THE RESPONSE OF LAYERS TO DIETARY SUPPLEMENTATION WITH ACTIVATED CHARCOAL

BY

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DECLARATION

I, Makarabo Makhaleme hereby declare that this Thesis is my original work, approved and supervised by my Supervisor Prof O.I.A. Oluremi, and carried out by me, and has not been submitted to any University or similar Institutions for the award of any degree or certificate.

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DEDICATION

This work is dedicated to God for providing strength and wisdom in all my life endeavours. Also, to my parents ‘Makhotso Makhaleme and Moshoeshoe Makhaleme, my family and friends whose constant support and guidance have shaped my journey.

THESIS APPROVAL

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TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

LIST OF ACRONYMS

VF – Volatile fatty acids

AFAs – Antibiotic feed additive

AGPs – Antibiotic growth promoters

NSP – Non starch polysaccharides

DNA – Deoxyribonucleic acid

HU – Haugh unit

CRD – Completely randomized design

SEM – Standard error of mean

NFE – Nitrogen free extract

ME – Metabolizable energy

AC – Activated charcoal

LM – Layer mash

CP – Crude protein

Ca – Calcium

P – Phosphorus

NaCl – Sodium chloride

ABSTRACT

The study was conducted at the Animal Farm of the Teaching and Research Farm, The National University of Lesotho Roma to determine the effect of graded levels of activated charcoal in the diets of laying hens on performance response, internal and external egg qualities. A total of 120 Hyline white hens, 44-week-old with a mean body weight of 2060.70 g and egg production level of 88.92% were randomly allotted into five treatment groups, with three replicates per treatment and 8 hens per replicate. Hens were fed *ad libitum* on a basal diet supplemented with activated charcoal at five different levels of 0, 0.5, 1.0, 1.5 and 2% to give treatments T1, T2, T3, T4 and T5, respectively for 42 days. Data collected on nutrient composition profile of the activated charcoal supplemented diets, performance response, and external and internal egg qualities were subjected to the One-Way Analysis of Variance (ANOVA). Supplementation of the basal diet of laying hens with 0, 0.5, 1, 1.5 and 2% activated charcoal produced significantly ($p<0.05$) increased dietary salt (NaCl) as the percentage of activated charcoal supplementation increased, but varied significant ($p<0.05$) sequence on CP, crude fat, ash, Ca and P. There was no significant ($p>0.05$) nutritional advantage of feeding activated charcoal to laying hens on the performance response, feed intake and final body weight. Activated charcoal supplementation significantly ($p<0.05$) improved egg weight, egg width and shell weight which are external egg indices at 2% level, and also significantly ($p<0.05$) improved some internal egg quality characteristics, yolk height, albumen weight and yolk colour at 2% level. The results of the study showed that activated charcoal supplementation of laying hens can be used at 2% level for enhancement of egg weight, egg width, shell weight, yolk height, albumen weight and yolk colour. Further research should be conducted to investigate the optimal supplementation level of activated charcoal which will not be adverse to the performance of hens and egg quality.

Keywords: Activated charcoal, laying hens, egg quality, performance

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Due to high inflation, disruptions in supply chains as it is the case because of the conflict between Russia and Ukraine, the world is currently experiencing a severe global food crisis. This occurs at a time when the stability of global food market is still recovering from economic crisis brought on by the Covid-19 pandemic and climate-related crop failures caused by hurricanes and drought. According to the update by FAO (2023), global food insecurity has worsened as the result of the conflict in Ukraine, which has involved two major Agricultural nations. Global Network Against Food Crises (2022) highlights that over a quarter of billion people are facing acute hunger with economic shocks and the Ukraine war contributing to the increase. The suffering is worst in 48 countries, many which are heavily dependent on imports from Russia and Ukraine, and around the globe, more than 828 million people go to bed hungry every night (Georgieva *et al*., 2022). Food insecurity has negative effects on the economy, including undernourishment and famine, a rise in food prices, environmental issues (soil erosion and water pollution) and civil unrest (Aminetzah *et al*., 2022). To address the effects, sufficient financing is essential for organizations that focus on food security, such as Food and Agriculture Organization of the United Nations and the World Food Program (WFP, 2022).

Animal agriculture is important for various reasons. It contributes to food security, economic development and sustainable agricultural development and sustainable agricultural systems. Livestock provide a source of high-quality food that improves nutritional status, and they also contribute additional resources such as manure for fertilizer, on-farm power, and other by-products, which can assist smallholder famers in developing nations diversify their source of income and lower poverty (Reynolds *et al*., 2015). Additionally, it plays a vital role in poverty reduction, food security and agricultural development and can contribute to preservation of biodiversity in certain environments (NSAC, 2002). Meat, milk, eggs, fish and other animal products are excellent sources of dietary protein and other nutrients. In addition, livestock may assist remove otherwise undesired agricultural leftovers and serve as a source of draught power, fertilizer, and soil conditioner. Direct sales of livestock products such as milk, eggs, wool, meat and hides and manure, as well as sales of live animals, and charges for draught power or transport services, can all provide money on regular basis. Furthermore, livestock in mixed agriculture systems assist in lowering crop production risks.

Nutrition education is a very important concept which plays a crucial role in fostering a paradigm shift towards healthier lifestyle. A paradigm shift in nutrition education is therefore a fundamental transformation in understanding of an attitude to healthy eating. The need to address the intricate issues surrounding sustainable and healthful diet is a motivation for food security. Aleta *et al* (2022) reported that the application of the latest advances in fields such as complex systems, network sciences, big data, and artificial intelligence is necessary to solve some of the more challenging problems in sustainable and healthy nutrition. The benefits of a paradigm shift in nutrition education are significant and encompass various aspects of individual and public health. A paradigm shift in nutrition education can lead to improved health outcomes for individuals. By emphasizing the vital connection between food and health, individuals can make more informed dietary choices, leading to better overall health (Wallace, 2023). A paradigm shift in nutrition education can be achieved through various approaches, including the application of technological advancements, innovative research, and a focus on holistic and sustainable solutions. It is necessary to adopt a new perspective that centers on food within a one health framework due to the development of the present food systems, which have been shaped by a limited concentration on economic efficiency to offer inexpensive food at any cost (Rushton, 2021). Therefore, in order to address the complex issues surrounding sustainable and healthful nutrition, a paradigm shift in nutrition education and food systems is required.

A few methodologies have been suggested towards tackling the high feed cost issue including the utilization of local feed materials, manipulation of feed forms and feeding methods and use of feed additives have been applied to reduce the cost of feeding (Ugwu and Okoli, 2017). Nutritionists and experts in animal production have also argued in favor of use of feed additives as a way to maximize the adsorption of nutrients from ultimate feed source materials and boost output (Terrence, 2005). Feed additives are compounds that are added to feed to increase the effectiveness and the acceptability of the feed, or better metabolism and animal health. According to Peter *et al* (2003), feed additives are introduced for a number of reasons which include addition of colour and flavour to the diet and to alter the efficiency and speed of growth of animals. The inclusion of feed additives into compounded feed is now a typical practice in animal feed industry. Feed additives must be approved by the appropriate authority before they are used in livestock or poultry feeds (EFSA, 2011). Feed additives tend to fall into specific classes which describe their activity in feed or animal. They include feed manufacturing additives, performance enhancing additives and feed additives that improve animal health.

1.2 Problem Statement

In poultry industry, ensuring the quality of eggs is crucial for both consumer satisfaction and economic profitability. Various feed additives have been explored to enhance egg quality, including nutritional supplements, natural products and use of antibiotics. The use of antibiotics and feed additives to enhance egg quality is limited in most countries due to the belief that these compounds accumulate in the human body and adversely affect individual health. Therefore, this study aimed to investigate the efficacy of activated charcoal as a feed additive in layer diets and its impact on egg quality parameters. The study sought to determine whether incorporating activated charcoal into the diet of laying hens can lead to a significant improvement in the egg quality parameters compared to the traditional feed additives.

1.3 Justification of the Study

The findings of this study will provide valuable insights into the feasibility and benefits of using activated charcoal in poultry nutrition, potentially leading to enhanced egg quality and better health outcomes for hens. The results of the study will provide poultry producers with a novel feed additive option that enhances egg quality, contributes to animal welfare, and meets consumer expectations for natural products. Therefore, investigating the use of activated charcoal in layer diets is both timely and relevant, offering potential advancements in poultry nutrition and egg production.

1.4 Objectives

1.4.1 General Objective

To determine the effect of incorporation of activated charcoal as a feed additive in layers diet.

1.4.2 Specific Objectives

a) Chemical composition of activated charcoal and supplemented diets.

b) To determine the effect of activated charcoal as a dietary feed additive on external egg qualities.
c) To determine the effect of activated charcoal as dietary feed additive on internal egg qualities.

1.5 Hypothesis

1.5.1 Null hypothesis (Ho): The use of activated charcoal as a feed additive in layer diets has no effects on egg quality.

1.5.1 Alternative hypothesis (Ha): The use of activated charcoal as feed additive in layer diets has effects on egg quality.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Egg Laying Chickens

The main purpose of raising egg laying chickens is to produce high quality eggs. Therefore, it is important to know the difference between egg laying chickens from other poultry and it is also crucial to check the flock’s output and sport underperforming hens that reduce or eliminate profits. Egg laying chickens, particularly those in commercial settings, are bred to produce a large number of eggs, which can deplete their store of calcium and lead to various welfare issues such brittle bones and declining productivity (Stuttgen, 2020). Egg-laying chickens, especially those raised for high output, exhibit a variety of biological and genetic features that have a substantial impact on their reproductive success and egg quality. The economic significance of egg-laying performance is well acknowledged in the poultry production, as it is directly correlated to the profitability of egg production systems. High-yield commercial laying hens, such as White Leghorns, are specifically bred to produce approximately one egg per day under ideal conditions (Wang and Ma, 2019). Local breeds, such as dual-purpose chickens, often produce fewer eggs and are rarely subjected to systematic breeding efforts to improve laying performance (Zhang *et al*., 2021). The ability of chickens to lay eggs is mostly determined by genetic factors (Azmal *et al*., 2019). Furthermore, it has been demonstrated that the expression of genes linked with hormonal activity and metabolism in the ovaries correlates with rates of egg production, emphasizing the complex relationship between reproductive physiology and genetics (Du *et al*., 2023). The significance of metabolic activities in egg formation is further highlighted by the liver's function in the synthesis of lipoproteins for yolk deposition (Ding *et al*., 2008). Furthermore, there are several features that assist in describing egg laying chickens. Laying hens (layers) are leaner and small. They are well adapted to regularly laying eggs. At their peak, layers can lay one egg every day (FAO, 2012). The laying cycle of a chicken flock typically covers a lifespan of about 12 months (Jacob *et al*., 2022). Egg production starts when the birds get to about 18-22 weeks of age, depending on the breed and season. Furthermore, compared to broiler hens, egg laying chickens are thinner and develop more slowly. They can continue to lay eggs on regular basis for nearly a year although their natural lives can be up to ten years, after that they are usually slaughtered. Layers have prominent and active eyes and bright red and large crowns. Sigh (2019) reported that in good laying chickens, the yellow pigment that accumulates in the eye lid and beak is repelled shortly after the egg begins to hatch and these spots gradually become white. Sigh (2019) further reported that a non-laying chicken's pubic bones are tight, fairly hard and small, preventing the fingers from fitting between them, while egg laying chickens have flexible pubic bones that are widely separated. Normally, the broad pubic bone area makes it easier for eggs to flow through. Egg laying chickens have a deep, soft belly and are free of body fat buildup, whereas a non-laying chicken has rigid, shallow abdomen. Linking the breast bone and the pubic bones is where the abdomen's depth is measured. Layers also have a clean-cut, robust polished heads. Egg laying chickens are often more brilliant than broilers and also the vent of egg laying chickens is wide, oval, moist and warm (Ibukun, 2022).

