrasts with prior research by Heflin *et al*. (2018), which found that eggs from hens in traditional housing systems did not possess elevated levels of potassium and manganese in comparison to those in alternative systems.

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5.4 Discussion on prediction of proximate and mineral content in eggs using NIR

5.4.1 NIR Spectra analysis and characteristic band determination

Figure 6.1 depicts the evident noise at both ends of the spectrum, which is due to the diffraction grating's efficiency waning as it approaches the edges. This phenomenon is attributed to the behavior of the diffraction grating, an optical component widely used in spectroscopy to disperse light into its constituent wavelengths. However, as one moves towards the edges of the spectrum, the efficiency of the diffraction grating diminishes, leading to an increase in noise levels. The NIR spectra of rice grains exhibit distinct absorption features, indicating the rice grains' interaction with specific wavelengths of light. Two prominent absorption peaks are observed within the spectrum. Beyond that, the NIR spectra of rice grains show two separate absorption peaks at wavelengths between 920 and 950 nm. In this wavelength range, there is a clear absorption peak, suggesting that rice grains possess a strong affinity for absorbing light in this particular region. The absorption at these wavelengths could be attributed to specific molecular or chemical properties inherent to rice grains. Also, another distinct absorption peak is observed within the NIR spectra, spanning the wavelengths of 1400-1500 nm. This peak signifies that rice grains also strongly absorb light in this range, potentially due to different molecular components or structural characteristics. There is another significant feature within the range of 1650 and 1700 nm. This range exhibits a distinct absorption peak, indicating that rice grains strongly absorb light in this particular spectral region as well. This absorption

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peak at 1650-1700 nm provides further insight into the interaction between light and rice grains in this specific wavelength range. A notable feature in the rice grain NIR spectra occurs at approximately 1670 nm. At this specific wavelength, there is a significant dip or valley in the spectrum, indicating that rice grains exhibit reduced absorption or may even scatter light in this region. Importantly, this feature is credited to the "first overtone of O-H stretching." In more precise terms, it suggests that the behavior of oxygen-hydrogen (O-H) bonds within the rice grains is linked to their interaction with light at this wavelength.

Chemometric analysis (PLS, iPLS, SiPLS) was utilized for model and examine the spectral data to investigate the model performance of the following wavelength ranges: 900 to 1700 nm, 920 to 950 nm, 1320-1390 nm, and 1570 to 1680 nm, respectively. Furthermore, the egg samples were split into two groups: samples in the calibration set and 27 samples in the forecast created by the SPXY method using the sample set partitioning approach with joint x-y distance. The leave-one-out method was also used for cross validation. In order to prevent prejudice when dividing the subset, every three samples were separated into a calibration set consisting of roughly two randomly selected samples and a collection of predictions generated by the leftover samples.

5.5 Discussion on bacterial and fungal load in eggs

5.5.1 Prevalence of Bacterial and Fungal Load

In this study microbial contamination on eggshell and egg contents from eggs from layers of the three different ages comparison made between

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the deep litter and battery cage housing systems. According to Eddin *et al*. (2019), eggs can be exposed to harmful microorganisms through different routes, with several foodborne pathogens able to penetrate the egg and remain viable until its expiration date. This collaborates with the results of the present study as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter*, *Listeria* and Yeast and Mould were isolated from the eggshell and content (main reason(s) for prevalence). A study by Awny and colleagues in 2018 found that the aerobic plate count is indicative of the hygiene practices followed during production, handling, and storage. The increased aerobic plate count is likely due to inadequate sanitation in the farms, particularly in the pens. The discovery of Staphylococcus aureus in eggs matches the results of Awny *et al*. (2018), where eggs from balady farm hens and ducks did not meet the Egyptian Standard for Nil levels in egg content.

5.5.4 Effects of Housing System on Bacterial and Fungal Load on Egg Quality

The results of the study are in line with the conclusions of Englmaierová *et al*. (2014), Sharma (2020), and Adegbenro *et al*. (2020). Sharma (2020) attributed the higher bacterial count to the fact that birds in range or litter may consume the soil or litter and as such the cloacal microflora may be changed. Smith (2000) also explained that eggs are mainly sterile when laid but become contaminated when they come in contact with dust, litter droppings and with conducive environment penetrate the content of the eggs. However, there was difference based on the earlier research conducted by (Oviasogie *et al*., 2016) which highlighted highest fungi count in battery

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cage rearing system (give reasons). They attributed the high microbial counts to poor hygienic conditions. Despite the higher bacterial and fungal load in the deep litter, housing did not have a notable impact on the amounts of microorganisms which contradicted the previous study by Chousalkar *et al.* (2021) where housing significantly affected fungal and mycotoxins penetration in eggs.

The E. coli and Staphylococcus isolation in both the eggshell and content confirms the findings of Adegbenro *et al*. (2020) and De Reu *et al.* (2008). Keller *et al.* (1995) explained that microbes in the content of the egg could potentially be due to a vacuum-effect which occurs when heat is loss in the egg after lay. Consequently, the microorganism will penetrate the shell and the membrane which may led to severe health effects when eggs are consumed raw or uncooked.

