

**AKENTEN APPIAH-MENKA UNIVERSITY OF SKILLS TRAINING
AND ENTREPRENEURIAL DEVELOPMENT**

**INFLUENCE OF GRADED LEVELS OF DIETARY CALCIUM ON GROWTH
PERFORMANCE, BLOOD PROFILE AND CARCASS CHARACTERISTICS OF
HELMETED GUINEA FOWL**

SAANI ALHASSAN MOHAMMED

2025

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BY

SAANI ALHASSAN MOHAMMED

(8212090010)

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requirements for the award of a Master of Philosophy degree in Biology

MAY, 2025

DECLARATION

Candidate's Declaration

I hereby declare that this thesis, with the exception of quotation and references contained in published works which have been duly acknowledged; is the result of own original work and that no part of it has been presented for another degree in this university or elsewhere.

Saani Alhassan Mohammed

Signature: Date:

Supervisor's Declaration

I hereby declare that the preparation and presentation of this work was supervised in accordance with the guideline for supervision of thesis as laid down by Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development.

Dr. Holy Kwabla Zanu

Signature: Date:

ABSTRACT

The objective of this study was to examine the growth response of grower helmeted Guinea fowls to different levels of dietary calcium. The experiment utilized a total of 150 unsexed day-old Guinea keets. On 16 days post hatch, 150 unsexed Guinea keets were weighed and then placed into 15 floor pens in a completely randomized design. Each pen was composed of 10 birds, and there were three dietary treatment groups with 5 replicates in each of the treatment groups. The birds were grouped into three groups and each group was given a different treatment with calcium concentrations of 0.36 %, 0.6 %, and 0.9 %. The diets were fed to the birds for a period of 10 weeks. The parameters measured include growth performance, gut pH, carcass traits, bone traits, haematology, and lipid profiles of blood. The results revealed that there were no significant differences ($p > 0.05$) observed among all the treatment groups for all the parameters. The dietary calcium amounts studied did not have a significant impact on the growth performance, gut pH, carcass characteristics, bone traits, haematology, and lipid profiles. The results indicate that varying dietary calcium levels of 0.36 %, 0.6 %, and 0.9 % do not cause any noticeable changes in the assessed physiological parameters of helmeted Guinea fowls during a 10-week period. Thus, the study suggests that Guinea fowl may not be affected by inadequate dietary calcium in the grower phase of production. A variety of calcium levels within the examined concentrations could be satisfactory for preserving their general well-being and performance.

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DEDICATION

This work is dedicated to Allah and my family.

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LIST OF ABBREVIATIONS

ADP	Active – Dicalcium Phosphate
BW	Body weight
Ca – to – NPP	Calcium to Non – Phytate Phosphorus
ECaC	Epithelial Calcium Channel
FGF	Fibroblast Growth Factor
GRA	Granulocytes
HCT	Hematocrit
HGB	Hemoglobin
IGF	Insulin – like Growth Factor
LO	Linseed Oil
LYM	Lymphocytes
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MID	Mid-sized Cells
MPV	Mean Platelet Volume
NARS	Non – Absorbed Reference Substance
NPP	Net Primary Production
NPP	Non – Phytate Phosphorus
PCT	Plateletcrit
PDW	Platelet Distribution Width

P-LCR	Platelet Large Cell Ratio
PLT	Platelets
PO	Palm Oil
PTH	Parathyroid Hormone
PUFA	Polyunsaturated Fatty Acid
RBC	Red Blood Cells
RDW-CV	Red Cell Distribution Width - Coefficient of Variation
RDW-SD	Red Cell Distribution Width - Standard Deviation
SFA	Saturated Fatty Acid
SO	Soybean Oil
WBC	White Blood Cells

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Calcium (Ca) is the predominant metal in terms of mass in numerous animal species. Calcium is a vital component of bones, teeth, and shells, where it is present in a form that cannot be dissolved. The passage of calcium ions into and out of the cytoplasm serves as a signal for numerous cellular activities. The indispensability of this element is indisputable, and signs of insufficiency manifest swiftly, particularly in juvenile, rapidly developing animals and in hens that have high requirements for the production of fresh bone mineralization or eggshell development (Leeson & Summers, 2009). Therefore, most diet formulations have a significant safety margin for calcium concentration, and lower amounts of Ca in the diet are not taken into consideration, despite the potential benefits for performance.

Calcium is a vital mineral for the health of birds, especially those like the helmeted Guinea fowl that produce eggs with hard shells (Orounladji et al., 2021). Calcium is essential for the processes of bone formation, muscle contraction, nerve impulse transmission, and blood coagulation (Pravina et al., 2013). Additionally, it plays a crucial role in maintaining the structural strength of eggshells, which has a direct effect on the reproductive success of certain avian species (Panyako et al., 2016).

Sufficient calcium intake is essential for the general well-being and efficiency of Guinea fowls. It is an essential nutrient that is crucial for many metabolic processes and the growth of bones in poultry (Veum, 2010). The primary sources of calcium in the diet of Guinea fowl are limestone and oyster shells. Both of these sources include calcium in the form of carbonate (Inoti, 2020; Barshan et al., 2019).

Many nutritionists believe that having a higher than planned dietary calcium concentration provides a greater level of safety without any drawbacks, as long as there is enough phosphorus present (Roberts et al., 2013).

Studies have shown that the intake of calcium is crucial for the health and performance of chickens, affecting different elements of their growth, reproduction, and overall well-being (Alagawany et al., 2021; Bryden et al., 2021). Insufficient calcium levels in laying hens can lead to decreased egg production, compromised shell quality, and an elevation in liver fat, as emphasized by Nys (2017). However, an excessive amount of calcium consumed by growing hens might have negative effects such as hindered growth, decreased efficiency in utilizing diet, and reduced absorption of minerals, as stated by Abdollahi et al. (2016). The dietary balance between calcium and phosphorus is crucial, since elevated calcium levels can have a detrimental influence on the digestion of phosphorus and the efficacy of phytase, an enzyme employed to improve the availability of phosphorus in poultry diets (Bedford & Rousseau, 2017).

In order to enhance the ability of chickens to tolerate calcium, it is of utmost importance to guarantee sufficient amounts of other necessary elements in their diet, as advised by Matuszewski et al. (2020). This well-balanced technique serves to mitigate the adverse consequences of both calcium deficiency and calcium overload. Furthermore, it is essential to closely monitor the levels of calcium in the diet in order to fully maximize the advantages of phytase supplementation, particularly when administering high dosages to enhance bird performance (Bedford & Rousseau, 2017). Poultry breeders may optimize the health, productivity, and general performance of their flocks by carefully managing the calcium-to-phosphorus ratio and taking into account the interplay between vitamins and minerals.