2.2 Importance of Egg Laying Chickens in Human Nutrition

Egg laying chickens provide eggs for human consumption. Eggs are high in vital nutrients, including high-quality proteins, vitamins, and minerals, making them an important nutritional component for many people. The nutritional composition of eggs changes depending on the diets of the chickens and their environmental conditions, which has a direct impact on the health advantages of egg consumption. For example, the yolk of an egg is known for its high nutrient content, which includes polyunsaturated fatty acids, vitamins A, D, and E, and vital amino acids that are required for human health (Sipos, 2023). Iannotti *et al*. (2014) stated that with undernutrition remaining a notable problem in several parts of the world, egg production may be observed as part of the solution to fight famine. Eggs are an affordable source of nutrients for a healthy diet, making them accessible to a wide range of consumers, including pregnant women, children and the elderly (Lei, 2021). Along with high value protein, eggs similarly include very important vitamins, minerals and essential fatty acids. Eggs contain higher concentrations (20-30%) of vitamin A, B and B12. According to Mayer *et al.* (2003), eggs were identified as the third-most significant source of n-3 polyunsaturated fatty acids (6%), after meat (71%). Both poultry meat and eggs can be enriched with omega-3 fats, which are beneficial for heart health and overall well-being. The role that eggs play in managing micronutrient deficiencies, especially in susceptible populations like children and women of reproductive age, highlights the significance of eggs in human nutrition. Eggs provide vital nutrients that contribute to better health outcomes throughout pregnancy and early childhood development, as demonstrated by studies, which also reveal that eggs can considerably enhance mother and child nutrition (Stark *et al*., 2021).

Furthermore, eggs contain a lot of lutein which lessens the incidence of cataracts and muscle deterioration in the elderly, especially in developing countries (Lei, 2021). Regarding total protein, essential amino acids, total lipid, phospholipids, phosphorus and iron contents, eggs are generally consistent (Rizzi and Marangon, 2012). The consumption of eggs is not harmful to human well-being, particularly for those who live in poverty. Eggs are crucial for their health and welfare, thus wherever feasible, intake of eggs should be promoted. Furthermore, the nutritional value of eggs goes beyond basic sustenance. They are related with a variety of health benefits, including increased cognitive function and a lower risk of chronic diseases. According to Miranda *et al*. (2015), frequent egg consumption may have little to no negative consequences on cholesterol levels for the majority of people, calling into question the long-held idea that dietary cholesterol has a significant impact on heart health. Instead, the emphasis has turned to the overall dietary pattern and the quality of fats consumed, with eggs providing essential elements that can help reduce the health risks associated with poor nutrition (Miranda *et al*., 2015). Rizzi and Marangon (2012) reported that one egg per day will not significantly affect cholesterol levels, and two eggs per day will not likely have much impact either for most people. In addition to their nutritional importance, eggs help to provide food security and economic stability in many communities. Egg production can offer a source of revenue for families, especially in rural areas where chicken farming is prevalent. Communities can improve their nutritional condition while simultaneously increasing household income and food security by promoting laying hen production (Habib, 2024). This dual advantage emphasizes the significance of including poultry production into overall nutrition and economic development plans. Furthermore, it is becoming more widely acknowledged that laying hen welfare plays a crucial role in egg production. Consumer preferences are shifting in favour of eggs produced under systems that put animal welfare first, which may have an effect on the eggs' nutritional value (Sharma *et al*., 2022). Studies reveal that hens raised in enriched settings typically yield eggs with superior quality characteristics, underscoring the connection between animal welfare, egg quality, and human nutrition (Sharma *et al*., 2022).

2.3 Nutrient Requirements of Egg-Laying Chickens

2.3.1 Crude protein

Protein requirement of egg laying chickens vary by breed, age and production stage. Generally laying hens need a diet containing 15 to 19% crude protein, influenced by factors like egg production rate and molting (Rollin *et al*., 1974). In compliance with the NRC (1994), a leghorn-type laying hen required 18.8, 15.0 or 12.5% crude protein for feed intake of 80, 100 or 120 g/hen per day respectively. Technically, laying hens do not have a requirement for protein. Nonetheless, the diet must contain enough protein to provide a sufficient amount of non-essential amino acids (NRC, 1994). Egg size and egg weight have improved in response to higher protein levels (Parsons *et al*., 1993). According to Leeson (1989), egg weight continued to rise linearly to 20 g level of protein intake, whereas egg size rise significantly while protein intake climbed from 13.1 to 20.7 g/bird per day. Furthermore, protein is important in many aspects of bodily functions and bird production. Pacheco *et al*. (2022) stated that dietary protein is very important in layer diets as it is required to build components of the egg (mainly the albumen or egg white). Dietary protein is used for hormone and enzyme making, immune cell function, tissue maintenance, oxygen transportation and many other functions.

2.3.2 Energy

Energy is the primary dietary factor that affects the performance of all species (NRC, 1994) and the amount needed varies based on breed, body weight, production phase, egg size and room temperature (Coon, 2002). Morris (2004) stated that when a diet has a low energy density, birds will increase their intake of feed until it satisfies their energy demand. In compliance with Harms *et al*. (2000), the energy use efficiency by laying hens depends on certain genetic characteristics of the bird. Faria and Santos (2005) stated that taking into account the metabolic weight and mass daily production of eggs, the energy needs of laying hens is directly proportional to body weight in the range of 1.2 to 2.5 kg. According to Albino and Barreto (2003), the birds in production prefer to eat more to accomplish their energy demands, a rise in diet energy levels results in a decrease in feed intake. Depending on the stage of production the hen is in, the calorie contents of its feed can vary from 2800 to 2900 kcal/kg. It is important to match the density of each nutrient to the energy levels (actual amount of feed intake). Albino and Barreto (2003) stated that one can work with increasing dietary energy levels along with posture cycle, but only after egg mass peak and within the levels indicated.

2.3.3 Fibre requirement

It is important to note that the appropriate impact of fibre used in layer diets was not clear for a long rime (Cruz and Rufino, 2017). This is because the majority of studies on this topic only focused on the negative effects (Goulart *et al*., 2016). Though recent studies have presented a new viewpoint, showing that birds benefit from accurate fibre measurements in terms of improved performance, high quality eggs and other factors (Incharoen and Maneechote, 2013). According to Montagne *et al*. (2003), fibre is the most crucial dietary component for gut health. It has advantages over commercial prebiotics (Goulart *et al*., 2016). In accordance with these novel concepts, it has been suggested that poultry diets include fibre to preserve a low energy density and physiological processes in the digestive system, allowing for control over feed intake, nutrient absorption egg deposition (Braz *et al*., 2011). This addition could regulate the amount of nutrients consumed, how well they are absorbed and the weight of the chicken. Furthermore, convective efficiency and microbial dynamics in the gut are significantly influenced by the structure of fibre and its interaction with water in the lumen (Incharoen and Manteechote, 2013). According to research by Rufino *et al*. (2017), there can be wide differences in the amount of fibre added to chicken diets, with moderate amounts being better for both positive and negative outcomes. Typically, these effects are triggered by interactions between the type of fibre (soluble and insoluble), birds age and nutritional quality of ingested non-starch polysaccharides, making fibre act in different forms on bird's metabolism (Mtei *et al*, 2019).

2.3.4 Fat requirement

Due to the greater energy density of fat compared with carbohydrates and protein, poultry diets usually include fats to achieve the needed dietary energy concentration (Oliveira *et al*., 2010). Kucukersan *et al*. (2010) investigated the influence of different dietary oil sources on the laying performance of Hisex brown layers. The results showed that the egg weight and egg production were significantly higher in hens supplied with 3% soybean oil. Rahman *et al*. (2011) also revealed that supplement of volatile fatty acids (VF) at 520 ppm at the last production cycle of laying hens would help to increase egg production, egg mass and eggshell thickness. The addition of fat and oil in layer diets has been illustrated to reduce the percentage of broken eggs (Bozkurt *et al*., 2012). This improvement might be associated with the measure of eggshell thickness following the oil/fat supplement.

2.3.5 Minerals requirement

The "ash content" of the feed is the organic mineral portion. Lesson and Summers (2005) reported that minerals are used by the chicken for bone development (calcium, phosphorus and magnesium), for electrolyte balance in the cells (sodium, potassium and chlorine) and trace amounts for chemical reactions (zinc, manganese, iron, copper, selenium and iodine). Furthermore, egg shell quality is mainly driven by dietary minerals in which the dietary calcium (Ca) and phosphorus are the key elements to produce the good egg shells. According to Halls (2005), either deficiency or excessive quantities of calcium in layer diets would result in poor egg shell quality. Swiatkiewicz *et al*. (2015) reported that diets with 3.2 to 3.5 % calcium have been recommended for laying hens to ensure egg production and high-quality egg shells during laying period. According to Pavlovski *et al*. (2012), the transition period when the pullets become laying hens is very important which will decide the production performance of the flock thereafter. Therefore, supplement of 2.5 to 3.5% Ca to the pullet diets about 2 to 4 weeks before the laying phase is recommended to ensure calcium balance and facilitate the medulla bone development in birds.

3.5.6 Vitamin requirements

The addition of vitamins in layer diets can enhance egg production and egg shell quality. According to Abdalla *et al*. (2009), increasing levels of vitamin A have been reported to significantly increase egg production, egg weight and egg mass. Provision of vitamin C at 200 mg/kg might increase the egg production and reduce the number of cracked eggs (Kilinc and Karaoglu, 2013). Furthermore, during the warm period, the supplement of Vitamin C and vitamin E would help laying hens maintain their laying performance and egg quality. Vitamin D is essential for calcium metabolism. This, diets with inadequate vitamin D resulted in a considerable reduction of egg shell weight (Halls, 2005). Sufficient levels of vitamin E increase the egg production and might diminish the effects of heat stress (El-Sheikh and Salama, 2010).

2.4 Importance of Feed Additives in Egg Laying Chickens

A number of strategies have been proposed to address the issue of excessive feed costs. These strategies include using non-traditional local feed materials (Okoli and Udedibie, 2017) and applying feed additives (Ugwu and Okoli, 2017) to lower the cost of feeding chickens. Feed additives have also been supported by nutritionists and animal production specialists (Terrence,

2005) as a way to maximize nutrient absorption from substitute feed basic materials and boost output. Feed additives are compounds that are added to feed with the intention of enhancing animal health and metabolism as well as efficiency of nutrient uptake and use (Okey, 2023). Both human and animals consume a diverse range of additives in their diets. The main purpose of feed additives in poultry diets are to enhance feed utilization, prevent illnesses and increase the effectiveness of the bird's development and laying capability. All feed additives must have approval before being used and they must be utilized according to the recommended inclusion levels and feeding period. Feed additives can have a significant effect on egg production and quality in hens that produce eggs. Egg laying chickens can benefit from feed additives in a number of ways including enhanced egg production. Yulianti and Muharlien (2020) indicated that supplementing feed with natural feed additives can boast laying chicken's egg output. Feed additives improve growth of birds. Probiotics, prebiotics, enzymes, organic acids and external emulsifiers are examples of alternatives that have been shown in studies to have some positive regulatory and antioxidant effects on intestinal flora of poultry. These compounds can also be thought of as growth stimuli if their toxic and therapeutic effects are assessed in addition to their interactions with medications (Jatinder and Gaikward, 2020). Furthermore, certain feed additives possess antibacterial qualities that can aid in preventing illnesses in hens that produce eggs.