The amount of Salmonella in eggshells was greater in deep litter systems but higher in the eggs themselves in battery cages. This outcome validates Chousalkar *et al.*'s (2021) discovery that the housing system has a function in affecting the overall Salmonella presence on an eggshell. Raspoet *et al.*, (2014) connected the ability of S. Enteritidis to survive in bird reproductive systems and remain viable in eggs to certain genes that control cell surface lipopolysaccharides and flagella, as well as those involved in stress responses, which are typically seen as minor alterations in several genes.

The isolation of Yeast and Mold species concurs with the conclusions of Awny *et al*. (2018) when they identified Molds from balady, farm hen egg and duck egg content. Joseph and Babatunde (2006) explained that eggs that

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are kept under relatively high humid condition may facilitate a high incidence of molds in egg content. According to USDA/AMS, (2000) Mold contamination triggers severe losses in egg industry in addition to incidence of public health hazard.

5.6 Discussion on PCR identification of microbes

Pathogenic bacteria such as *Salmonella, E. coli, Listeria* etc in egg samples frequently turn out to be the cause of food poisoning in humans. As a result, a rapid, subtle and precise detection technique is required to determine the bacteria contained in the foods (Laude *et al*., 2016) so that doctors and health professionals will successfully treat the victims. This study used the one Tag Quick-load 2x master mix with standard buffer (50 rxns (50ul. vol) and Quick load purple 2-Log DNA Ladder (0.1 - 10.0kb) 10ug/ml from New England BioLabs. The primer pair for the forward and reverse were enhanced in an annealing temperature range 58 °C– 78 °C. Accurate temperature is important because inaccurate temperature can lead to primer attachment errors.

Data obtained showed the incidence of exact DNA bands of *Salmonella typhi*, *Salmonella enteritidis*, *E. coli, S. aureus*, *Campylobacter coli*, *Campylobacter jejeni*, Listeria, yeast and mould with amplicon size of 204bp, 304bp, 625bp, 450bp, 287bp, 159bp, 497bp and 300bp, 400bp, 700bp respectively, an indication that the DNA of the microbes has been amplified successfully. The result is determined by the size of the amplicon in the amplification result, consistent with the primer design findings from (Nurjayadi *et al*., 2019). Figures 9.1 - 9.5 and 9.6 reveal that the DNA band

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appeared thickest and brightest at temperatures of 58 °C and 72 °C, indicating optimal amplification through annealing suitable for PCR use (Nurjayadi *et al*., 20219). In their research on identifying E. coli O157 and Salmonella species in raw chicken meat cuts in Ismailia province, Egypt, Solikhah (2020) discovered E. coli with a 625bp band, consistent based on the results of the current research

Scallan *et al*. (2015) indicated that Salmonella has been behind food-borne diseases associated with higher rates of illness and death globally. In the USA it was ranked the 2nd most logged bacterial foodborne illness in 2011. The confirmation of Salmonella typhi and Salmonella enteritidis corresponds to the result of Nurjayadi *et al*. (2019). Nurjayadi *et al*. (2019) isolated Salmonella typhi bacteria in contaminated eggs while Kubo *et al*. (2020) detected Salmonella enterica in egg yolk by PCR. On the contrary, AS and HA (2019) could not detect Salmonella species in balady hen's egg content. De Buck *et al*. (2004) attributed the difference to the layer's health condition at transovarial transmission of Salmonella to eggs, the magnitude of shell contamination, and the succeeding penetration of the shell. In addition, Gast *et al*., (2022) mentioned Salmonella enterica may be isolated from shells and contents because it has a strange capacity to colonise in the tissues of layers and spread vertically to exist in the contents of unbroken shell eggs

Kareem *et al*. (2020) isolated Staph aureus at 450bp, Chen *et al*. (2017) also isolated Listeria at 497bp and isolated yeast and mould at a range of 300bp to 700bp. These isolations confirm the results of the present research. As stated by Raso *et al.,* (2011), efficiency in quantitative PCR methods relies

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on consistency, reproducibility, and dynamism. Inefficiency (<90%) may be caused by tag inhibitor contamination, a high annealing temperature, or faulty primer design, while high efficiency (>110%) may result from non-specific primer dimer or amplicon formation. Taylor *et al*. (2010) credited poor pipetting techniques for causing both high and low efficiency.

5.7 Discussion on sensory quality of eggs

5.7.1 Effects of the strain of layer on the sensory quality of eggs

The significant effects of yolk taste, flavour and mouth feel (texture) in the present research align with the results of Hussain *et al*. (2018) where White Leghorn eggs scored significantly higher ($P \leq 0.05$) in terms of yolk taste, yolk flavour, and yolk mouth feel than Aseel eggs. The better rating for appearance/colour of Lohmann brown eggs than that of Lohmann white eggs agrees with the studies of (Berkhoff *et al*., 2020) who found consumers rating brown shell eggs more favourable than white shell eggs. The difference in appearance as explained by (Wan *et al*., 2019) may be attributed to the influence of strain. According to Titcomb *et al*. (2019), the variation in yolk color can be attributed to multiple factors, such as the origin and types of pigmentation (natural or artificial), the combinations of xanthophylls used, the durability and presence of xanthophylls in the diet, the makeup of the diet, stress levels, genetic factors, and the health status of the laying hens. These various factors can influence the pigments present in the yolk and contribute to the diverse range of yolk colors observed in eggs. Previous study by Zurak *et al*. (2022) suggested that the color variation in the yolk is due to the absorption and levels of carotenoids. As a result, birds that have access to feeds that are

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higher in pigments like carotenoids found in grasses will have darker yellow shades.