1.2 Problem Statement

Studying the needed components for a balanced diet in wild birds is considerably more challenging than studying confined birds. The complexity of this research is influenced by factors such as species variety, age, size, weight, seasonal requirements, population sizes, behavior, and weather. An area of limited research pertains to the calcium intake in wild avian species, which plays a vital role in egg production and the proper development of offspring.

Birds do not retain the surplus calcium required for egg-laying in their skeletal structure, as it would augment their mass, diminish their agility, and render them more susceptible to predators. They depend on obtaining calcium from their diet. Insufficient calcium in birds can result in the production of faulty eggshells, which can lead to the abandonment of the

clutch and unsuccessful hatching of the eggs (Ritchison, 2023). Studies have demonstrated that insects and seeds, which serve as key sources of nourishment, may only supply 10% of the necessary calcium for the process of laying eggs, presenting a notable obstacle throughout the reproductive period (Babarinde et al., 2021).

Female avian species must accumulate a significant quantity of calcium within a limited timeframe, frequently ingesting it shortly before dusk to guarantee its availability for the production of eggshells (Reynolds & Perrins, 2010). Inadequate calcium intake can impede the reproductive efficacy of wild birds, resulting in reduced clutch sizes or thinner eggshells (García-Campa et al., 2020).

On the other hand, Guinea fowls, which have traditionally been raised in developing countries using extensive methods, have difficulties such as inadequate nourishment, significant keets mortality, worm infestations, predation, and low egg hatchability (Moreki & Radikara, 2013). Nevertheless, with the transition of Guinea fowl production to intensive methods, they are currently being provided with diets specifically designed for chickens, which contain high levels of calcium. It is uncertain whether Guinea fowl in these systems necessitate the same elevated quantities of calcium as chickens, considering their capacity to thrive on reduced calcium intake in their natural habitat.

This study hypothesized that Guinea fowl reared in intensive production systems will not have negative consequences due to reduced calcium intake in their diet, and will exhibit similar performance to those fed with sufficient or greater levels of calcium. Although

Guinea fowl is increasingly being commercialized, particularly in European countries where they rank as the second-largest provider of poultry meat and eggs, there remains a lack of extensive research on their precise nutritional needs. This study aims to fill this knowledge gap by examining the calcium requirements of Guinea fowl in intensive farming settings.

1.3 Main Objective

The main objective of this study was to determine the growth responses and carcass characteristics of helmeted Guinea fowl to different dietary calcium levels.

1.4 Specific Objectives

The specific objectives of this study were to assess the effect of different levels of dietary calcium on the:

1. growth performance of helmeted Guinea fowl.
2. gut pH of helmeted Guinea fowl.
3. carcass traits of helmeted Guinea fowl.
4. haematological parameters of helmeted Guinea fowl.
5. lipid profile helmeted Guinea fowl.

CHAPTER TWO

LITERATURE REVIEW

2.1 Calcium Metabolism and Physiology in Poultry

Calcium metabolism in poultry is distinguished by exceptionally efficient intestinal absorption mechanisms and finely tuned hormonal regulation that together satisfy the high mineral demands of egg production. In laying hens, up to 70% of dietary calcium is absorbed in the duodenum and jejunum through active transcellular transporters—such as TRPV6 channels, calbindin, plasma membrane Ca^{2+} -ATPases, and $\text{Na}^{+}/\text{Ca}^{2+}$ exchangers—and passive paracellular diffusion via claudin-mediated tight junctions (Sinclair-Black, Garcia, & Ellestad, 2023). This absorptive capacity fluctuates with the oviposition cycle and declines with age, compelling older birds to draw increasingly on bone calcium reserves (Garcia-Mejia et al., 2024). Endocrine control involves parathyroid hormone (PTH) stimulating renal 1α -hydroxylase to elevate circulating 1,25-dihydroxyvitamin D_3 , which in turn upregulates intestinal calcium transport proteins. Concurrently, estrogen at sexual maturity promotes medullary bone formation—a specialized trabecular network serving as a rapid calcium reservoir—and enhances expression of vitamin D-binding protein and calbindin in both gut and kidney (Garcia-Mejia et al., 2024). In contrast, avian calcitonin exhibits relatively minor effects on calcium homeostasis, reflecting species-specific receptor sensitivity (Sinclair-Black et al., 2023).

The mobilization of skeletal calcium for eggshell mineralization is equally remarkable. With the onset of lay, elevated estrogen levels induce deposition of medullary bone, which supplies up to 40% of the calcium required during the approximately 20-hour shell formation process in the shell gland (Garcia-Mejia et al., 2024; Sinclair-Black et al., 2023). Under the combined influence of PTH and 1,25-dihydroxyvitamin D₃, osteoclast activity in medullary bone surges nearly ninefold, liberating calcium for the concentric deposition of calcium carbonate atop an organic matrix that constitutes the eggshell (Sinclair-Black et al., 2023). When medullary stores are depleted, cortical bone is catabolized, predisposing hens—particularly aged birds—to osteoporosis and compromised shell quality. Emerging research also implicates fibroblast growth factor 23 (FGF23) in the coordination of calcium and phosphorus balance, underscoring the multilayered endocrine control of mineralization in laying flocks (Sinclair-Black et al., 2023).

Embryonic skeletal development further exemplifies poultry's specialized calcium economy, relying on maternal mineral provisioning and temporally distinct extraembryonic pathways. Early in incubation, the yolk sac expresses calbindin and vitamin D-binding protein to mobilize yolk-derived calcium, whereas between days 12 and 16, the chorioallantoic membrane (CAM) upregulates proton secretion (via carbonic anhydrase II) and calcium transporters such as TRPV6 and SGK1 to dissolve eggshell matrix and transfer Ca²⁺ to the embryo (Halgrain et al., 2022). The absence of yolk-type calbindin in the CAM highlights complementary roles for these tissues in ensuring proper mineral delivery for ossification. Collectively, these findings illustrate the dynamic, highly regulated nature of calcium metabolism in poultry, from intestinal uptake and bone

remodeling in laying hens to specialized embryonic transfer mechanisms that secure skeletal integrity.

Li et al. (2017) summarized the key findings of their study on the physiology and metabolism of calcium in chickens. Calcium (Ca) is essential for various physiological processes in chickens, such as bone development, enzyme cofactor creation, coagulation, eggshell formation, muscle function, and nerve function.

Cereal grains, which are commonly consumed by non-ruminant animals, contain a significant amount of phosphorus but have a low calcium content. The phosphorus is predominantly bound as phytate, which is believed to be biologically inaccessible. Plant feed ingredients, especially wheat, may contain substantial levels of naturally occurring phytase activity. The steam pelleting procedure results in the loss of this activity, hence impacting the availability of phosphorus in the diet. Parathyroid hormone and vitamin D are crucial in chickens for maintaining the balance of calcium and phosphorus levels, a process mostly controlled by the endocrine system. This system plays a crucial role in the precise regulation of calcium and phosphorus levels in the body. The findings emphasize the significance of calcium and phosphorus in poultry diet and the intricate regulatory mechanisms required to maintain their equilibrium for optimal health and output.