2.4.1 Common feed additives in egg production

2.4.1.1 Antibiotics

Antibiotic feed additives (AFAs) have been used in poultry production for about 80 years to improve productivity and efficiency (Suresh *et al.*, 2018). Several studies indicate that the use of antimicrobials has resulted in increased productivity and decreased cost for consumers (Van Boeckel *et al*., 2015). Antibiotic growth promoters (AGPs) are commonly utilized in poultry production due to their low cost and ease of application. This approach has improved intensive poultry production by improving gut health, lowering subclinical infections, and boosting growth, productivity, and feed conversion efficiency. Low dosages of antibiotics can improve gut health, reduce pathogen load, and prevent subclinical infections in poultry, even on well-managed farms. Antibiotics can thicken the gut, resulting in better nutritional absorption (Cox and Dalloul, 2015). Broiler chickens are now administered low-dose antibiotics to encourage rapid growth (Costa *et al*., 2017). Tylosin, penicillin, oxytetracycline, erythromycin, and neomycin are a few examples of mostly used antibiotics (McEwen and Fedorka-Cray, 2002). Sub-therapeutic antibiotic use in developing economies has negatively impacted gut microflora, resulting in antibiotic residues in tissue and the development of drug-resistant pathogenic bacteria through mutation or plasmid transfer (Castanon, 2007). This resistant bacteria population enters into the human body through consumption and handling of meat and eggs contaminated with such pathogens that are resistant to antimicrobial drugs (Van den Bogaard and Stobberingh, 2000). Resistant bacteria (superbugs) can colonize the host's intestinal tract and pass on antibiotic resistance genes to other bacteria in the host's microflora, causing bacterial infections to take longer to treat (Ripon *et al*., 2019). In 2005, the European Union Commission banned the use of antibiotics in animal feed as growth enhancer. Antibiotic resistance is being actively examined, and many countries are considering banning antibiotic growth enhancers. This has led to an increased interest in finding suitable antibiotic substitutes for layer and broiler production (Alam and Ferdaushi, 2018). Alternatives are being studied to replace antibiotics in animal agriculture because of the pressure antibiotics impose on intestinal microbiota to select for antimicrobial resistance, which has serious implications for animal and public health (M'Sadiq *et al*., 2015).

2.4.1.2 Probiotics

Probiotics are defined as living organisms and compounds that affect the gut bacteria population's balance to improve animal's productivity and growth. According to FAO/WHO (2001), probiotics are living bacteria that give the host health benefits when ingested in sufficient amounts. Probiotics help an animal's gut bacteria community grow and establish itself in a way that is beneficial. Direct consumption of dissolved organic material mediated by these microbes has been linked to its capacity to improve poultry performance and health (Ezema and Ugwu, 2014). Probiotics assist eliminate stress-induced gastrointestinal abnormalities and restore normal gut activity (Kutlu and Gorgulu, 2001). Probiotics reduce oxidation-reduction potential in the gut, inhibiting aerobic pathogens like Staphylococcus aureus, *Escherichia coli*, *Salmonella Enteritidis*, *S. Typhimurium*, *Clostridium perfringens*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Candida albicans* (Dhama *et al*., 2014). Probiotics and competitive exclusion approaches have been utilized to manage endemic and zoonotic illnesses in poultry (Rafiq *et al*., 2022). Exclusion of competition refers to competition for binding sites on the intestinal mucous membranes, which prevents harmful germs from populating the digestive system. The third mechanism is competition for nutrients (Patterson and Burkholder, 2003). Probiotics promote avian health by lowering harmful amine and ammonia levels, producing digestive enzymes, B-complex vitamins, and stimulating hunger (Ferdous *et al*., 2018). Kabir *et al*. (2004) observed significant body weight gain and higher antibody production in vaccinated and non-vaccinated birds fed with probiotics time dependently. Certain non-pathogenic species of bacteria, fungi, yeasts, or their combination have been used as probiotics. Probiotics can be classified into colonizing species (*Lactobacillus sp*. *Enterococcus sp*. and *Streptococcus sp*.) and free, non-colonizing species (*Bacillus sp*. and *Saccharomyces cerevisiae*). The microbes generally used to develop probiotics are *Lactobacillus acidophilus, L. sporogenes, L. bulgaricus, L.* casei*, L. plantarum, L. cellobiosus, L. salivarius, Streptococcus faecium*, S. *thermophiles*, *Bacillus coagulans*, *B. licheniformis, Bifidobacterium bifidum* and *Aspergillus oryza*, which have beneficial effects on broiler performance (Dhama *et al.,* 2014).

2.4.1.3 Enzymes

Enzymes are proteins that facilitate specific chemical reactions. After completion of the reaction, the enzyme disassociates and becomes available to assist in further reactions. Although animals and their associated gut microflora produce numerous enzymes, they are not necessarily able to produce sufficient quantities of specific enzymes or produce them at the right locations to facilitate absorption of all components in normal feedstuffs or to reduce anti-nutritional factors in feed that limit digestion (Panda *et al*., 2000). Some cereal grains (rye, barley, wheat, sorghum) have soluble long chains of sugar units (referred to as soluble non-starch polysaccharides – NSP) that can entrap large amounts of water during digestion and form very viscous (thick gel-like) gut contents. Enzymes that are harvested from microbial fermentation and added to feeds can break these bonds between sugar units of NSP and significantly reduce the gut content viscosity (Creswell, 1994). Creswell (1994) further reported that lower viscosity results in improved digestion as there is more interaction of the digestive enzymes with feeds and therefore more complete digestion; improved absorption as there is better contact between the digested feed nutrients and the absorptive surface of the gut; and improved health as the moisture and nutrient levels in the manure are reduced which reduces the nutrients available for harmful gut microflora to proliferate and challenge the birds (e.g. necrotic enteritis, a chronic intestinal disease caused by *Clostridium perfringens*, resulting in reduced performance, mortality).

2.4.1.4 Minerals

Minerals are crucial components of feed which are required as activator of hormone, enzymes, skeletal and structural maintenance of bone, egg formation and for maintenance of acid-base balance and osmotic homeostasis (Ravindran, 2010). Animals require them for optimal health and healthy physiological processes. Poultry therefore need both macro and micro or trace minerals in their diet. The most abundant elements in the body are macro minerals, such as phosphorus (P) and calcium (Ca) (Esonu, 2006). Included in this category are also the elements chloride (Cl), magnesium (Mg), potassium (K), sodium (Na), and sulfur (S), each of which has a dietary requirement of more than 100 mg/kg feed (Ravindran, 2010). The growth of chickens depends on trace elements like manganese (Mn), selenium (Se), copper (Cu), iron (Fe), and zinc (Zn), which are needed as co-factors of enzymes in several metabolic pathways. In poultry, they are necessary in trace amounts, typically 0.01% (Ravindran, 2010). The physiological processes that are essential for maintaining life, such as development, reproduction, immune system activities, energy metabolism, and bone formation, depend on trace minerals (Dibner *et al*., 2007). Calcium (Ca) is a major mineral in poultry nutrition as an important component of bones, shells, blood-clot formation and muscle contraction (Talpur *et al*., 2012). Lack of calcium ions in bones led to the deterioration of the skeletal structure and reduction in bone strength (Kwiatkowska *et al*., 2017)

2.4.1.5 Vitamins

According to Alagawany *et al*. (2021) vitamins are important dietary supplements meant for optimal health and physiological processes including development, maintenance, and reproduction. They perform catalytic functions that speed up the synthesis of nutrients, regulating metabolism and having an impact on poultry health and performance. They are divided into two categories: fat soluble (A, D, E, and K) and B1, B2, B6, B12, folic acid, pantothenic acid, biotin, niacin, and vitamin C are water-soluble vitamins. Deficiency of any of the vitamins manifest as cessation of growth, incoordination, weakness, ataxia, xerophthalmia and blindness occur in chicken due to deficiency of vitamin A (Alagawany *et al.,* 2021). Vitamin B complex shortage results in polyneuritis, perosis, and impaired feed consumption, whereas a vitamin E deficiency causes exudative diathesis and encephalomalacia. Anemia can result from shortages in vitamins like vitamin B12, folic acid, pantothenic acid, and biotin acid, which are necessary for the proper growth of hemopoietic organs and erythropoiesis (Alagawany *et al*., 2021). Diets supplemented with vitamins play an important role in disease treatment and prevention, because it enables an animal to make use of protein and energy for health improvement, FCR, growth and reproduction (McDowell and Ward, 2008). Ferdous *et al*. (2018) reported that the addition of vitamin in water improved on the hematological and serum biochemical profiles without any detrimental effect on broiler chickens. It may also improve the development of intestinal mucosa and protect enterocytes from oxidative stress (Hassanpour *et al*., 2016).

2.4.1.6 Amino acids

Animals depend on amino acids for many physiological processes since they are the structural and functional building blocks of proteins (Debnath *et al*., 2019). They are classified into two groups: non-essential (synthesized in the body) and essential amino acids (cannot be synthesized rapidly enough to meet the metabolic requirements. Lysine, methionine, threonine, arginine, isoleucine, leucine, histidine, phenylalanine, and valine are ten examples of necessary amino acids; lysine, methionine, and threonine are known as limiting amino acids in poultry (Rehman *et al*., 2019). According to Siegert and Rodehutscord (2019), tyrosine and cysteine are considered semi-essential amino acids as they may be produced from methionine; however, glycine and serine are the non-essential limiting amino acids in the diet of fowl. After absorption, amino acids are assembled and metabolized to form proteins that are used to build different body tissues (Alagawany *et al*., 2021). Beski *et al*. (2015) reported that dietary synthetic amino acid supplementation to poultry diets improved feed conversion efficiency and reduced nitrogen excretion. Additionally, the inclusion of herbal essential oils and organic acids in diets has been demonstrated to improve laying performance, particularly during heat stress (Ozek and Kucuk, 2011). According to Ozek and Kucuk, (2011), hens fed diets with herbal supplements produced considerably more eggs than control groups during hot summer months; this shows that nutritional interventions can counteract the negative effects of environmental stressors on laying performance.