5.7.2 Effects of housing system on the sensory characteristics of eggs

The notable impact of the housing system on the smell and flavor of egg white and yolk in this research is akin to the results of Sharif *et al*. (2017). Aroma and taste, according to Sharif *et al*. (2017), are related. Sharif *et al*. (2017) explained that because the aroma is a volatile compound it is perceived by the odour receptors of olfactory tissues of the nasal cavity during the mastication process which releases a pleasant smell that makes the food delicious. This, thus infers that the aroma of the individual egg can influence the differences in taste. Schneider *et al*. (2013) discovered that there were no noticeable variations in the appearance and texture of eggs from various production methods. This aligns with the results of the present research. The differences in appearance according to (Schneider *et al*, 2013), may result from the decrease in the egg's internal quality when eggs are stored. Water and CO2 are lost in storage through the eggs shell causing a rise in the albumen's pH level, leading to a decrease in its freshness (Alleoni & Antunes, 2005). Dietary additives (Janist *et al*., 2019) are also believed to influence the appearance of eggs. Berkhoff *et al*. (2020), Küçükyilmaz *et al*. (2012) and Lordelo *et al*. (2017) both observed that farm eggs had a richer yolk color compared to eggs from industrial production systems, supporting the current study's findings where panelists slightly preferred the color of deep litter eggs (Albumen 6.18/yolk 6.10) over battery cage eggs (Albumen 5.97/yolk 5.98).

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5.7.3 Effects of age of birds on the sensory characteristics of eggs

The significant effect of age on taste and texture contradicts the finding of Kraus and Zita (2019) who indicated that the internal quality of eggs declines with age. Vlčková, Tůmová, Míková, Englmaierová, Okrouhlá, & Chodová, (2019) attributed the difference in the texture of eggs to age and accumulation of ammonia from the droppings. He explained that as the birds age there is a loss of CO2 and moisture through the shell which softens the texture of the albumen.

5.7.4 Interactive Effects of age, housing system and strain on the sensory characteristics of eggs

Taste according to Mouritsen and Styrbæk (2017) is one of the most essential senses that humans depend on to steer us towards food that is palatable and nourishing. The combined impact of age, housing type, and breed on albumen and yolk taste support Cardona *et al*.'s (2023) discovery of a link between panelist and treatment on aroma, flavor, and overall rating. The difference in texture according to Vlčková, *et al.*, (2019) may be a result of high environmental temperature. Sasaki *et al*. (2018) found no statistical interaction effect on aroma and taste between rice-feeding and farm which disagrees with the findings in this study.

5.8 Discussion on Antibiotics Residue Analysis

5.8.1 Effects of age on antibiotic residue in eggs

In relation to the effects of age on antibiotic residue in eggs, the results suggest that the age of the hens (grouped as Shell and A + Y) affects the existence of antibiotic remnants in the eggs. The observed variations in

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antibiotic susceptibility between the two groups, particularly in response to specific bacterial species (EC and SP), could imply differences in how antibiotics are metabolized or excreted in older versus younger hens. These findings highlight the importance of considering the age of the hens when evaluating antibiotic usage in poultry farming to minimize the risk of antibiotic residues in eggs, promote food safety, and address concerns related to antibiotic resistance.

The study is consistent with Donoghue, Hairston, Gaines, Bartholomew, & Donoghue (1996) who found that drug residues were more prevalent in less mature yolks compared to larger preovulatory yolks, implying that eggs released later may have higher residue content even after drug withdrawal. Similarly, Roudaut and Moretain (1990) noted that the excretion of drugs in the yolk generally lasted longer, with spiramycin being the antibiotic most commonly excreted in eggs. Additionally, Roudaut (1989) stated that detectable residues in eggs were only found with DHS administered through the intramuscular route, and such residues persisted in the whole egg for up to 8 days.

5.9.2 Effects of strain on antibiotic residue in eggs

The study compared the antibiotic residue content in eggs between two types of hens that lay eggs, Lohmann White and Lohmann Brown, and observed significant differences in susceptibility to various antibiotics between Escherichia coli (EC) and Salmonella enterica serovar Enteritidis (SP) bacterial species within each strain. The results highlight the influence of strain-specific factors on antibiotic residue content in eggs and emphasize the

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need for tailored antibiotic treatments based on both bacterial species and the specific strain of hens to ensure safe egg production and address concerns related to antibiotic resistance.