Stanford (2006) conducted a study on the physiology and metabolism of calcium in hens. Metabolic calcium anomalies are commonly seen in the poultry industry, leading to reduced output and growth issues such as tibial dyschondroplasia in broiler chickens.

Additionally, it possesses a highly efficient homeostatic mechanism that promptly responds to the increased calcium requirements during egg production and the rapid growth of young birds. Birds maintain calcium levels by the action of parathyroid hormone (PTH), vitamin D metabolites, calcitonin, estrogen, and prostaglandins on various organs including the kidney, liver, gastrointestinal tract, and bone. Birds exhibit a more rapid response to hypocalcemic conditions compared to mammals. Domestic chickens have the ability to rapidly utilize their calcium reserves in order to produce eggshells. The calcium metabolism of birds relies on vitamin D₃, which also regulates mineral absorption and exhibits feedback mechanisms in response to calcium requirements. African grey parrots have a high susceptibility to calcium metabolism diseases; hence it is crucial to adhere to proper husbandry practices to meet their calcium requirements. These findings highlight the need of understanding and regulating calcium metabolism in hens to ensure optimal health, growth, and production.

However, Jansen et al. (2020) present detailed insights into the physiological and metabolic aspects of calcium in poultry, with a specific focus on laying hens. The publication provides the following key conclusions regarding the physiology and metabolism of calcium in poultry: In order for laying chickens to produce eggshells and maintain strong bones, they require calcium. Modern laying hybrids have a significant need for calcium, which is met through increased bone resorption and enhanced absorption in the gut. Estradiol-17 β affects the formation of medullary bone in birds, which serves as a readily available supply of calcium. This specific type of osseous tissue facilitates the process of eggshell calcification by ensuring a very effective provision of calcium.

Osteoporosis in laying hens is a result of the excessive release of calcium from bones, leading to a decrease in bone quality and an increase in the likelihood of fractures. The differences in how chicken layer lines respond to a lack of dietary calcium can be ascribed to genetic factors and their evolutionary history. Brown-egg lines may demonstrate greater resilience to calcium deficiency in comparison to white-egg lines. The process of selecting hens with high egg production efficiency may result in abnormalities in the allocation of resources, which might possibly impact physiological characteristics like calcium metabolism and bone health. The results underscore the complex interplay between calcium metabolism, genetic variables, and performance qualities in poultry, underscoring the significance of comprehending and controlling calcium needs for optimal well-being and efficiency in laying birds.

2.2 The Role of Calcium in Bone Formation and Health in Avian Species

Calcium is essential for birds to have robust and healthy bones. It facilitates bone development, durability, and overall structural strength, constituting around 35 % of the mineral content in bones. In a study conducted at Stanford University, Stanford (2006) presented significant data regarding the role of calcium in the bone formation and health of avian species. Calcium is essential for providing structural strength to the avian skeleton through the formation of calcium salts. Avian species possess a very efficient homeostatic mechanism that regulates calcium levels to fulfil the demands of bone development, especially during periods of fast growth and egg production. The medullary bone contains a labile calcium pool that is regulated by the activities of 1,25-dihydroxycholecalciferol and estrogen. This calcium pool is essential for birds to make their eggshells. The D₃

vitamin is essential for bone metabolism as it controls the absorption of minerals and responds to calcium needs through feedback mechanisms.

Li et al. (2017) thoroughly investigated the various significant findings related to the role of calcium in the well-being and bone formation of avian species. Calcium is essential for the formation and strength of bones in avian species because it combines with phosphorus to create hydroxyapatite, the mineral responsible for bone stiffness. Avian bone development is meticulously controlled, and the maintenance of bone health mostly relies on calcium metabolism. Avian species experience continuous bone turnover, highlighting the active involvement of calcium metabolism in the process of bone remodeling. The crucial significance of calcium in avian physiology is shown by its necessity for both eggshell formations in laying hens and bone growth. A new review has examined the process of eggshell creation and the growth of bones in birds. Calcification elucidates the complex mechanisms underlying the utilization of calcium for maintaining bone health and facilitating egg production. These findings emphasize the significance of calcium in promoting bone health, growth, and the creation of eggshells in birds. They show the complex relationship between calcium metabolism and physiological processes that are crucial for the well-being of birds.

Furthermore, according to Ricardo de Matos (2008), calcium is essential for bone formation and overall bone health in various bird species. Calcium is essential for providing structural support and enhancing strength, particularly in bones and eggshells. The body's biochemical functions, such as blood coagulation, hormone secretion

regulation, and the conduction of muscles and neurons, are crucial. Avian calcium metabolism is highly efficient and closely regulated due to the high needs of eggshell calcification and the rapid growth rate of juvenile birds. Calcium-regulatory hormones such as parathyroid hormone, calcitonin, and 1,25-dihydroxyvitamin D₃ (calcitriol) have a role in regulating calcium metabolism in birds. Prostaglandins, androgens, and estrogens all have a substantial role in the calcium metabolism of birds. The findings emphasize the crucial significance of calcium in avian species, specifically in the process of bone development and overall well-being.

Hormones play a crucial role in the process of restructuring and repairing bones. Thomas N. Tully's (2002) book contains crucial details about the role of calcium in the growth and health of bird species' bones. Bird skeletal systems provide both structural support and serve as a reservoir for calcium, which is crucial for important physiological processes, especially during egg development. The primary calcium storage site for egg development is the medullary bone reserve. Collagen, a crucial constituent of bones, plays a vital role in providing strength and structure to the skeletal system. Birds possess two primary categories of bone: cancellous bone, which serves as a lightweight supportive structure, and cortical bone, which is located in the shafts of long bones. The organic fibre of bone is capable of withstanding bending and twisting forces, while the mineral component of the bone is resistant to compression. Hormones have a crucial role in the restructuring and recovery of bones in bird species. Estrogen-treated birds have exhibited changes in callus strength and stability at fracture sites, illustrating the influence of hormones on the healing of bird bones. Growth hormone has a crucial role in controlling bone growth after hatching

in birds. It indirectly enhances the production of insulin-like growth factor 1 (IGF-1), which promotes the growth of cartilage cells, creation of the extracellular matrix, and development of bone tissue. In addition to growth hormone and IGF-1, fibroblast growth factor and transforming growth factor- β also contribute to the stimulation of chondrocyte proliferation and differentiation in the growth plate. The findings emphasize the complex systems involved in calcium management, hormone control, and growth factors that contribute to the creation and health of bones in bird species.