2.5 Dietary Effect on Egg Laying Chicken's Performance

The dietary composition of laying hens significantly influences egg-laying chicken performance including egg production, egg quality and overall health. A balanced diet rich in protein, vitamins, and minerals promotes optimal egg production (Khatibi *et al.,* 2021). Certain dietary components can have undesirable impact on laying hen performance. For instance, the inclusion of white lupin seeds at levels of 25% and 30% in the diet resulted in decreased egg mass and production rates compared to a control diet devoid of lupin (Park *et al*., 2016) indicating that while some ingredients may offer nutritional benefits, their excessive inclusion can lead to adverse effects on egg production metrics. The dietary energy and protein level must be modified to ensure that hens consume sufficient nutrients required for development and the start of egg production. Egg laying chickens generally modify their intake based on their energy needs. Harms *et al*. *(*2000) stated that chickens fed diets high in energy lay larger eggs. According to Bhatti and Sharma (1989), reducing dietary protein of egg laying chickens from 17% to 13% caused an increase in mortality, a drop in egg weight and hen-day egg output. Furthermore, vitamin and mineral supplementation is crucial to improve the productivity of poultry. Higher levels of vitamins in the diet may enhance the laying efficiency and egg quality of aged hens (Gan and Zhao, 2020). Furthermore, dietary addition of Vitamin A and K improve egg shell quality and yolk colour as well as antioxidative status on the egg shell gland. Minerals are important and responsible for proper biological activities of a chicken to sustain the ideal growth and productivity. The availability of phosphorus is another important component that affects the performance of laying hens. Nie *et al*. (2013) research revealed that diets lacking in non-phytate phosphorus had a negative effect on feed conversion ratios and egg production. Furthermore, the addition of microbial phytase has been demonstrated to improve production performance in hens fed non-phytate phosphorus in the diet (Chaidhury and Koh, 2019). Similarly, studies have shown that appropriate quantities of accessible phosphorus, around 0.22%, are critical for increasing egg production (Lim *et al*., 2020). Moreover, the effect of dietary calcium should not be underestimated. High calcium diets have been revealed to have varying properties on egg production, with some research suggesting that excessive calcium may limit egg production (Chang *et al*., 2019). This emphasizes the importance of balanced feed formulations that suit the specific nutritional needs of laying hens while remaining within recommended levels of key minerals. Fiber also forms an important part of layer diets. According to Montagne *et al*. (2003), fibre is the furthermost crucial dietary component for gut health. Inclusion of fibre in diets may regulate the amount of nutrients consumed, how well they are absorbed, and the weight of the chicken. According to Rufino *et al*. (2017), there can be a wide range of effects from moderate to high levels of fibre added to poultry diets with both positive and negative outcomes possible where reasonable levels tend to be better recommendation.

2.6 Charcoal Production and Distribution

Charcoal production and distribution include several steps and processes. Charcoal is formed by heating wood in airtight ovens or retorts, in chambers with various gases, or in kilns supplied with limited and controlled amount of air (Baldwin, 1949). All approaches to high-temperature heating result in the breakdown of the wood into gases, a tar mixture that is too thin, and the well-known solid carbon substance that is called charcoal. Carbonizing ovens of plants intended for recovery of products other than charcoal are heated externally, and the wood is not in direct contact with the heat source. The production process is labour-intensive, employing a large number of people at different phases of the process and distribution. Up to 25% of the overall production costs from the forest to the wholesale market can be attributed to distribution phase, which involves the packaging, loading, and transportation of charcoal from the kiln to the location of wholesale distribution or large-scale industrial application. FAO (1993) reported, that because charcoal has a calorific value that is roughly double that of dry fuel wood, carrying charcoal is far less expensive in terms of heat energy units than shipping fuel wood. Planning systems for the manufacture of charcoal requires careful consideration of transportation issues.

2.7 Nutrient Composition of Activated Charcoal

The nutrient composition of activated charcoal itself is mainly carbon, with minimal amounts of ash and moisture. Its specific effects on nutrient digestibility can vary; for example, a study by Gerlach and Schmidt (2012) revealed that it may reduce the digestibility of certain nutrients like crude fats in some livestock, depending on the dosage used. Generally, activated charcoal is added at low levels in diets of different livestock (0.050 g/kg of live weight) to optimize its benefits without negatively impacting nutrient absorption (Lavrentyev *et al*., 2021). Furthermore, activated charcoal is mostly utilized as an absorbance to enhance feed quality and animal health; it typically has little protein and no fat. Its main function in the digestive tract is to bind toxins and mycotoxins, improving the absorption of nutrients and general health in animals like pigs and turkeys (Lavrentyev *et al*., 2021). Hinz *et al*. (2019) reported that activated charcoal at low doses in pig's diets can yield positive results in growth and health without significantly altering the protein and fat composition of the feed. In accordance with Alkathari *et al*. (2020), activated charcoal itself is not an important source of nutrients, but it can contribute to dietary fibre depending on the raw materials and manufacturing process used. Steam activated, unlike chemical activation help preserve the natural fibre content. The fibre content helps provide bulk and may contribute to the absorbent properties of activated charcoal in animal feeds and supplements. Alkathari *et al*. (2020) reported that activated charcoal powder arranged utilizing zinc chloride activation had the highest content of C=N, which are characteristic of nitrogen-containing functional groups similar to those found in proteins and amino acids. Gonzalo *et al*. (2018) reported that activated charcoal used in animal feeds, particularly for pigs has shown effects on phosphorus and calcium levels. Gonzalo *et al*. (2018) indicated that use of activated charcoal can increase inorganic phosphorus levels by approximately 4%-8.8% and total calcium levels by 1.5%-4.4% in the blood of experimental animals compared to control group. These findings suggest that activated charcoal may enhance mineral absorption and overall mineral absorption and overall nutrient digestibility in livestock diets. Because charcoal has a high concentration of vital plant macronutrients like potassium and phosphorus, it may also have extra utility as fertilizer (USDA, 2002).

2.8 Utilization of Charcoal in Farm Animal Production

Charcoal, particularly biochar and activated charcoal are utilized in animal production for various purposes. The majority of studies confirmed at least some efficiency for charcoal to enhance measured parameters, including rises in body weight gain, feed intake and digestibility, carcass enhancement, reduction in antibiotic residues and pathogens as well as effect in reducing enteric methane emissions (Toth and Dou, 2016). Charcoal has been used as a feed ingredient to prevent and cure infectious or feeding-related diarrhoea in animals as well as an emergency therapy for animal poisoning. Research suggests that activated charcoal can improve feed conversion ratios (FCR) and body weight gain in poultry, swine and ruminants. For example, research indicates that broiler hens given activated charcoal diets had advanced body weight and feed efficiency compared to control groups (Raphael *et al*., 2010). Furthermore, research on activated charcoal as feed additive for pigs has demonstrated prospective advantages on mineral metabolism and rates of growth and development (Lavrentyev *et al.*, 2021). Lavrentyev *et al.*, (2021) further reported that the use of activated charcoal throughout the rearing and fattening of pigs contributes to an increase in the average daily gain in live weight, decrease in fattening period, a rise in massiveness and churn index. TAP NOSB (2002) reported that activated charcoal can be used as a feed supplement to stimulate feeding on unappetizing or poisonous plants and has been shown to reduce suffering and save many lives of animals. According to Gerlach and Schmidt (2012), toxins such as dioxin, glyphosate, mycotoxins, pesticides and polycyclic aromatic hydrocarbons are effectively bound by the charcoal, preventing any adverse effects on the gut flora and gastrointestinal system. Furthermore, they further indicated that foot pad dermatitis in chickens and ducks can be relieved. Volkmann (1935) observed an efficient reduction on excreted oocysts through adding charcoal to the feed of pets with coccidiosis or coccidial infections. Activated charcoal has also been revealed to control lactic acid concentration and assists to maintain pH level and microflora in the rumen of steers during and after acute overloading with barley (Hoshi *et al*., 1991). Reports from Gerlach and Schmidt (2012) showed that charcoal inclusion improved dairy cow health and vitality and decreased mortality, reduced diarrhoea in affected animals, decreased milk protein and fat, increased fecal ammonium and reduced nitrate excretion amongst other benefits. Overall, charcoal is utilized extensively and in variety of ways in farm animal production, offering advantages such as better nutrient intake efficiency, enhanced animal health and toxin absorption.

2.9 Limitations to the Use of Charcoal in Animal Production

While, charcoal has some benefits in farm animal production, it can also come with limitations because excessive use of charcoal in animal production can have advantageous and harmful effects. According to Toth and Dou (2016), charcoal can improve an animal's intake of dry matter, make fibre easier to digest and facilitate digestion. However, excessive feeding of charcoal may lead to constipation and affect meat quality in animals (Ayankoso *et al*., 2023). In the gastrointestinal system, charcoal has the ability to bind with certain nutrients. This might result in decreased nutrient absorption and have an impact on the animal's general health (Hinz, 2019). One of the key concerns is activated charcoal's nonspecific adsorption capabilities. While it is excellent in binding specific pollutants, it can also adsorb vital nutrients, vitamins, and minerals, resulting in nutritional deficits in animals. According to studies, excessive doses of charcoal supplementation (more than 10%) can have a deleterious impact on feed conversion rates and overall nutrient absorption because activated charcoal can indiscriminately bind both hazardous chemicals and good nutrients (Zhang *et al*., 2022). This non-selective binding can hamper performance, notably in broiler hens fed mycotoxin-contaminated diets, where activated charcoal has been shown to absorb not just toxins but also essential nutrients (Santos and Eerden, 2021). Moreover, the effectiveness of activated charcoal as a detoxifying agent varies greatly depending on a number of parameters, plus the kind of toxin, the timing of administration, and the precise formulation of the charcoal utilized. While some studies show that activated charcoal effectively sequesters mycotoxins such as deoxynivalenol (DON), others show conflicting results, showing that not all activated charcoal products are equally efficient (Ahn *et al*., 2022). Adsorption capability varies due to changes in the source material, activation procedure, and unique chemical structure of the toxins involved (Wardani *et al*., 2022). In addition to these biological factors, using activated charcoal in animal production poses practical challenges. High-quality activated charcoal can be prohibitively expensive for some farmers, and its introduction into feed necessitates careful formulation to ensure that it does not negatively impact feed palatability or animal acceptability (Ross *et al*., 2021).

Furthermore, charcoal can pose potential health risks to animals. Its production and usage in animal feeds are associated with specific adverse health outcomes including respiratory diseases, cardiorespiratory and neurological diseases, cancer, DNA damage, carbon monoxide poisoning, physical injury, sick house syndrome, unintentional weight loss, body mass index reduction, increased blood pressure, and carbon-monoxide death (Idowu *et al*., 2023). Charcoal can have negative effects on the animal. Black stools, digestive slowing or destruction, regurgitation into the lungs and dehydration are some of the negative effects of charcoal. Charcoal made from various materials including wood and plants has a wide range of absorptive capacities. Idowu *et al.* (2023) indicated that charcoal production and usage are linked with respiratory diseases such as cancer, oral cavity cancer, COPD and asthma. Activated charcoal can cause allergic reactions to some animals, which could lead to respiratory problems and other health issues. Higgs (2023) reported that before giving activated charcoal to animals, it is important to consult with a veterinarian since it may not be appropriate in all situations and it can be fatal if handled improperly. Charcoal production can lead to sick house syndrome which is characterized by nausea, vomiting and symptoms of poisoning in animals (Idowu, 2023).