Consistently, the results of the present study support the findings of various researchers. Hakimzadegan, Khalilzadeh and Hasseini (2014) found that 30% of chicken eggs in Tabriz City were contaminated with antibiotic residues, with macrolides being the most common. In similar vein, McReynolds, Caldwell, McElroy, Hargis and Caldwell (2000) found that enrofloxacin treatment in egg-producing chickens resulted in detectable antibiotic residues in eggs. Additionally, Shahbazi, Hashemi, Afshari, & Karami, (2015) found that 3.3% of commercial eggs in Kermanshah, Iran had antibiotic residues, with tetracycline being the most common. Also, Cornejo, Pokrant, Figueroa, Riquelme, Galdames, Di Pillo, ... & Hamilton-West (2020) found that eggs from small-scale chicken farming operations in the central region of Chile were positive for different families of antimicrobials, indicating a lack of biosecurity procedures and a risk for human consumption.

5.9.3 Effects of housing system on antibiotic residues in eggs

The study compared battery cage and deep litter housing systems and revealed significant differences in antibiotic content between the two bacterial species, Escherichia coli (EC) and Staphylococcus aureus (SP), within each housing system. In the Shell group, significant differences were observed for multiple antibiotics, including AMP/EC, AMP/SP, CIP/EC, CRX/EC, MEM/EC, TET/EC, VAN/EC, COX/SP, CTX/EC, and AMK/EC. Similarly, in the A + Y group, significant differences were found for AMP/EC, AMP/SP,

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CIP/EC, CIP/SP, COT/EC, COT/SP, CRX/SP, CTX/EC, AMK/EC, COX/SP, and AUG/SP. These findings underscore the importance of the housing system in influencing antibiotic residue levels in eggs and emphasize the need to consider housing conditions in poultry farming practices to ensure food safety and responsible antibiotic use.

In conformity with the current study, Álvarez-Fernández Dominguez-Rodriguez, Capita, & Alonso-Calleja (2012) discovered that conventional cage, barn, and free-range housing systems showed higher resistance to antimicrobials compared to organic and domestic systems. On the other hand, Cornejo, Pokrant, Figueroa, Riquelme, Galdames, Di Pillo, F., & Hamilton-West (2020) found that eggs from backyard poultry production systems in Chile contained residues of various antimicrobial families, suggesting a lack of biosecurity measures and designing these systems vulnerable to the spread of remnants of antimicrobial substances. However, Matt, Veromann and Luik, (2009) did not observe a significant difference when there are traces of pesticides leftovers in organic and conventional eggs. Lastly, Holt, Davies,

Dewulf, Gast, Huwe, Jones, & Willian (2011) suggests that transitioning from conventional cages to alternative housing systems may have implications for egg safety and quality, including the presence of pathogens or chemicals

5.9.4 Interactive effects of age, strain and housing system on antibiotic residue in eggs

The study presents insightful findings on the effects of age, strain, and housing system interactively on antibiotic residue content in eggs. The study

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analyzed various antibiotics and bacterial species (EC and SP) under different scenarios. In the Shell group, significant interactions were observed between age and strain (S X A) for EC and SP content, indicating that hens' age, along with their genetic background, influences antibiotic residue levels. Additionally, significant interactions between strain and housing system (S X H) for EC content suggested that housing conditions can modify antibiotic residues depending on the hen's strain. Three-way interactions (S X A X H) further highlighted the combined impactss of age, strain, and housing system on EC content. Similar significant interactions were found in the A + Y group, emphasizing the joint impact of age, strain, and housing system on antibiotic residues in eggs. The study underscores the complexity of antibiotic usage in poultry farming and emphasizes the need for a comprehensive approach to ensure safe egg production and responsible antibiotic use.

The study is in line with Alvarez-Fernández, Dominguez-Rodriguez, Capita, & Alonso-Calleja (2012) who noted that the housing system had a significant impact on the prevalence of antimicrobial resistance in Escherichia coli strains found in eggs. In same vein, Vlčková, Tůmová, á & Chodová (2018) discovered that the housing system and storage time had the biggest impact on both the presence of microorganisms on eggshells and the entry of microbes into eggs. Similarly, Jones and Anderson (2013) found that Different housing systems showed varying levels of egg microbial content due to stress, emphasizing the importance of considering egg safety when selecting hen strains for each housing system. Lastly, Cornejo, Pokrant, Figueroa, Riquelme, Galdames, Di Pillo, F., & Hamilton-West (2020) reported that eggs produced

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by small-scale poultry farms in Chile tested positive for at least one antimicrobial residue, indicating a lack of biosecurity measures and the potential for antibiotic-related illnesses and antimicrobial resistance.

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CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATION

6.1 Introduction

This is the concluding chapter of the study. It summarizes the study process and outcomes, draws conclusions, and gives recommendations.

6.2 Summary

The study's main focus was meant to evaluate the quality of eggs using destructive means and nondestructive means. The destructive phase of the study focused on using conventional methods to assess the quality parameters of eggs. The nondestructive means investigated the feasibility of building a quantitative model for quick identification and quantification of egg quality. Destructively the study evaluated the physical (internal and external), the nutritional, microbiological, organoleptic and antibiotic properties of eggs from different strains of layers of different ages raised under different housing systems. In addition, the NIR identified and quantified egg freshness, mark date of lay and proximate values of eggs.

In order to generate accurate data, quantitative methods were used in the study. The study employed experimental design in determining the physical, nutritional, microbiological, organoleptic and antibiotic residual properties of eggs. Moreover, spectra data acquisition of the individual eggs using the NIRS assisted in building the models for quantification and identification of the eggs.