Similarly, Fleming (2008) provides significant observations regarding the role of calcium in the growth and well-being of birds' skeletal structure. The following are significant findings: Genetic variations have a significant impact on the strength and structure of chicken bones. Adding limestone particles to the diet has been proven to enhance eggshell thickness, strength, and bone strength and structure. Dietary sources of particulate calcium can improve overall bone health by reducing the number of active osteoclasts in the body. Broiler chickens' fast bone growth can lead to deformities in their legs and gait. To maintain the health of our legs, it is crucial to maintain a proper balance of calcium and phosphorus. The starting diets provide around 10 g of calcium and 4.5 g of bioavailable phosphorus per kilogram of diet. Maintaining this equilibrium is crucial in order to prevent skeletal deformities in grill chickens. Egg-laying hens are greatly affected by osteoporosis. Sufficient amounts of calcium, vitamin D, and phosphorus are crucial for maintaining optimal bone health in laying hens. To avoid osteoporosis, it is advisable to increase the dietary inclusion rates of calcium to levels similar to those seen in adult diets well in before of sexual maturity. The efficacy of most dietary interventions diminishes once hens

have already started laying eggs, underscoring the significance of early nutritional management. The findings emphasize the crucial significance of calcium, in addition to other nutrients, in preserving bone health and averting skeletal diseases in avian species.

2.3 Calcium Requirements for Optimal Eggshell Production in Poultry

Factors including as age, breed, and stage of production influence the optimal calcium intake for egg production. Due to the growing interest in this field, an et al. (2016) presented significant findings on the calcium needs for optimal eggshell production in poultry. These conclusions were based on the provided information and new knowledge in poultry nutrition. Studies have shown a considerable correlation between the quality of eggshells produced by laying hens and their consumption of calcium-rich diet. A positive association exists between increased dietary calcium levels and improved eggshell strength, thickness, percentage, and weight per unit of surface area.

In order to optimize the quality of eggshells in chickens, it is essential to ensure that their diet is adequately supplemented with calcium. Calcium plays a vital part in the production of eggshells by supplying the essential element needed for the strength and integrity of the shell. Inadequate calcium levels can result in eggshells that are thin or fragile, hence elevating the likelihood of egg breakage and compromising egg quality. Adding phytase to various meals has been demonstrated to have a beneficial impact on the digestion and uptake of calcium. Although there is no consistent reporting on the effects of dietary phytase on eggshell quality, certain research has suggested that including phytase in the diet can have a positive impact on eggshell quality. The calcium needs for achieving proper

eggshell synthesis are affected by the interactions with other essential nutrients, including phosphorus, vitamin D, and protein. Ensuring a proper balance of these nutrients in the food is crucial for optimizing calcium absorption and enhancing the quality of eggshells in laying hens.

Utilizing precision feeding techniques can customize calcium supplements to cater for the unique requirements of each bird and enhance eggshell development. Regularly monitoring the parameters that determine the quality of eggshells and making necessary adjustments to calcium levels will help maintain a regular level of egg production and ensure high-quality eggs. Although numerous studies affirm the significance of dietary calcium in the creation of eggshells, there can exist inconsistencies in the scientific literature regarding the precise levels of calcium necessary for achieving maximum eggshell quality. Additional research is necessary to improve calcium recommendations and gain a deeper understanding of the factors that affect calcium utilization in poultry. Ultimately, it is imperative to address the calcium needs of laying hens in order to guarantee the utmost efficiency and excellence in eggshell production and quality.

Stanford (2006) conducted a study on the calcium requirements for hens to generate high-quality eggshells. The study concluded that calcium is crucial for the formation of strong and durable eggshells in chickens. Approximately 10 % of the body's overall calcium stores are required for egg production within a 24-hour timeframe. Consequently, it is imperative to ensure that laying hens are provided with sufficient calcium in their diet to meet their requirements for eggshell formation. The production of eggshells in laying hens

relies on a readily available calcium reserve in the medullary bone and increased absorption of calcium in the intestines. Medullary bone facilitates the acquisition of calcium necessary for shell formation. Estrogen activity plays a significant role in regulating medullary bone homeostasis. in controlling the movement of calcium for the purpose of producing eggshells in laying hens.

According to Jing et al. (2022), Vitamin D₃ is crucial for the absorption and utilization of calcium in poultry, which is necessary for the efficient metabolism of calcium and the development of high-quality eggshells. The accessibility of dietary vitamin D₃ is essential for promoting indoor poultry production, guaranteeing that laying hens receive sufficient vitamin D₃ for calcium metabolism and the quality of eggshells. Effective regulation of dietary calcium levels, supplementation of vitamin D₃, and exposure to UV radiation for the natural synthesis of vitamin D₃ are crucial elements in fulfilling the calcium needs for good eggshell development in chicken. It is crucial to monitor and modify the amount of calcium in the diet according to the specific stage of egg production and the calcium requirements of laying hens. This is necessary to ensure the construction of strong eggshells and optimal reproductive performance in poultry. These findings emphasize the need of satisfying the calcium needs of laying hens to achieve optimal eggshell production in poultry. They highlight the relationship between dietary calcium, vitamin D₃, and hormonal management in sustaining eggshell quality and reproductive success in poultry farming.

2.4 Utilization and Absorption of Calcium in the Avian Digestive System

The absorption rate of calcium in the small intestine is influenced by several factors, such as pH, vitamin D₃, and the presence of other dietary components. The gizzard facilitates the extraction of calcium from the swallowed meal for subsequent processing. In Wasserman's (2004) study, the context provided thorough information regarding the utilization and absorption of calcium by the avian digestive system. The saturable transcellular system that enables active calcium transport in the avian duodenum involves three primary mechanisms: intracellular calbindins (specifically avian calbindin-D28k), a basolateral membrane calcium pump activated by ATP, and epithelial calcium channels (CaT1 and ECaC). 1,25-dihydroxycholecalciferol [1,25(OH)₂D₃], which is the active form of vitamin D, stimulates the synthesis of these compounds and induces the formation of calbindins. In addition, calcium is absorbed through diffusion, where it moves across the cellular membrane of the intestine by taking the paracellular path between neighboring enterocytes. This process necessitates precise levels of calcium ions within the lumen and is enhanced by the presence of vitamin D.

The majority of calcium that is consumed is absorbed in the ileum, which is the lowest portion of the small intestine in birds. Vitamin D is essential for the avian digestive system to absorb calcium. It stimulates the active transport of calcium and increases the total rate at which calcium moves across cell membranes. The duration of passage and residence durations in the avian gastrointestinal system also have a role in the overall absorption of calcium, with the ileum exhibiting a comparatively lengthy duration of passage compared to other sections of the small intestine. These findings emphasize the significance of

vitamin D and the precise mechanisms involved in calcium absorption in the avian digestive system.