2.10 Effects of Supplementation of Egg Laying Chicken's Diets with Charcoal

Studies have been done on charcoal as a possible feed ingredient for animals. According to some investigators, feeding broiler chicks and laying hens charcoal or dietary wood can increase body weight gain, carcass weight, and feed conversion ratio (Kutlu *et al*., 2001). The addition of beneficial feed additives such as activated charcoal to the diet can help improve the intestinal integrity, improve gut health and thus increase nutrient availability and absorption which result to increased laying performance (Awad and Bohm, 2009). According to studies, 1–1.5% charcoal in the diet can result in higher egg production, increased laying rates, and heavier eggs. This is explained by charcoal's capacity to improve the digestive system, aid in nutritional absorption, and have the potential to bind toxins, all of which can improve the general health and wellbeing of hens. A mixture of bamboo charcoal powder and bamboo vinegar has been revealed to make a substantial increase in egg production by stimulating intestinal functions of laying hens in the early phase of production (Yamauchi, 2010). These were credited to the beneficial effects of the product in promoting intestinal functions which may help to absorb and assimilate more nutrients. Dietary charcoal supplementation has a significant effect on egg quality. The quality of eggs, especially eggshell strength and thickness, is critical for marketability and consumer acceptance. Activated charcoal incorporation in poultry diets was found to improve eggshell quality, which is critical for safeguarding egg contents and lowering the danger of microbial contamination (El-Naggar *et al*., 2022). Additionally, charcoal's adsorptive qualities may aid in the binding of toxins and toxic compounds in the intestines, potentially leading to better eggs (Obaid, 2023). This is especially important in the context of mycotoxin contamination, as charcoal can reduce the harmful effects of toxins such as aflatoxin B1 in poultry diets (Obaid, 2023). Kutlu *et al*. (2001) studied the effects of dietary oak charcoal on laying performance using Hyline breed fed a standard commercial layer diet and supplemented with 0, 10, 20 and 40g ground wood charcoal per kg. There was no effect on egg weight, albumen weight, yolk weight, shell weight, shell thickness and shape index. However, activated charcoal (AC) supplement reduced the number of broken eggs in a dose-related manner, which is attributed to the absorption capacity of charcoal for dietary fat and its excretion. Improved fat excretion promoted by charcoal probably enhanced mineral use, particularly calcium which promotes shell formation in the shell gland. Charcoal supplementation induced a non-significant reduction in feed intake, egg production and FCR and the lower feed intake was possibly due to higher bulk density and blackening of the diets by charcoal and decrease in palatability (Jindal *et al*., 1994). Other studies have found no important effects of charcoal on animal health, welfare, or performance (Hinz *et al*., 2019). Activated charcoal, however, has been shown in certain studies to enhance mineral absorption and litter quality in layers (Okey *et al*., 2021). Furthermore, the physiological benefits of charcoal nutrition extend beyond development and egg production. According to studies, charcoal can impact the intestinal microbiota of laying hens, resulting in a healthier gut environment. This shift in gut microbiota can result in increased nutrition utilization and lower harmful bacteria populations, which is critical for bird health and productivity (Nair, 2023). For example, Prasai *et al*. (2016) found that biochar supplementation altered the intestinal microbiota and reduced Campylobacter load in layer chickens, indicating charcoal's potential as a natural feed addition for enhancing gut health. Overall, research on the impact of charcoal in animal diets is still ongoing to identify any more potential advantages or disadvantages.

2.11 Egg Quality

According to Kramer (1951), quality is the characteristics of any particular meal that affect a consumer's decision to accept or reject it. Egg quality is a complex term that includes a range of internal and external factors that have significant effects on marketability and customer acceptability. The yolk height, yolk diameter, yolk weight, albumen height, albumen weight, yolk index, haugh unit (HU) and yolk colour are the main internal quality marker while egg weight, egg width, shell weight, shell thickness and egg shape index are the main external variables. Shell quality and internal egg quality for shell eggs, as well as interior egg quality for further processed eggs, are all quality indicators. The egg quality after it is laid cannot be enhanced. As a result, the majority of its maintenance is preventive in nature. While, egg white (albumen) cleanliness and viscosity, air cell size, yolk form, and yolk strength are all considered aspects of internal quality, shell cleanliness, texture, and shape are considered aspects of external quality. Yolk quality is determined by its colour, texture, firmness, and smell, while albumen quality is determined by its cleanliness, viscosity, size of the air cell, yolk shape, and yolk strength (Dilawar *et al*., 2021). Yolk colour is another key quality indicator that is frequently linked to consumer preferences. According to research, the hen's diet influences yolk pigmentation, particularly the incorporation of carotenoid-rich feed (Ariza *et al*., 2019). For example, using ground annatto seeds in layer diets has been proven to improve yolk pigmentation, which is frequently viewed as a sign of increased nutritional content by consumers (Garcia *et al*. 2010). Furthermore, the hen's genetic background can influence yolk colour variation, as various breeds have varying pigment absorption and deposition capabilities (Ariza *et al*., 2019). The consistency of the albumen is one of the indicators of the freshness of the egg, and it decreases over time due to an increase in pH, which leads to degradation of the binding of the proteins ovomucin and lysozyme, making the albumen increasingly fluid (Chukwuka *et al*., 2011). Along with bird age, sickness, egg age, temperature, humidity, handling, and storage, albumen quality also drastically declines (Rossi and Reu, 2011). The Haugh unit (HU) is an important pointer of albumen quality since it represents the height of the thick albumen in relation to the egg's weight. A greater HU suggests better egg freshness and quality (Zhao *et al*., 2012). According to studies, the HU drops dramatically with increased storage duration and temperature, reducing the interior quality of eggs (Quan and Benjakul, 2017). For example, eggs kept at room temperature show a significant decrease in HU over time, highlighting the necessity of correct storage conditions in maintaining egg quality (Quan and Benjakul, 2017). Furthermore, the nutritional composition of eggs, especially the presence of important lipophilic substances, can be altered by the age of the laying hens and their dietary consumption, which further affects the overall quality of the eggs produced (Ko *et al*., 2020). In general, an egg's interior quality plays a significant role in determining its freshness and suitability for consumption. External egg quality is measured by eggshell quality, strength, thickness, integrity, and soiling. he external quality of eggs, particularly the eggshell, is critical in preserving the internal contents from microbial infection and physical harm. The eggshell's structure, including thickness and strength, is critical for sustaining quality during storage and transportation (Ketta and Tumova, 2016). Eggshell quality can be strongly influenced by hen's diet, age, and environmental conditions (Moraleco *et al*., 2019). Furthermore, surface cleaning approaches have been demonstrated to maintain internal quality indicators such as albumen pH and yolk index, which are vital for maintaining egg safety and quality (Keerthirathne e*t al*., 2020).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Site

The study was conducted at the National University of Lesotho Farm, Roma. Roma is about 34 km southeast of Maseru, the capital city of Lesotho and it is located in the Foothills agro-ecological zone (AEZ) of Lesotho located in the western parts of the country. The Foothills gradually rise up to the higher elevations of the mountains, which stretch across much of the eastern and central parts of Lesotho (Guy *et al*., 2024). Its climate alternates between warm summers and cold winters. Summer temperatures can range from 10 ^{0}C to 30 ^{0}C, while winter temperatures can vary from below 0 ^{0}C to 15 ^{0}C (World Bank, 2021). Roma valley experiences four distinct seasons; summer, autumn, winter and spring. Summer is between December to February and is a warm to hot season and also the wettest time of the year, with regular rainfall. Autumn extends from March to May with a decrease in the summer temperature and rainfall. Winter falls between June to August, it is cold and a dry season with clear skies and minimal rainfall. Spring is from September to November and can be described as a transitional season with the cold winter temperatures gradually increasing and also experiences a slight increase in rainfall compared to winter (World Bank, 2021).

3.2 Experimental Animals' Management and Design

A total of 120 point of lay chickens were used for the study and were kept for a period of six weeks. A completely randomized design (CRD) was used in the experiment to avoid bias. The birds were randomly distributed into five treatment groups T1, T2, T3, T4 and T5 in three replicates each, with eight birds per replicate. The control group T1 was fed layer mash only, while the other groups T2, T3, T4 and T5 were fed the same layer mash but to which activated charcoal at 0.5, 1.0, 1.5, and 2%, respectively were added as a supplement. The birds had comparable initial live body weight and egg production level of 88.92%. The birds were reared in battery cages, and during the feeding trial, the birds had free access to clean water and fresh feed. The poultry house was three-quarter walled to mitigate the effect of prevalent strong wind. All the chickens were raised under the same management conditions and practices of feeding, watering and hygiene. The five treatments were arranged as shown in Table 1.

Table 1: Arrangement of Experimental Animals

Treatments	Replicates/No of birds		
	Replicate A	Replicate B	Replicate C
T1	8	8	8
T2	8	8	8
T3	8	8	8
T4	8	8	8
T5	8	8	8

3.3 Feed Supplement and Experimental Diets

The feed supplement used in the experiment was ground activated charcoal (AC) 0.02 mm size from Oos vrystaat kaap (OVK) Ficksburg. The activated charcoal was then mixed thoroughly with a commercial layer mash (LM) at a percentage ratio of 0 AC:100.0 LM (T1), 0.5 AC:99.5 LM (T2), 1.0 AC:99.0 LM (T3), 1.5 AC:98.5 LM (T4) and 2 AC:98 LM (T5).

3.4 Chemical Analysis of Activated Charcoal and Layer Mash

The proximate analysis of Makhulo brand layer mash was done using Sup NIR-2700 (Near Infra-Red Analyzer Series). The proximate analysis of activated charcoal was determined using the standard methods (AOAC, 2000). Metabolizable energy contained in the experimental diets and activated charcoal was computed (Pauzenga, 1985). The calcium content of activated charcoal was determined using Talapatra *et al*. (1940), and phosphorus content was determined using AOAC (2000).

3.4.1 Determination of dry matter

Dry matter of AC was determined as recommended by AOAC (2000). Clean moisture discs were dried at 105 ^{0}C in Series 2000 oven for 2 hours and then cooled in a desiccator. The oven dried moisture discs were weighed (W_1) using precision scale Model XY10002C. About 2 grams of activated charcoal was added to the moisture discs and the new weight recorded (W_2). The sample was oven dried for 24 hours, cooled in a desiccator for 30 minutes and the weight of the desiccator cooled AC sample determined (W_3). The dry mater content was calculated using the formulae:

% Dry matter = $\frac{w2-w1}{w3-w1}$×100

W_1= Weight of empty moisture disc

W_2= Weight of sample + moisture disc

W_3= Weight of sample + moisture disc after drying

3.4.2 Ash determination

The ash content of AC was determined by AOAC (2000). A crucible that was previously dried was weighed (W_1) using an electronic weighing balance Model XY10002C. About 2 grams of the sample was weighed into the crucible and the new weight taken (W_2). The crucible plus sample were placed in the oven at 600 ^{0}C for three hours. After heating was complete, both crucible and the sample were placed in a desiccator to cool. The weight of the desiccator cool ash plus the crucible was taken (W_3). The ash content was calculated using the formulae:

% Ash = $\frac{W3-W1}{W2-W1}$×100

W_1= Weight of empty crucible

W_2= Weight of sample + crucible

W_3= Weight of sample + crucible after ashing

3.4.3 Crude protein determination

Digestion

Crude protein content of the activated charcoal was determined using the procedure by AOAC (2000). About 2 grams of activated charcoal was weighed using a precision electronic balance Model XY10002C and placed in a 500 ml macro-kjedahl digestion flask. Two kjedahl tablets and 20 ml of concentrated sulphuric acid (H_2SO_4) were added to the flask. The flask was first heated moderately to prevent foam from rising up the neck of the flask or escaping from the flask. The mixture was then moderately heated with occasional swirling until the mass was cleared and the foam had disappeared. The heat was then increased until the liquid boiled steadily. After the liquid became clear, it was allowed to cool. The digest was diluted with 50 ml of distilled water, and transferred to a 250 ml volumetric flask and filled to the mark with distilled water.