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Descriptive and inferential statistics of GENSTATS and MATLAB were employed to analyse all data collected and the results presented in tables and graphs.

6.2.1 Key Findings

The following findings were made from the study:

Firstly, the study's findings suggest that egg quality is affected by various factors such as layer strain, age of layers, and somewhat by the housing system. The choice of layer strain is crucial, as it significantly impacts the inherent quality of eggs, particularly in relation to freshness, as measured by Haugh units. This suggests that poultry producers and consumers seeking eggs with specific internal quality attributes should carefully consider the strain of layers used. Additionally, the age of the hens that are laying eggs plays a role in freshness, emphasizing the importance of timing in egg production. However, reassuringly, the study demonstrates consistency in certain external egg qualities across different conditions, suggesting that producers can make choices based on other criteria without compromising these specific attributes. In essence, the study highlights the multifaceted nature of egg quality determination and underscores the value of informed decisions in poultry management and egg selection to meet desired quality standards.

Secondly, the nutritional properties data demonstrated no noticeable ($p<0.05$) influence from the strain on dry matter, moisture, protein, fibre, carbohydrate and sodium. There was, however, a significant ($p<0.05$) housing impact with deep litter birds producing eggs with higher proximate and mineral contents compared to those on the battery cage system. The finding

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also showed that the age of the hens had a significant impact on the proximate and mineral composition of the eggs produced. Proximate and mineral contents in mid-aged and older eggs were higher than in early-stage eggs.

Thirdly, the third objective which evaluated bacterial and fungal loads of table eggs from different layer strains under different housing system using conventional means revealed that the mean bacteria count for the target microbes were higher in deep litter housing system compared to the battery cage system. Specifically, the mean count for Salmonella, Escherichia Coli, Staphylococcus aureus, Campylobacter, Listeria and Yeast and Mold in the egg shell and egg content were 1.36 x 104 / 5.2 x 104 cfu/ml, 2.06 x 104 / 8.7 x 104 cfu/ml, 9.9 x 10 4 / 8.2 cfu/ml, 1.62 x 103 / 4.8 x 105 cfu/ml, 7.3 x 105 / 1.45 x 104 cfu/ml and 9.8 x 10 4 / 8.6 x 10 4 cfu/ml respectively. Although age, strain and housing system had no significant effect on the presence of Salmonella, Escherichia Coli, Staphylococcus aureus, Campylobacter, Listeria and Yeast and Mold on the egg samples; however, isolation of these microbes from both the eggshells and the content of eggs confirms the urgent need for improvement of hygienic conditions in farms. Moreover, PCR examination successfully identified *Salmonella typhi*, *Salmonella enteritidis*, *E. coli*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Campylobacter coli*, *Listeria monocytogenes* and Yeast and Mould in the eggshell and content of the egg samples.

The fourth objective which evaluated the sensory properties of eggs revealed age and strain of layer significantly affected the sensory characteristics of eggs. Nevertheless, only the taste/flavor of the albumen and

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yolk, as well as the aroma of the yolk, were significantly influenced by the housing system ($P > 0.05$). The dark yellow yolk colour and brown egg-shelled colour was the most preferred characteristic. These findings suggested that old lay eggs from Lohmann brown strain reared in either of the housing systems have superior sensory characteristics over Lohmann white.

The fifth objective assess antibiotics residues in eggs from different strains of birds raised under different housing systems using conventional (destructive). The findings indicated that housing system and strain of layers significantly affected the antibiotic residues in the yolk and albumen as well as on the eggshell. The findings show residues in eggs from birds raised in the battery cage system where above birds raised in the deep litter system.

The utilization of handheld near-infrared spectroscopy (NIRs) in determining egg freshness and date when laid yielded promising results. The LDA model demonstrated an identification rate above 95% in both calibration and forecast sets for eggs stored in room temperature and refrigerated storage conditions. In particular, the LDA successfully identified eggs stored in room temperature with a 95.54% accuracy using 5 principal components, and achieved a 100% accuracy in determining the lay date of marked eggs stored in cold storage with 5 principal components. In addition, the PLS-R model yielded good results with an R value of 0.87 and RMSEI of 2.57 for ambient storage, and an R value of 0.88 and RMSEI of 2.66 for cold storage in the separate dataset. These findings demonstrate that handheld spectrometers combined with multivariate analysis offer a rapid and non-destructive

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approach for accurately measuring egg freshness. This innovative solution holds promise for ensuring egg integrity throughout the value chain.

The study highlighted the effectiveness of portable NIR spectroscopic techniques as a quick and non-invasive technique for simultaneously analyzing eggs. It successfully classified categorizing egg freshness and estimating the date they were laid. The PCA-MSC-LDA model achieved an impressive identification rate of over 95% in both sets of eggs were calibrated and predicted while stored in varying conditions of ambient and cold temperatures. Additionally, the MSC-PLSR model exhibited good predictive performance with $R = 0.83$ and above for all storage conditions studied. These results indicate that handheld NIR spectroscopy can be a valuable tool for determining egg freshness and storage duration, regardless of whether the eggs are kept in cold or ambient storage conditions.