Bar (2009) provides crucial information about the utilization of Ca in the digestive system of birds. The effects of restricting dietary Ca or phosphorus, as well as the reliance on vitamin D for intestinal calcium absorption mechanisms, have been investigated using in situ-tied or perforated intestinal loops (Fleet et al., 2010). While the effects of various nutritional or physiological conditions on actual absorption in living organisms have not been thoroughly investigated, this research has provided insight into the ability to absorb calcium. The dynamics of body-calcium compartments, such as bone, and the rate of calcium absorption have been assessed by various in vivo methods, such as administering radioactive calcium through a single intubation or continuous feeding. Unlike mammals, birds employ a balancing strategy that prioritizes mineral retention above net absorption. This is primarily because they have difficulties in differentiating between renal and intestine excretion. Both in vivo and in vitro investigations have determined that the upper intestine is the primary location for calcium absorption in birds.

The intestinal calcium absorption capacity of laying birds correlates with their calcium requirements during growth, maturity, and reproduction. Factors like as rapid growth, hormonal regulation, and homeostatic reactions affect the active transcellular absorption of calcium in laying birds. These findings emphasize the intricate interaction of elements that affect the utilization and absorption of calcium in avian species, especially in relation to

the development of eggshells and the specific physiological needs of birds during egg laying.

In their study, Proszkowiec-Weglarz and Angel (2013) provided valuable insights on the utilization and absorption of calcium by the avian digestive system. Vitamin D is crucial for calcium absorption in hens. The intestinal mucosa can synthesize calbindin D28K in response to vitamin D, hence enhancing the absorption of calcium. Chickens that are fed a diet lacking in vitamin D have reduced levels of calcium in their blood, as well as decreased production of calbindin d28k mRNA and reduced protein expression in the kidney and gut. The expression of calbindin d28k and the absorption of calcium both rely on sufficient levels of vitamin D. The regulation of calcium and phosphorus metabolism in broilers is controlled by feedback mechanisms including parathyroid hormone (PTH), active vitamin D₃, and calcitonin, within a specific physiological range. These systems aid in the regulation of plasma calcium and phosphorus levels. Phosphatonins, such as FGF23 and FGF7, control the balance of phosphate in mammals. Renal phosphate absorption is inhibited by factors such as sFRP-4 and MEPE. FGF23 and sFRP-4 have a substantial impact on the regulation of 1, 25(OH)₂D₃ production in mammals. The amounts of total and ionized calcium in growing chickens are comparable to those found in mammals. Vitamin D₃ is crucial for maintaining calcium balance, whether it is produced in the skin or acquired through the diet. These findings emphasize the complex connection between vitamin D, the absorption of calcium, and the homeostatic systems that control calcium and phosphorus metabolism in chicken.

2.5 The Impact of Varying Calcium Levels on Poultry Growth and Performance

Both elevated and reduced calcium levels can affect the growth and functioning of chickens. Insufficient calcium intake can lead to skeletal abnormalities, reduced egg production, and poor feed consumption. Excessive intake of calcium can impede growth and disrupt the assimilation of minerals. An et al. (2016) provided valuable insights into the impact of varying calcium levels on the growth and performance of chickens. Increased dietary calcium intake can lead to improved eggshell quality. A study conducted by Batres (2022) revealed that supplementing older laying hens with elevated levels of calcium, up to 4.7 %, in their food during the last third of their laying cycle resulted in enhanced eggshell quality and reduced incidence of cracked eggs. There was no significant effect on the concentration of blood calcium and phosphorus, tibial breaking strength, or ash calcium due to linear or quadratic trends in dietary calcium and phosphorus contents in the tibia. Nevertheless, there was an inclination for the tibial breaking strength to augment when the dietary calcium levels increased. There was no significant impact on the total amount of food consumed or the laying performance of older laying hens due to variations in dietary calcium levels.

Talpur et al. (2012) conducted a study examining the impact of dietary calcium on the performance of commercial chickens. This study provided some significant findings about the impact of varying calcium levels on the growth and performance of poultry. Broilers that were fed a diet with 1 % calcium devoured a much greater quantity of feed compared to those fed diets with 2 % and 3 % calcium. The birds in the 1 % calcium group had a significantly higher water consumption compared to the groups with 2 % and 3 % calcium.

The impact of the 3 % calcium group on live body weight was more significant when compared to the groups with 2 % and 1 % calcium.

Gautier et al. (2017) examined the intricate relationship between various calcium levels and their impact on the growth and performance of chickens. The presence of dietary calcium and phosphorus is crucial for metabolic processes and maintaining the structural integrity of the skeleton in chickens. These minerals have a significant impact on the financial aspects of chicken production. When formulating diets for poultry, it is essential to carefully monitor the ratio of non-phytate phosphorus (NPP) to calcium (Ca). Imbalanced ratios could adversely affect the growth of broilers and their ability to retain nutrients. When comparing the effects of weight growth on the body, bone mineralization relies more heavily on the amounts of NPP and Ca in the diet. Heightened intake of dietary calcium might hinder growth and the ability to retain nutrients, highlighting the importance of controlling the absolute levels of dietary calcium and non-protein phosphorus (NPP), as well as their relative ratio. Elevated levels of calcium in broiler diets can enhance bone ash content, but they may also impede bird development performance and disrupt mineral absorption. Elevated calcium-to-net phosphorus production (Ca-to-NPP) ratios in the diet can hinder the efficiency of phosphorus digestion and absorption.

The ideal dietary calcium content for optimizing bone construction and function may exceed the amount necessary for optimal performance. For broilers, it is advised to have a Ca-to-NPP ratio of 2:1. Increasing the Ca-to-NPP ratio in diets may result in a decrease in broiler growth performance. The ratio of calcium (Ca) to non-phytate phosphorus (NPP)

may have a greater impact on bird performance than the individual amounts of Ca or NPP in the diet (Gautier et al., 2017). This is because dietary Ca can interfere with the production of phytate complexes. These findings highlight the significance of meticulously evaluating the quantities and proportions of calcium and non-phytate phosphorus in poultry diets to enhance development, bone structure, and overall performance.

In a similar vein, Xing et al. (2019) investigated the impact of varying calcium levels on the growth and performance of chickens in this particular scenario. The study utilized three different calcium sources and amounts: distilled water (SS), acetic acid-cleansed shell powder (SCAC), and active dicalcium phosphate (ADP). A total of thirty-two broiler chickens, aged one day, were randomly separated into six groups. They were then fed a basal diet that was supplemented with either 0.95 % or 1.05 % ADP, SCAC, and SS treatments until day forty-two. When comparing broiler hens that were fed SCAC and SS to those that were fed a diet supplemented with 1.05 % ADP, the latter group exhibited a reduced feed conversion ratio and an increased average daily weight gain. The breast muscle rate of the group undergoing high-calcium ADP treatment was 16.44 % higher compared to the group receiving the low-calcium SCAC treatment. The high-calcium ADP therapy group exhibited significantly elevated thymus and bursa indexes in comparison to the other groups, suggesting a potential influence on immune function. These data indicate that the calcium source and quantity in poultry feed can have a substantial effect on the growth performance, carcass features, and immune organ indexes of grill chickens.