Distillation

For distillation, a 250 ml distillate flask containing 25 ml of boric acid solution mixed with indicator (bromocresol green and methyl red) as a collecting solution was placed so that the condenser tube was immersed below the surface of the absorbing solution. In order to make the solution alkaline, 25 ml of the digested sample was slowly added to a solution containing 40% sodium hydroxide (about 80 ml) down the side of the flask. The distillation equipment was immediately attached to flask, which was distilled at a boiling rate of approximately 7. Until at least 150 ml of distillate were collected in the flask. A distillate flask was removed from the unit, then the condenser tube was rinsed with distilled water after the flask was removed.

Titration

Following distillation, the distilled solution was titrated with a standard hydrochloric acid solution (0.1N HCL), which resulted in a light pink final colour at the end of titration. The titre value was then recorded. The % nitrogen content in each sample was calculated as follows, and then multiplied with 6.25 protein factor and dilution factor. The crude protein content was calculated using the following formulae:

$$\% \text{ Nitrogen} = \frac{(S-B)\times N\times 0.014\times D}{Ws\times V}$$

% Crude protein = N % × 6.25

Where: Ws = Weight of the sample

B= Blank titration reading

S= Sample titration reading

N= Normality of HCL

D= Dilution of sample after digestion

V= Volume taken for distillation

0.014= Milli equivalent weight of nitrogen

3.4.4 Crude fat determination

Fat in activated charcoal was determined by the method described by AOAC (2000). 150 ml round bottom flask was rinsed with petroleum ether, drained, dried in an oven at 105 ^{0}C for 30 minutes. About 5 grams of activated charcoal was weighed with precision electronic balance Model

XY10002C and placed into the thimble (W_1) and cotton wool was placed inside the thimble. The thimble was then placed in a Soxhlet liquid/solids extractor. A 150 ml round bottom flask that had been well cleaned and dried was carefully weighed (W_2) and then filled with around 90 ml of petroleum ether. The suction unit was mounted over an electric heating source. The solvent in the flask was heated until boiling. The extraction continued for six hours. Then the Soxhlet liquids/solids extractor was taken from the heat source and detached. The flask was returned to the heat source to evaporate the solvent. The flask was then placed in an oven and the contents dried at 105^0C for 30 minutes. The flask was placed in a desiccator to cool and the flask and its contents were weighed (W_3). The ether extract was calculated with the following formulae:

$$\%\text{Ether extract} = \frac{W3-W2}{W1} \times 100$$

Where: W_1 = Weight of sample

W_2 = Weight of flask

W_3 = Weight of flask with fat

3.4.5 Crude fibre determination

Crude fibre determination in activated charcoal was discovered by the method described by AOAC (2000). A precision electronic balance Model XY10002C was used to weigh 5 g of homogenous fat free samples of AC (W_1). 1.25% of sulphuric acid and samples were placed in a conical flask (250 ml) and mixed by shaking until the sample was evenly distributed. The mixture was boiled on a hot plate for 30 minutes. After 30 minutes, the mixture was filtered using vacuum filtering system to obtain the filtrate which was washed with hot water to remove the remaining acid and any soluble components. The filtrate was then collected in a conical flask (250 ml) with 1.25% of sodium hydroxide and mixed evenly by shaking slowly. The mixture of filtrate and NaOH was boiled on a hot plate to evaporate any excess water. The crucible with fibre was further dried in a hot air oven at 105 ^{0}C for 2 hours and cooled in desiccator for 30 minutes and weighed (W_2). The crucible with fibre was heated in a muffle furnace for 1 hour at 550 ^{0}C to incinerate fibre, then cooled for 30 minutes and weighed (W_3).

$$\%\ \text{Crude fibre} = \frac{W2-W3}{W1} \times 100$$

Where: W_1 = Weight of the sample

W_2 = Weight of crucible with fibre

W_3 = Weight of crucible with ash

3.4.6 Determination of nitrogen free extract

Nitrogen free extract was determined by difference as:

%NFE=100% - (%H_2O + %CP + %CF + %Ash)

Where: H_2O = Moisture

CP = Crude protein

EE = Ether extract

CF = Crude fibre

3.5 Data Collection

A total of 60 eggs were collected from the experimental birds per week for both external and internal egg characteristics evaluation in the Animal Science Laboratory. Four eggs per replicate were randomly picked in a treatment per week at the rate of two eggs on each of Thursdays and Sundays for a total of six weeks. The eggs were first weighed separately, and external and internal quality indices determined. The eggs were carefully broken with a spatula at the equatorial region into a clean, smooth plain surface to determine the following internal characteristics; yolk diameter, albumen height, egg yolk index and yolk height, as well as their respective weights and haugh unit. The external characteristics of eggs determined were egg weight, egg length, egg width, egg shell thickness, egg shell weight and egg shape index (Moraleco *et al*., 2019).

3.5.1 Egg external quality indices

a) **Egg weight**

   Egg weight (in grams) of each egg was taken using precision electronic weighing balance Model XY10002C having a sensitivity of 0.01 g.

b) **Shell weight**

   Inner shell membrane was manually and carefully removed from the shells and the shells were put in an oven at 65 ^{0}C for 48 hours to dry. All the dried egg shells were weighed with precision electronic weighing balance Model XY10002C having a sensitivity of 0.01 g.

c) **Egg length**

Electronic digital vernier calipers Model SR070438 with accuracy of 0.01 mm was used to determine the egg length. It was taken as the longitudinal distance between the narrow and the broad ends.

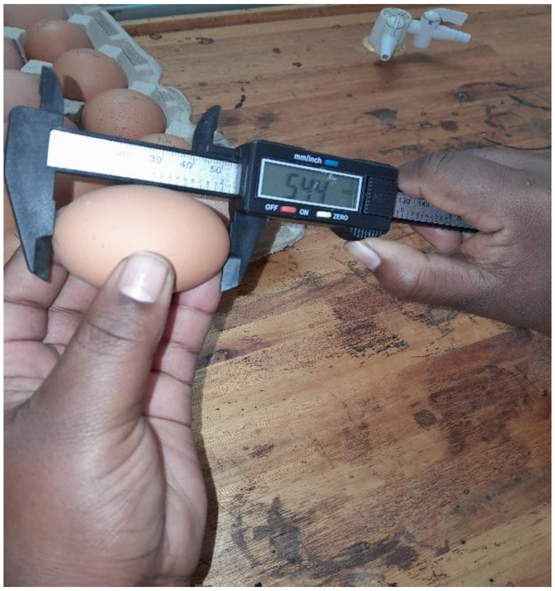

Figure 1: Measuring egg length

d) **Egg width**

It was measured to the nearest 0.01 mm with an electronic digital vernier calipers Model SR070438. The egg width was taken as the diameter of the widest cross-section region.

e) **Egg shell thickness**

Egg shell thickness was measured using a micrometer screw gauge Model PGA5024 after drying the shell in an oven at 65 ^{0}C for 48 hours. Three measurements were taken one each from both the narrow and broad ends, and in the middle of the egg. The shell thickness of each egg was determined by the arithmetic mean of the measurements.

Figure 2: Measuring shell thickness

f) Egg shape index

It was calculated as the percentage of the ratio of breadth (egg width) to egg length. The formula is as follows:

Egg shape index = $\frac{\text{Egg width}}{\text{Egg length}} \times 100$

3.5.2 Egg internal quality evaluation

Each egg was gently cracked open with a spatula on a clean flat surface to evaluate the following internal qualities of an egg:

a) Albumen height

The height of the albumen was measured with a micrometer screw gauge Model PGA5024 at approximately one centimeter away from the egg yolk.

b) Yolk height

Digital vernier calipers Model SR070438 was used to measure the height of the egg yolk from the surface of egg white. The caliper was placed perpendicular to the surface.

c) Haugh unit

The Haugh Unit (HU) was calculated with the formular:

HU =100 × log (H-1.7W$^{0.37}$+7.6)

Where:

HU = haugh unit

H = height of the albumen in millimeters

W = weight of the egg in grams

d) Yolk weight

The yolk of each egg was carefully separated with 15 ml (1 table spoon), carefully rolled on a damp paper to remove any adhering albumen and weighed on precision electronic weighing balance Model XY10002C with an accuracy of 0.01 g.

Figure 3: Yolk separation from albumen

e) Yolk diameter

Yolk diameter was measured using electronic digital vernier calipers Model SR070438 along the axis bisecting the short and the long sides of the yolk.

Figure 4: Yolk diameter measurement

f) Albumen weight

Albumen weight (g) was calculated as the difference between the weight of the entire egg and the combined weight of the yolk and the egg shell (g).

Albumen weight = Egg weight - (shell weight + yolk weight)

g) Yolk index

Yolk index was calculated by dividing the yolk height by the yolk diameter.

Yolk index = $\frac{\text{Yolk height}}{\text{Yolk diameter}}$

3.4 Data analysis

The data obtained were subjected to a one-way analysis of variance (ANOVA) using SPSS (2011) version 20. Significant differences between treatment means were compared and separated using Duncan's multiple range test of the same package at a probability value of $p \leq 0.05$.

CHAPTER FOUR

4.0. RESULTS

4.1 Chemical Composition of Activated Charcoal Supplement

The result of the nutrient analysis of activated charcoal is in Table 4.1. The activated charcoal used as a dietary supplement contained 90.38% dry matter, 0.06% crude protein, 7.56% crude fibre, 0.00% crude fat, 8.59% ash and 83.79% nitrogen free extract. Calcium content was 0.13% and phosphorus was 0.02%, while calculated metabolizable energy was 2976.75 kcal/kg.

4.2 Nutrient Composition of Activated Charcoal Supplement Diets

The nutrient composition of the five experimental diets T1, T2, T3, T4 and T5 supplemented with 0, 0.5, 1, 1.5, 2% of activated charcoal, respectively is presented in Table 4.2. Activated charcoal supplementation significantly ($p<0.05$) affected crude protein, crude fat, ash calcium, phosphorus and salt content in the experimental diets, while dry matter, crude fibre and metabolizable energy were not significantly ($p>0.05$) affected by the activated charcoal supplementation. T2 had significantly ($p<0.05$) higher crude protein compared to T1, T4 and T5 with values 17.56, 17.50 and 17.64% respectively. Crude protein was highest in T2 at 18.73% and lowest in T1 at 17.56%. Crude fat decreased linearly with increasing levels of activated charcoal, from 3.10% in T1 to 1.28% in T5. All treatments were significantly ($p<0.05$) different from each other. Ash content was highest in T2 at 14.10% and lowest in T5 at 13.53%. T2 had significantly ($p<0.05$) higher ash content compared to T1, T4 and T5. Calcium was significantly ($p<0.05$) higher in T2, T3, T4 and T5 (4.33 to 4.24%) compared to control group T1 (3.34%). Phosphorus was significantly ($p<0.05$) higher in T2, T3, T4 and T5 (0.79 to 0.97%) compared to control group T1 (0.56%). Inclusion of activated charcoal in the diet increased salt linearly with increasing activated charcoal levels, from 0.48% at T1 to 0.53% T4 and T5. T3, T4 and T5 had significantly ($p<0.05$) higher salt compared to T1 and T2.