6.4 Conclusions

In conclusion, the study demonstrated that handheld NIR spectroscopy is an effective non-invasive tool for assessing egg quality. It achieved over 95% accuracy in classifying egg freshness and predicting laying dates, with strong predictive performance across storage conditions. Raising Lohmann White layers under the deep litter system and limiting extended laying periods are recommended to improve physical egg quality. Nutritional analysis showed variations in proximate and mineral content based on bird age, strain, and housing system. Eggs from younger hens are suitable for processors due to higher moisture content, while those from older hens appeal to consumers for their higher mineral content. Birds raised in deep litter systems had better

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overall nutritional profiles. Microbial contamination was detected on eggshells and contents, including pathogens such as Salmonella spp. and E. coli. These findings highlight the need for strict biosecurity, proper handling, and contamination prevention measures to protect consumers. Sensory evaluation revealed a preference for dark yellow yolks from brown-shelled eggs produced by older layers, emphasizing the importance of dietary xanthophyll and optimal farming practices to meet consumer demands. Antibiotic residues detected in eggs pose health risks, necessitating strict monitoring and management to ensure food safety. In conclusion, the findings highlight practical recommendations to improve egg quality, safety, and mark*et al*ignment, supporting the value chain from production to consumption.

6.5 Recommendations

Based on the study results, the following recommendations were put forward.

1. If larger egg sizes are desired, the Directorate of Animal Production should encourage the adoption of the deep litter system among farmers.
2. To enhance egg freshness, it is recommended to raise Lohmann White layers using the deep litter method and avoid keeping them in lay for extended periods.
3. The Directorate should also encourage Farmers to do target marketing in order to sell eggs from younger hens and eggs from older birds to consumers (table eggs).
4. The Food and Drugs Authority and Veterinary Service department should intensify their surveillance to ensure on-farm biosecurity,

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reduced cross-contamination, inspection of eggs and poultry feed to protect eggs against contamination

5. In order to protect the integrity of egg along the value chain, it is recommended the model from the study be developed into a mobile app for accessibility to all stakeholders.

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APPENDICES

APPENDIX A

Proximate and Mineral Composition

Weighing of eggs

Analysis of minerals

Analysis of minerals

Analysis of minerals

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Oven

Drying of samples

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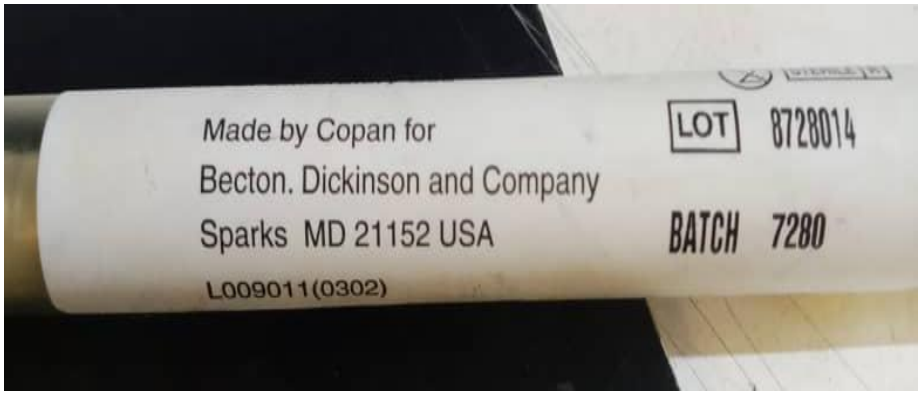
Chemical Residues in eggs



Microbes in their cases

E. coli and Staphylococcus. Aureus

E. coli and Staphylococcus. Aureus

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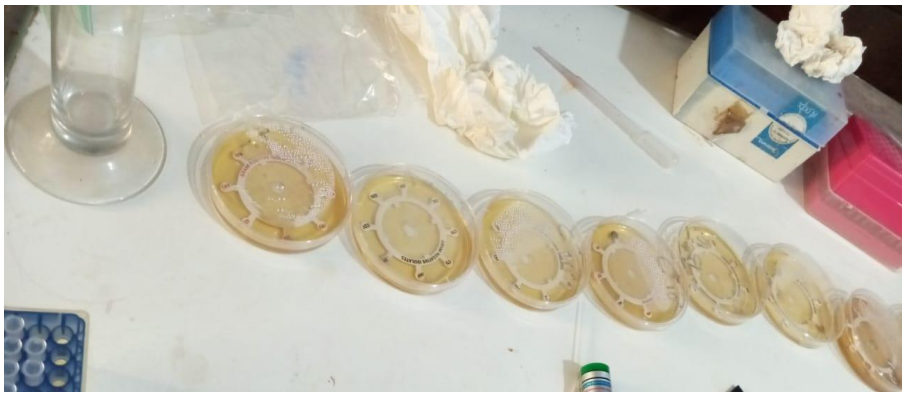
Control