Calcium is crucial in the diet of poultry species to ensure their optimal growth and skeletal development, which are necessary for their overall performance. Proper calcium supplementation in the food is essential for bone mineralization, eggshell development, muscular function, and metabolic functions in chickens. Varying levels of calcium intake in the diet can impact the integrity of eggshells, the strength of bones, and the overall health and productivity of chicken populations. Chickens can experience bone abnormalities, reduced egg production, and metabolic problems due to imbalances of calcium in their food, which can have negative effects on their growth and overall well-being. Chicken diets necessitate targeted calcium supplementation as their calcium needs fluctuate depending on age, breed, reproductive state, and environmental conditions (Reynolds & Perrins, 2010). Ensuring the calcium levels in chicken diets are at their best is crucial for achieving maximum growth rates, feed efficiency, and overall performance outcomes in commercial poultry production. Optimizing growth, bone health, and production parameters in birds can be achieved by monitoring and modifying dietary calcium levels according to the individual needs of chicken flocks. The results emphasize the substantial influence of different calcium levels on the growth and performance of chicken, emphasizing the necessity of accurate control of dietary calcium to promote the health, productivity, and welfare of poultry in commercial production systems.

In a study conducted by Li et al. (2017), the researchers examined the impact of varying calcium levels on the growth and performance of chickens. They provided a comprehensive analysis of their findings. Broilers may tolerate a wide variety of calcium concentrations in their food as long as the meal contains sufficient bioavailable phosphorus

or is supplemented with phytase when bioavailable phosphorus is lacking. It is essential to optimize phosphorus consumption in poultry diets as reduced levels of bioavailable phosphorus in the diets result in decreased phosphorus excretion in manure. The lack of thorough research on the phosphorus requirements of modern laying hen breeds has led the poultry industry to implement large safety margins when it comes to phosphorus levels in their diets. This is done to prevent any shortages during production. Establishing the appropriate calcium and phosphorus levels in food poses a challenge in the commercial sector. It is important to ensure that the phosphorus levels in bird feed matches their nutritional needs, taking into account the mineral concentrations in the feed ingredients. This highlights the necessity for biologically determined data and evaluation systems specifically for calcium and phosphorus. These findings highlight the significance of maintaining a proper equilibrium between calcium and phosphorus levels in poultry diets in order to enhance growth, performance, and nutrient utilization, while simultaneously reducing environmental consequences and production costs.

2.6 Factors Influencing Calcium Requirements in Helmeted Guinea Fowl

The calcium requirements of helmeted Guinea fowl are influenced by factors such as age, body weight, reproductive status, and environment. While mature hens require additional calcium for the formation of their eggshells, newborn chicks have greater calcium needs for the growth and development of their skeletal system. The study conducted by Gilber (1983) analyzed the supplied data and identified the key findings on the variables that influence the calcium requirements in helmeted Guinea fowl. The quantity of calcium in the diet is a significant determinant of calcium intake. In contrast to carnivorous birds that

gain calcium from their diet of fish, crustacea, or shelled mollusks, herbivorous or omnivorous birds like Guinea fowl may struggle to acquire sufficient calcium through their usual dietary sources. Food consumption is essential for the provision of calcium. Guinea fowl may adjust their daily food consumption to control the amount of calcium they receive, which affects the production of their shells.

Guinea fowl have a particular preference for calcium and can easily take large amounts of calcium carbonate when it is offered as an alternative. They adjust their calcium consumption based on their output needs. The parathyroid hormone potentially regulates the specific craving for calcium in Guinea fowl. Diets containing excessive amounts of calcium can negatively impact egg production, highlighting the importance of closely monitoring and controlling calcium levels in the diet of helmeted Guinea fowl. The reproductive process in domestic chicken results in a substantial depletion of resources, and calcium needs are vital for both egg production and the creation of the eggshell (Xia et al., 2019). Guinea fowl possess mechanisms to monitor the correlation between the loss of calcium output and the intake of food, potentially involving pituitary gonadotrophins and the amounts of calcium circulating in the body. In contemporary breeding lines, the need for increased production rates might diminish the efficacy of inherent defense mechanisms, resulting in possible complications such as limb weakness and "cage layer fatigue" syndrome. The results emphasize the intricate interaction between nutritional, physiological, and hormonal aspects that affect the calcium needs of helmeted Guinea fowl. It is crucial to comprehend these dynamics in order to achieve optimal production and maintain bird health.

In contrast to helmeted Guinea fowl, Rao et al. (2002) specifically focus on the calcium requirements of White Leghorn layers and commercial broilers. However, it is widely understood that the genetic potential of helmeted Guinea fowl poultry is a significant factor in determining their calcium requirements. This genetic potential directly impacts their ability to utilize calcium effectively, which in turn determines the amount of calcium they need. Studies have shown that the activity of serum alkaline phosphatase and bone resorption in chickens can be influenced by the calcium levels in their diet. Poultry may necessitate varying quantities of calcium depending on their age and phase of production. White Leghorn layers may have varying calcium requirements during their peak production phase, in contrast to broilers at different growth stages. The calcium requirements of chickens may be influenced by factors such as the ambient temperature and other environmental conditions. The interplay between calcium and other essential nutrients, including phosphorus, vitamin D, and phytase, can impact the total utilization and needs of calcium in poultry.

2.7 Health Consequences of Calcium Deficiency in Guinea Fowl

Inadequate calcium levels in guinea fowl can result in weakened skeletal structure, thinner eggshells, and an increased susceptibility to fractures. Additionally, it could potentially affect overall health and the functioning of muscles. Julian's (2005) study offers significant insights into the health consequences of calcium deficiency in chickens, particularly in layer hens and broilers. While the paper does not explicitly mention Guinea fowl, potential health consequences can be inferred by drawing on our fundamental knowledge of calcium deficiency in poultry. A calcium deficiency can lead to skeletal problems such as

osteoporosis, brittle bones, and fractures. Layers may have cage layer fatigue due to the physical demands of egg production, resulting in weakened bones. Calcium is essential for the development of robust skeletal structure and fragments of eggshells. Insufficient consumption of calcium can cause eggshells to become thin or fragile, which can negatively impact the quality of eggs and potentially result in health problems associated to eggs. Insufficient calcium levels can have a negative effect on the reproductive health of Guinea fowl, leading to decreased egg production and reduced hatchability. During the process of eggshell development, the occurrence of hypocalcemia might result in paralysis or mortality. Calcium plays a vital role in facilitating muscle contraction and maintaining proper nerve function.