4.1: Nutrient composition of Activated Charcoal Supplement

Nutrients	% DM
Dry matter	90.38
Crude protein	0.06
Crude fibre	7.56
Crude fat	0.00
Ash	8.59
Nitrogen free extract	83.79
Calcium	0.13
Phosphorus	0.02
Metabolizable energy kcal/kg	2976.75

Metabolizable energy = (37× %CP) +(81.8× %EE) + (35.5× %NFE) ………. (Pauzenga, 1985)

Table 4.2: Nutrient Composition of Activated Charcoal Supplement Diets

Nutrients (%)	T1	T2	T3	T4	T5	SEM	*P*-value
Dry matter	88.20	89.18	89.01	88.99	88.75	0.14^{ns}	0.16
Crude protein	17.56^{c}	18.73^{a}	18.63^{ab}	17.50^{bc}	17.64^{bc}	0.52	0.02
Crude fibre	6.80	7.46	7.10	7.87	7.51	0.21^{ns}	0.59
Crude fat	3.10^{a}	2.53^{b}	1.92^{c}	1.60^{cd}	1.28^{d}	0.18	0.00
Ash	13.69^{b}	14.10^{a}	13.81^{ab}	13.71^{bc}	13.53^{c}	0.08	0.02
NFE	47.37	46.68	47.98	50.24	49.02	0.38^{ns}	0.25
Calcium	3.34^{b}	4.33^{a}	4.22^{a}	4.28^{a}	4.24^{a}	0.11	0.02
Phosphorus	0.56^{b}	0.79^{a}	0.93^{a}	0.98^{a}	0.97^{a}	0.04	0.00
Salt (sodium chloride)	0.48^{b}	0.50^{b}	0.53^{a}	0.53^{a}	0.53^{a}	0.01	0.01
ME (Kcal/kg)	2586.43	2547.56	2546.08	2511.96	2498.19	12.38^{ns}	0.16

$^{a, b, c, d}$Means with different superscripts in the same row differed significantly ($p<0.05$), ^{ns}Not significantly different, SEM = Standard Error of Mean, NFE = Nitrogen Free Extract, ME = Metabolizable Energy, AC = activated charcoal, LM = layer mash

T1 = Diet with 0 AC:100 LM (control)
T2 = Diet containing 0.5 AC:99.5 LM
T3 = Diet containing 1 AC:99 LM
T4 = Diet containing 1.5 AC:98.5 LM
T5 = Diet containing 2 AC:98 LM

4.3 Effect of Activated Charcoal Supplementation on Performance Response of Laying Hens

The effect of activated charcoal supplement on the performance response of laying hens is presented in Table 4.3. The experimental diets had no significant ($p > 0.05$) effect on the final body weight and feed intake of laying hens. The final body weight varied from 2046.70 g/bird to 2063.30 g/bird and feed intake varied from 106.44 g/bird to 107.42 g/bird. The egg production percentage varied from 86.00 to 88.00%.

4.4 Effect of Activated Charcoal Supplementation on External Egg Quality Indices of Hens

The effect of activated charcoal as a feed supplement on external egg quality is presented in Table 4.3. The inclusion of activated charcoal as a supplement in the experimental diets had a significant ($p<0.05$) effect on egg weight, egg width and shell weight. The experimental diets had no significant ($p>0.05$) effect on egg length, shell thickness and shape index. The egg weight increased significantly with higher inclusion levels of activated charcoal, and thus T5 group had the highest egg weight of 68.03 g compared to T1 (63.94 g), T2 (63.64 g), T3 (64,68 g) and T4 (62.53 g) which did not differ significantly ($p>0.05$). Similar to egg weight trend, egg width also increased significantly ($p<0.05$) with higher levels of activated charcoal, and hence T5 had the highest egg width of 46.77 mm compared to the control group T1 (44.61 mm), T2 (44.36), T3 (44.63) and T4 (44.69). The egg shell weight also increased at higher supplementation level of activated charcoal, resulting in T5 having a shell weight of 6.27 g and it was significantly ($p<0.05$) higher compared to other treatment groups T1 (5.87 g), T2 (5.98 g), T3 (6.05 g) and T4 (6.00 g).

Table 4.3: Effect of Experimental Diets on Growth Performance of Layer Chickens

	Experimental Diets					SEM	P-Value
	T1	T2	T3	T4	T5		
Initial body weight(g/bird)	2096.70	2056.70	2086.70	2036.70	2036.70	0.01^{ns}	0.20
Final body weight(g/bird)	2063.30	2046.70	2050.00	2046.70	2050.00	0.01^{ns}	0.98
Feed intake(g/bird/day)	107.37	107.42	106.81	107.38	106.44	00.41^{ns}	0.05
Egg production level (%)	88.00	86.00	87.00	87.00	87.00		

^{ns}Not significantly different, SEM = Standard Error of Mean, AC = activated charcoal, LM = layer mash
T1 = Diet with 0 AC:100 LM (control)
T2 = Diet containing 0.5 AC:99.5 LM
T3 = Diet containing 1 AC:99 LM
T4 = Diet containing 1.5 AC:98.5 LM
T5 = Diet containing 2 AC:98 LM

4.5 Effect of Activated Charcoal Supplementation on Internal Egg Quality Indices of Hens

The effect layer mash supplementation with activated charcoal has on the internal egg quality of hens is presented in Table 4.4. The experimental treatments had significant ($p<0.05$) effect on yolk height, albumen weight and yolk colour with percent increase in the activated charcoal, but did not affect significantly ($p>0.05$) yolk diameter, yolk weight and yolk index, albumen height and haugh unit. Yolk height significantly increased from T1 (18.49 mm) to T5 (19.20 mm), albumen weight tended to increase from T1 (40.07 g) to T5 (43.60 g) and the yolk colour became deeper from T1 (3.40) to T5 (9.65).

Table 4.4: Effect of Activated Charcoal Supplement Diets on External Egg Quality Indices of Hens

Indices	Activated Charcoal Supplement Diets					SEM	P-value
	T1	**T2**	**T3**	**T4**	**T5**		
Egg weight (g)	63.94^{b}	63.64^{b}	64.89^{b}	62.53^{b}	68.03^{a}	0.58	0.00
Egg length (mm)	55.96	56.56	56.75	56.46	57.18	0.17^{ns}	0.26
Egg width (mm)	44.61^{b}	44.36^{b}	44.63^{b}	44.69^{b}	46.77^{a}	0.26	0.00
Shell weight (g)	5.87^{b}	5.98^{b}	6.02^{b}	6.00^{b}	6.27^{a}	0.04	0.00
Shell thickness(mm)	0.36	0.36	0.36	0.37	0.36	0.00 ^{ns}	0.07
Egg shape index	79.90	78.79	78.94	79.12	79.18	0.22^{ns}	0.60

$^{a,\ b}$Means with different superscripts in the same row are significantly different ($p<0.05$), ^{ns}Not significantly different, SEM = standard error of mean, AC = activated charcoal, LM = layers mash
T1 = Diet with 0 AC:100 LM (control)
T2 = Diet containing 0.5 AC:99.5 LM
T3 = Diet containing 1 AC:99 LM
T4 = Diet containing 1.5 AC:98.5 LM
T5 = Diet containing 2 AC:98 LM

Table 4.5: Effect of Activated Charcoal Supplement Diets on Internal Egg Quality Indices of Hens

Parameters	Activated Charcoal Supplement Diets					SEM	*P*-value
	T1	**T2**	**T3**	**T4**	**T5**		
Yolk height (mm)	18.49^{c}	18.65^{bc}	18.80^{bc}	19.01^{ab}	19.20^{a}	0.08	0.00
Yolk diameter (mm)	42.08	41.86	42.03	41.82	42.24	0.09^{ns}	0.65
Yolk weight (g)	17.96	17.72	18.03	17.78	18.26	0.12^{ns}	0.68
Albumen height (mm)	9.35	9.42	9.51	9.49	9.60	0.03^{ns}	0.22
Albumen weight (g)	40.07^{b}	39.83^{b}	40.44^{b}	39.37^{b}	43.60^{a}	1.48	0.01
Yolk index	0.44	0.44	0.44	0.45	0.45	0.00^{ns}	0.23
Haugh unit	95.70	95.30	95.95	96.47	95.80	0.18^{ns}	0.40
Yolk colour	3.40^{d}	6.95^{c}	8.15^{b}	8.51^{b}	9.65^{a}	0.58	0.00

$^{a, b, c, d}$Means with different superscripts in the same row are significantly different ($p<0.05$), ^{ns}Not significantly different SEM = standard error mean, AC = activated charcoal, LM = layers mash
T1 = Diet with 0 AC:100 LM (control)
T2 = Diet containing 0.5 AC:99.5 LM
T3 = Diet containing 1 AC:99 LM
T4 = Diet containing 1.5 AC:98.5 LM
T5 = Diet containing 2 AC:98 LM

CHAPTER FIVE

5.0 DISCUSSION

5.1 Chemical Composition of Activated Charcoal Feed Supplement

The nutrient analysis provided indicates that the activated charcoal supplement has a high dry matter content of 90.38%, implying that it is low in moisture and consequently has long shelf-life. The dry matter content in this study is slightly lower compared to 90.96% for activated sheabutter charcoal (Ayanwale *et al*., 2006). The supplement has negligible amounts of key nutrients like crude protein and fat, suggesting that it is not a good source of dietary protein and fat. The primary causes of activated charcoal's low protein and zero fat content can be due to its production process and intended use. Carbon-rich materials, such as wood, coconut shells, peat or sawdust are burned at very high temperatures when gas is present to create activated charcoal. This technique increases its surface area and porosity but does not contribute to protein content. As a result, activated charcoal is mostly made of carbon and contains few organic components, including proteins (Al-Hamed and Kharoufa, 2022). The crude fibre content of the charcoal supplement in the current study is within the range of 6.89% and 7.56% reported by Ayanwale *et al*. (2006). The crude fibre content in this study is moderate. Crude fibre is a traditional measure of fibre content in feeds, and research shows that its presence can improve digestive health by increasing gut motility and faecal bulk (Cruz-Requena *et al*., 2019). Furthermore, it has been reported that increasing fibre consumption might result in faster transit times in the digestive system, which is advantageous to overall gut health (Savaci and Karaca, 2023). Both calcium and phosphorus are present in low amounts in the activated charcoal compared to other feed ingredients. While these minerals are essential for various physiological functions, their low levels in activated charcoal indicate it can only be relied upon as a source of supplemental Ca and P. However, it has been reported that Ca in activated charcoal can increase immunological function and gastrointestinal health, resulting in greater growth performance and egg-laying efficiency in layers (Muadifah *et al*., 2023). Also, according to Felicia *et al*. (2006), the calcium content in activated charcoal may have an impact on the balance of other minerals, such as phosphorus, which is necessary for healthy bones and the production of eggs. For effective layer performance, a precise ratio of calcium to phosphorus is needed. The nitrogen-free extract (NFE) obtained indicates a significant amount of readily available carbohydrates, which may be used as an energy source, to complement other dietary energy sources for layers. The metabolizable energy content of activated charcoal is high, which suggests that it may contribute a significant quantity of energy, required by layers for production and survival. This high energy content of activated charcoal, along with its detoxifying characteristics, make it an important addition in animal nutrition, especially when animals are exposed to mycotoxins or other harmful substances (Tahir *et al*., 2016).