Plates

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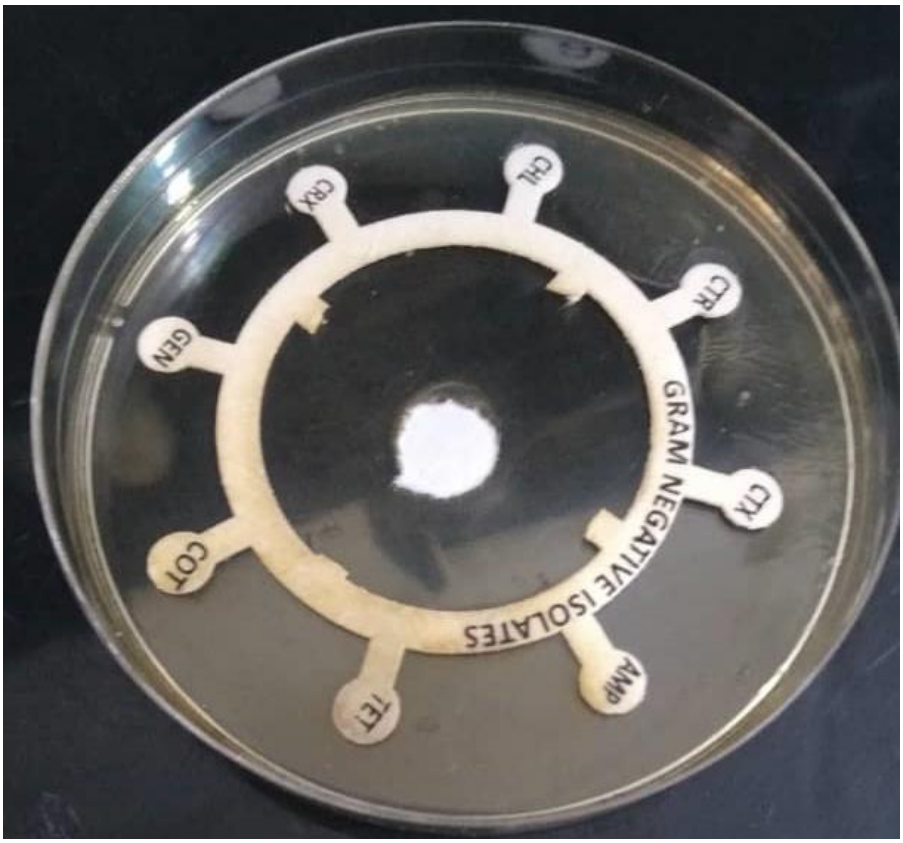

E.coli Lohmann Brown battery cage serial 1

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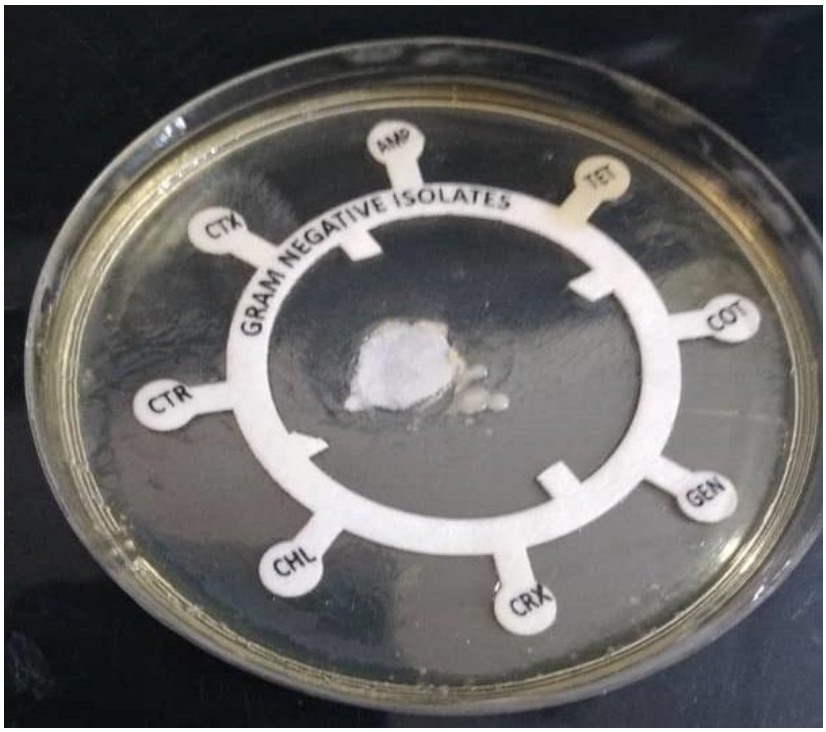
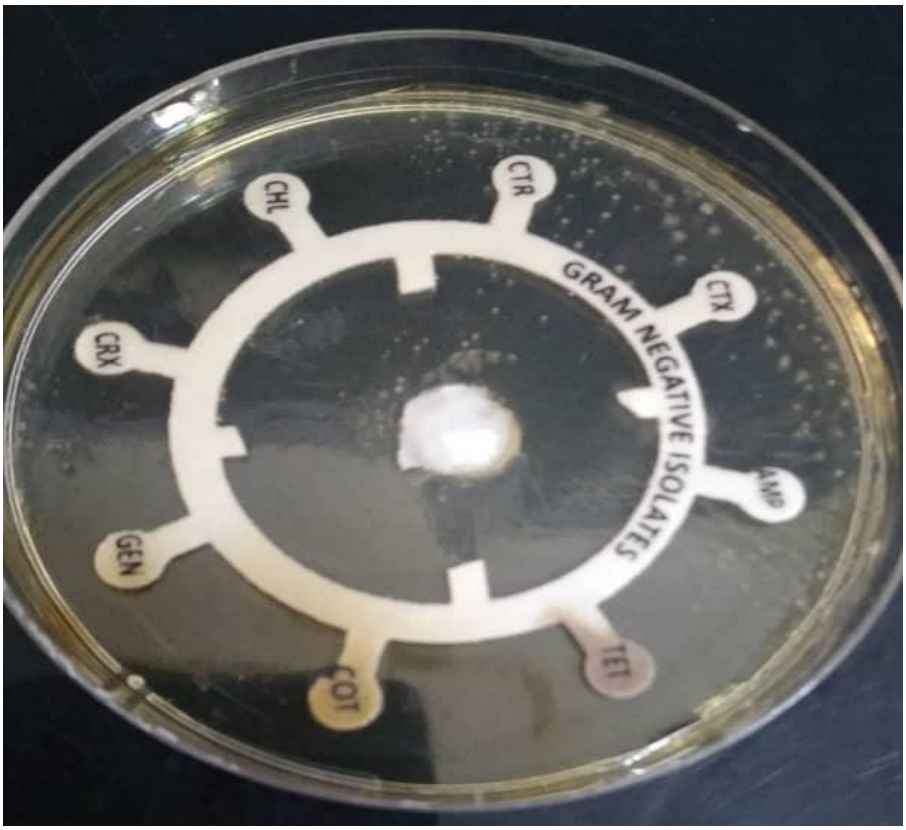