Inadequacy can lead to muscular debility, convulsions, and further neuromuscular complications. In order to combat calcium insufficiency in Guinea fowl, it is crucial to offer a well-balanced diet that contains sufficient amounts of calcium, phosphorus, and vitamin D. This will help promote healthy bones, enhance egg production, and improve overall health and vitality. By closely monitoring the amount of calcium consumed and making necessary adjustments to the feed formulations, it is possible to prevent the adverse health effects that can arise from calcium insufficiency in chicken.

In a similar vein, Okaeme et al. (2003) said that their study did not particularly investigate the health consequences of inadequate calcium intake in Guinea fowls. However, a comprehensive comprehension of the adverse health consequences of a calcium deficiency in guinea fowl indicates that such a shortfall can lead to the production of low-quality

eggshells in Guinea fowl. These eggshells are thin or fragile and have a higher likelihood of cracking. Reproductive problems such as reduced fertility and hatchability can occur due to a calcium deficiency, as calcium is essential for proper muscle function and egg formation. Calcium is essential for the development and durability of bones. Inadequate calcium levels can lead to skeletal issues such as weak bones and deformities. Moreover, calcium is essential for the proper functioning of nerves and muscles. A deficiency can lead to muscular weakness and reduced mobility in Guinea fowl.

2.8 Effects of Excessive Calcium Consumption on Avian Physiology

Excessive intake of calcium can lead to a condition called hypercalcemia, which can damage the kidneys and cause the hardening of soft tissues as well as hinder growth. Furthermore, it can disrupt the assimilation of zinc and phosphorus, along with other minerals. Bar (2009) elucidated the impact of elevated calcium consumption on avian physiology. Excessive intake of calcium can disrupt the metabolism of birds, leading to anomalies in their calcium levels. This imbalance can negatively impact physiological processes such as eggshell production and bone health. Birds possess a unique excretory system that allows them to eliminate both urine and faeces through a single opening called the cloaca. Excessive calcium intake might hinder the differentiation between the excretion of calcium in the intestines and the kidneys in birds, hence affecting the overall calcium balance in the body. Elevated dietary calcium levels can have a significant effect on the skeletal well-being of birds, potentially resulting in illnesses such as osteoporosis or skeletal deformities. Ensuring a correct equilibrium of calcium is essential for preserving the robustness and soundness of bones in avian species. Overconsumption of calcium can

also have an impact on the quality of eggshells in laying birds. Disruptions in calcium levels can cause irregularities in the development of eggshells, resulting in problems like thin or fragile shells. These issues can have negative effects on the reproductive success of birds. Birds possess regulatory mechanisms to control the levels of calcium in their bodies. Consuming too much calcium can put strain on these regulatory mechanisms, which may result in disturbances in the processes of absorbing, using, and getting rid of calcium. These findings emphasize the significance of maintaining a well-balanced calcium diet in avian species to promote overall health, reproductive success, and physiological functions. Overconsumption of calcium can have important consequences for the physiology of birds and should be closely controlled to avoid negative impacts on their health and well-being (Bryden et al., 2021).

In a similar vein, Proszkowiec-Weglarz and Angel (2013) conducted a thorough analysis and provided insights into the impact of elevated calcium consumption on avian physiology. Here are some key findings from their document. Excessive calcium intake can disrupt calcium homeostasis in birds. Maintaining an optimal calcium intake is crucial for normal physiological functions. Elevated calcium intake levels may have an effect on the renal function of birds. In order to prevent any possible renal issues, it is essential to monitor the dietary intake of calcium. Calcium is essential for bone formation and health, but excessive amounts can lead to problems in bone mineralization. Optimal bone structure and strength necessitate adequate quantities of calcium. Excessive calcium intake can affect the regulation of parathyroid hormone (PTH) in birds. PTH plays a crucial role in maintaining the balance of calcium in the body and regulating bone health. The correlation

between calcium consumption and vitamin D levels plays a crucial role in preserving calcium equilibrium in avian physiology. Imbalances in vitamin D levels might affect the body's ability to absorb and use calcium. These findings highlight the significance of maintaining adequate calcium levels in bird diets to promote optimal physiological activities and prevent potential disturbances in calcium balance and associated metabolic processes.

However, an et al. (2016) did not extensively discuss the impact of increased calcium intake on avian physiology. Nevertheless, a number of crucial data regarding the potential impacts of elevated calcium intake on avian physiology were presented, relying on a comprehensive comprehension of chicken nutrition. Excessive calcium intake in poultry, especially in male birds, can lead to the formation of urinary calculi, often known as stones. These calculi can obstruct the urinary tract, leading to health issues and decreased functionality. Excessive intake of dietary calcium may impede the absorption of other crucial minerals such as phosphorus, zinc, and magnesium. This imbalance may have negative effects on both nutritional consumption and avian health. Soft tissue calcification, a condition characterized by the accumulation of calcium deposits in soft tissues, can be caused by an excessive dietary consumption of calcium. This can hinder the functioning of organs and overall physiological systems. If the dietary calcium levels are very high, birds may decrease their feed intake, resulting in reduced nutritional intake and consequent performance problems. Although calcium is necessary for maintaining healthy bones, consuming too much calcium can disturb the equilibrium between calcium and phosphorus, resulting in skeletal problems such irregular bone growth or fragile bones.

Poultry breeders must meticulously maintain the calcium levels in the meal to ensure that the birds' nutritional needs are met without negatively impacting avian physiology. It is essential to closely monitor the amount of calcium consumed and carefully evaluate the overall balance of minerals in the poultry's diet as part of effective nutrition management.

Xing et al. (2019) conducted a study to investigate the impact of increased calcium consumption on the physiological processes of grill chickens. The study found that the birds' immune system and organ development were dramatically affected by different dietary calcium sources and levels. Specifically, an association was found between elevated calcium intake and deviations in the thymus and bursa indices, which are indicators of immune function and organ growth. This study also examined the influence of calcium on mineral ions and serum biochemical components. The findings revealed that the type of calcium used had a significant impact on the levels of high-density lipoprotein. The findings suggest that the intake of high levels of calcium can affect various aspects of avian physiology, including serum biochemical components, organ development, and immune function.

Michael (2006) investigated the influence of excessive calcium intake on avian physiology and discovered that birds can develop hypercalcemia, a condition characterized by high levels of calcium in the blood, as a result of consuming too much calcium. This excessive calcium consumption can have a deleterious effect on avian physiology. Excessive intake of calcium in the diet can disrupt the balance of calcium in birds, leading to hypercalcemia. This condition can cause metabolic irregularities and potential health issues. Birds can

experience kidney damage, soft tissue calcification, and impaired organ function as a result of long-term exposure to elevated levels of dietary calcium. Excessive intake of calcium can also hinder the body's capacity to assimilate and utilize other essential minerals, such as phosphorus, hence disturbing the equilibrium of nutrients and physiological processes in birds. Avian health can be negatively affected by high calcium, leading to symptoms such as fatigue, lethargy, reduced feed intake, and diminished reproductive success in poisoned birds.