5.2 Nutrient Composition of Activated Charcoal Supplemented Diets

The addition of activated charcoal to layers basal diet produced varying effect on the nutrient composition of the supplemented diets T1, T2, T3, T4 and T5 and seemed not to have any adverse effect on crude protein, crude fat, calcium, phosphorus and NaCl which are critical nutrients required for good production performance, despite significant variations observed across the diets. The crude fat content in the diet decreased progressively with increasing levels of activated charcoal. Zhang *et al*. (2022) reported that activated charcoal is a highly porous structure and its large surface area allows it to attract and trap various substances including fats and fat-soluble vitamins. When added to feed, activated charcoal can bind dietary fats, reducing its absorption in the diet. This binding effect reduces the total availability of fats for digestion and assimilation, resulting in a decreased fat level in the final product (Healthline, 2023). Kawashima *et al*. (2022) discovered that combining activated charcoal with a high-fat diet increased the excretion of neutral lipids, cholesterol, and bile acids, implying that activated charcoal lowers fat absorption and increases faecal fat elimination. The addition of activated charcoal appeared to improve crude protein levels up to a certain point, as seen by significantly greater crude protein content in T2 (0.5% AC) compared to T1 (control). However, this trend tended to significantly diminish with increased AC supplementation as evidenced in T4 (1.5% AC) and T5 (2% AC). This showed that moderate dose of activated charcoal (0.5%) may improve dietary protein in layers diet, while higher doses may cause a decline. Increased protein levels in the diet results in higher egg production, egg weight, and overall egg quality. Studies revealed that layers fed diets with high crude protein (CP) levels produced more eggs and have higher feed conversion ratios (Ribeiro *et al*., 2024). The result of the current study is not in line with that reported by Kim *et al*. (2006) that activated charcoal incorporation with feed increased crude protein content. On the other hand, activated charcoal has been demonstrated to have a strong affinity for proteins and may efficiently bind to them, thereby resulting in the decrease of dietary CP at higher levels. The small variations in dry matter across treatments, as well as the non-significance, indicate that the incorporation of activated charcoal did not affect dry matter content of the diets. This is consistent with the findings of Kim *et al*. (2006), who found that nutritional supplementation of activated charcoal has no effect on feed dry matter content. Crude fibre of the AC supplemented diet was lower than 10% maximum crude fibre recommended by Nigerian Industrial Standards (1989). Adding activated charcoal to animal feed has no substantial effect on crude fibre content (Alzawqari *et al*., 2021) and this is in agreement with current dietary formulations. The ash content also varied significantly between treatments and increased from T1 to T2 and thereafter significantly decreased to T5. This showed that at high AC supplementation, dietary ash decreased. Ash content generally indicates the overall mineral content, and activated charcoal may be affecting the availability or retention of minerals such as calcium and phosphorus. Both dietary calcium and phosphorus contents showed significant increase across treatments. Calcium levels in the diets are higher than 3.25% minimum, and P lower than total available P of 1% (Nigerian Industrial Standards, 1989). Higher calcium and phosphorus levels in the diet promote strong eggshells in laying hens. Calcium is the major mineral necessary for eggshell formation, while phosphorus is essential for bone growth and general hen health (Pavan *et al*., 2005). Salt (NaCl) tended to increase with increasing activated charcoal levels in the diets. The salt content reported in this study is higher than 0.28 – 0.48% recommended by NRC (1994). Increased dietary NaCl can negatively affect eggshell quality, including thickness and strength (egg shell integrity). The metabolizable energy content was similar among treatments, with no significant variations. The dietary metabolizable energy is also adequate for layers performance and within the level of 2500 kcal/kg (Aduku, 2012) and 2600 kcal/kg (Olomu, 2011) This showed that the addition of activated charcoal had little effect on the energy content of the diet, which is consistent with that reported by Wang *et* al. (2020) that non-caloric additions such as charcoal have little effect on the total energy value of diets. Villalba *et al*. (2002) highlighted that various dietary regimen, including those containing activated charcoal, had no significant variations in metabolizable energy values, supporting the observation of this study that activated charcoal has no significant effect on metabolizable energy.

5.3 Effect of Activated Charcoal Supplementation on Performance Response of Laying Hens

The initial body weight of hens across the different treatment groups T1 to T5 were comparable and ranged from 2036.70 to 2096.70 g/bird. This showed that the hens had similar live body weight at the start of the trial, which is crucial for verifying that any observed effects on performance metrics are due to nutritional treatments rather than pre-existing variations in body weight. The final body weights of hens were similarly not significant across the treatment groups. This suggests activated charcoal had no deleterious effect on hen's overall health and body weight stabilization over the period of the study. The findings of this study agree with the work of Saleh *et al*. (2021), which indicated that, while some dietary oils improved specific performance metrics, they did not significantly alter body weight, further supporting the finding that not all dietary modifications lead to enhanced performance. The result of the current study is in line with an earlier study by Rattanawu *et al*. (2014). Feed intake also did not vary significantly among the hen groups, and this finding agrees with Mahata *et al*. (2020) who discovered that various dietary inclusions of miana plant flour had no significant effect on feed consumption or egg production in laying hens. This supports the idea that certain dietary supplements such as activated charcoal may not always improve performance in laying hens, especially when baseline nutritional requirements are satisfied.

5.4 Effect of Activated Charcoal Supplementation on External Egg Quality of Hens

The use of activated charcoal as dietary supplement in laying hens produced changes in egg weight, egg width and shell weight across treatment groups, while the egg length, shell thickness, and egg shape index were not affected. Egg weight is an important metric in poultry production, and the result showed that hens given the T5 diet produced heavier eggs (68.38 g) than other treatments T1, T2, T3 and T4, which produced similar mean egg weight from 62.53 g to 64.89g. Studies have demonstrated that feed supplementation with activated charcoal can boost egg production parameters by improving nutrient absorption and metabolic efficiency (Majewska *et al*. 2012), and better poultry health and production (Raphael *et al*., 2010). These could help explain the observed rise in egg weight in this study. The hens in T5 had a significantly larger egg width (46.77 mm) than the hens in T1, T2, T3 and T4 with comparable mean egg width (varying from 44.36 mm to 44.69 mm). This increase in egg width may represent an overall improvement in the hens' nutritional state as a result of the detoxifying capabilities of activated charcoal, which can lead to improved nutrient utilization according to Firdus *et al*. (2020). The potential of activated charcoal to adsorb toxins and enhance gastrointestinal health has been recognized, implying that it may promote greater nutritional absorption, leading to increases in egg dimensions (Firdus *et al*., 2020).

Furthermore, there was no significant variation in egg length among the treatments, demonstrating that while activated charcoal supplementation may improve specific quality indices, it does not impact other elements of egg quality. Santos and Eerden, (2021) have discovered varying impacts of dietary supplements on egg quality parameters, supporting the pattern of egg quality result in this study. Kim *et al*. (2019) have shown that charcoal supplementation improves egg production and quality, whereas others found that the benefits vary depending on the dietary composition and environmental conditions. The study's findings demonstrated a substantial increase in shell weight with increasing dietary levels of activated charcoal (T5), while shell thickness was comparable across the treatment groups. This is supported by Award *et al*. (2009), who found that activated charcoal can improve shell quality by increasing calcium absorption. A study by Kutlu *et al*. (2000) indicated that including activated sheabutter charcoal in the diets of laying hens significantly increased egg shell weight. Specifically, the results demonstrated that as the inclusion levels of activated charcoal increased, the mean egg shell weight rose, reaching up to 7.76 g at the maximum level of supplementation (40%) as compared to lower levels and the control group. Egg shape index in the current study were similar across treatments. This is in line with earlier studies that showed that, while nutritional supplements can change some egg quality metrics, they may not significantly affect other indices (Zhao *et al*., 2023).

5.5 Effect of Activated Charcoal Supplemented Diets on Internal Egg Quality of Hens

Yolk height of chickens fed the basal diet supplemented with graded levels of activated charcoal varied significantly among the treatment groups. Hens receiving the T5 diet containing 2% activated charcoal supplement had highest yolk height than the other treatment groups. The result in this study is not in line with that reported by Kalus *et al*. (2020) of a non-significant effect on yolk height of eggs of layer hens fed different doses of activated charcoal. Kalus *et al*. (2020) said that addition of biochar (form of activated charcoal) to the diet of laying hens positively influenced laying performance and shell quality but did not significantly affect the yolk traits. The inclusion of activated charcoal or biochar in hen diets can affect the nutritional composition of feed. Changes in dietary components, particularly protein and fats levels can significantly influence yolk traits including height (Gama *et al*. 2024). Yolk diameter and yolk weight were comparable to T1, (the control group), suggesting that the nutrient intake by the hens on 0.5, 1.5 and 2% AC dietary supplementation have no effect on the yolk diameter and yolk weight. The albumen weight increased significantly at 2% of activated charcoal dietary supplement. This result is not in agreement with Okey *et al*. (2021) who reported no significant effect on albumen weight. In a study by Zhang *et al*. (2022), dietary supplementation with activated charcoal at dosages of up to 100 mg/kg (1%) had no significant effect on serum immunoglobulin levels, which are associated with albumen synthesis. Both yolk index and haugh unit did not vary significantly from the control group. This is an indication that incorporation of activated charcoal as a feed supplement in layer diets from 0 to 2% did not enhance nor reduce the quality of albumen and yolk. This showed that the haugh unit and yolk index, related to egg freshness and overall quality remained stable despite charcoal supplementation up to 2%. This result is in agreement with Kutlu *et al*. (2000) and Ayanwale *et al*. (2006) that activated charcoal supplementation does not markedly affect these internal egg indices. However, the results are not in line with that reported by Kim *et al*. (2006) that activated charcoal enhances haugh unit scores when combined with wood vinegar. A progressive significant increase in yolk colour was observed from 1% AC to 2% AC supplementation. Thus, T5 has the highest yolk score of 9.65. This showed that higher activated charcoal levels can enhance yolk pigmentation. Kutlu *et al*. (2000) stated that major mineral constituent of activated charcoal are calcium, potassium and magnesium and these minerals are likely to promote the absorption and deposition of colour in egg yolk.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results obtained from this study which evaluated the response of layers to dietary supplementation with activated charcoal showed that the activated charcoal is moderate in crude fibre, low in crude protein, low in phosphorus and calcium, negligible in crude fat but high in dry matter, ash and metabolizable energy. Crude protein, crude fat, ash, calcium, phosphorus and salt (sodium chloride) tended to increase as the activated charcoal supplementation increased from 0% to 2% in the diets. Activated charcoal significantly increased the egg weight, egg width and shell weight (external egg qualities) and yolk height, albumen weight and yolk colour (internal egg qualities) with increased level of dietary supplementation.

6.2 Recommendations

a) The study has shown that activated charcoal can be used as a supplement in hens' diets at a level of up to 2% for the enhancement of egg weight and yolk colour, without any adverse effect on the other external and internal qualities of eggs produced.
b) Further research should be conducted to investigate the optimal inclusion level of activated charcoal supplementation in the diet of layers without adverse effects on egg quality.

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