E.Coli Lohmann Brown battery cage serial 3



E. Coli Lohmann Brown deep litter serial 1

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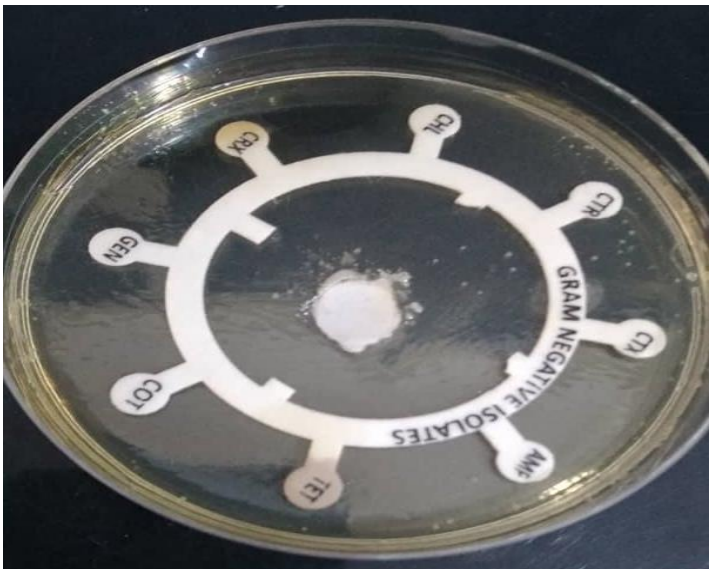

E. Coli Lohmann Brown deep litter serial 3

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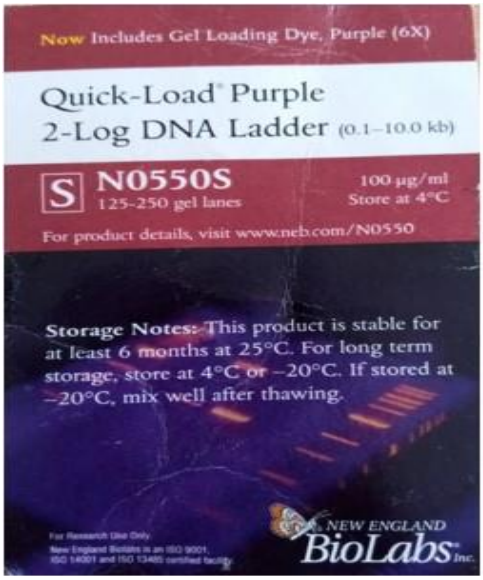

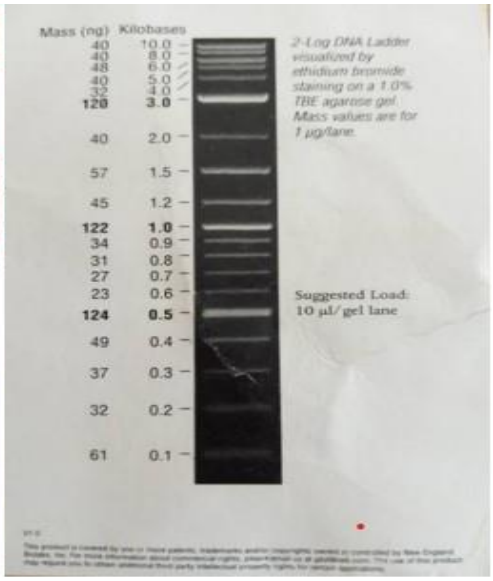

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