Effective regulation of dietary calcium levels is essential to mitigate the detrimental impacts of high calcium intake on avian physiology and promote optimal health and well-being in birds. To prevent calcium-related illnesses and preserve physiological balance in avian species, it is crucial to monitor calcium intake, change dietary formulations, and provide a well-balanced diet. These findings emphasize the need of avoiding excessive calcium intake in birds to prevent potential health issues and preserve adequate physiological function in avian species. Ensuring proper dietary management and closely monitoring calcium levels are crucial for achieving optimal health and performance results in birds.

2.9 Calcium Homeostasis and Regulation Mechanisms in Birds

Birds maintain calcium homeostasis by an intricate interaction of hormonal control and physiological processes involving the parathyroid gland, kidneys, and intestines. Multiple studies have been undertaken to examine it. Xing et al. (2019) conducted a study to

examine how various sources and quantities of calcium affect calcium homeostasis and control mechanisms in broiler chickens.

Different calcium levels and sources had a substantial impact on the thymus and bursa indices. Group 4 exhibited the greatest indices relative to the other groups. The study concluded that the kind and amount of calcium did not have a significant impact on the blood biochemical components, except for the high-density lipoprotein levels, which were notably higher in the low-calcium SCAC therapy group. The research discovered variations in the levels of detrimental bacteria (*E. coli* and *Salmonella*) and beneficial bacteria (*Lactobacillus* and *bifidobacteria*) in the caecum of broiler chickens, depending on the treatment of calcium source. This suggests that the choice of calcium source influences the composition of the intestinal microbiome. The findings indicate that the type and amount of calcium can greatly impact the development of organs, levels of biochemical components in the blood, and the composition of the intestinal microbiota in grill chickens.

Tordoff (2001) offers unique insights into the physiological and behavioral aspects of calcium balance in birds, specifically in relation to calcium homeostasis and control mechanisms.

The calcium needs of growing hens are approximately 100 mmol per day, whereas adult hens require 2.5-5.0 mmol per day. Research has shown that birds, whether they are in their natural habitat or kept as pets, display distinct actions in order to acquire food that is high in calcium. Several avian species enhance their diet by ingesting calcium-rich

substances such as bones, owl pellets, mortar, grit, and shells from different organisms. The concept of calcium appetite is widely acknowledged in avian literature.

The quality of housing can impact the amount of calcium that birds consume (Bryden et al., 2021). When hens are kept together, they tend to eat more sources of calcium compared to chickens that are housed individually. Boredom is believed to be a factor that affects their consumption. Studies by Anita et al. (2021) have demonstrated that birds lacking calcium exhibit a preference for meals that include calcium, suggesting that learning plays a significant part in their calcium intake behavior. Birds are capable of linking the visual characteristics of a diet rich in calcium with the positive outcomes it provides, which in turn affects their selection of food. These findings emphasize the intricate interaction of physiological systems, behavioral responses, and environmental factors in controlling calcium balance in birds. Comprehending these factors is crucial for maximizing calcium consumption and upholding general well-being in bird populations.

Bar (2009) offers comprehensive information on the mechanics and regulation of calcium homeostasis in birds. Key findings indicate that birds possess specialized mechanisms in their intestines for efficient calcium absorption. These mechanisms encompass both transcellular and paracellular routes, facilitating the effective absorption of dietary calcium. Calcium transportation in birds is facilitated by a range of proteins and channels, such as ATPase, calbindin, and epithelial calcium channels. These components are crucial for the processes of calcium absorption, transport, and utilization in the body. Hormones such as vitamin D and gonadal hormones play a crucial role in closely regulating calcium

homeostasis in birds. These hormones have crucial functions in regulating calcium equilibrium, promoting bone integrity, and facilitating the development of eggshells in avian species. Birds gradually develop the ability to absorb calcium, with changes in absorption capacity occurring as they grow and mature. Comprehending these developmental changes is crucial for maximizing calcium intake and utilization in avian species. Birds may have diurnal fluctuations in calcium absorption, resulting in varying rates of absorption at different times of the day. The circadian process of calcium absorption can have an effect on the overall balance of calcium in the body and the various physiological activities in birds. Vitamin D is essential for maintaining calcium homeostasis by controlling calcium absorption in the intestine and promoting bone health. The vitamin D receptor has a role in preserving the integrity of the intestinal mucosal barrier, which in turn affects the absorption of calcium. Calcium homeostasis in birds can be affected by nutrition and dietary variables.

Ensuring appropriate feed composition, which includes sufficient calcium levels and supplementation, is crucial for maintaining calcium balance and fulfilling the physiological requirements of the birds. These findings emphasize the intricate regulatory mechanisms involved in calcium balance in birds and emphasize the significance of hormonal, developmental, and nutritional factors in maintaining ideal calcium levels for bone health, eggshell formation, and overall physiological functions in avian species.

Similarly, Shi et al. (2020) reported the main discoveries on calcium homeostasis and the mechanisms of regulation in birds. Calcium is an essential component for the development

of eggshells in laying hens, as the eggshell is composed of 95% calcium carbonate. Ensuring adequate calcium supplementation is crucial for preserving the quality of eggshells. Ensuring the appropriate amount of calcium supplementation is essential for healthy eggshell calcification and development in laying hens. Research has demonstrated that just increasing the amount of calcium supplements may not consistently lead to enhanced egg quality. This suggests that a well-rounded approach to managing calcium levels in chicken diets is necessary. The solubility of calcium sources, such as limestone, can affect the strength and thickness of eggshells in laying hens. Administering calcium supplements with protective properties may result in improvements in the strength and thickness of eggshells, hence reducing the occurrence of fractured eggs. Factors such as dietary calcium levels, particle size of calcium sources, and the presence of other nutrients like phosphorus influence calcium absorption and metabolism in birds. Ensuring a proper equilibrium of these components is crucial for the maintenance of calcium homeostasis in avian species. These findings emphasize the complex connection between calcium balance, eggshell quality, and the addition of calcium to the diet in birds. This underscores the significance of customized nutritional approaches for maximizing chicken health and egg production.

Stanford and Micheal (2006) conducted a comprehensive study on the homogenous investigation and reporting of calcium homeostasis and regulatory mechanisms in birds. Avian serum has three components of calcium: ionized calcium, calcium that is bound to proteins, and calcium that is complexed with different anions. Ionized calcium is the biologically active portion that plays a role in maintaining bone balance, muscle and nerve

activity, blood clotting, and hormone control. Most of the calcium that is attached to proteins is considered biologically inert, therefore alterations in serum albumin levels have a direct impact on the overall calcium levels. Alterations in pH can also impact the portion of calcium that is bound to proteins. Avian calcium control is governed by the interplay of vitamin D₃, parathyroid hormone (PTH), and dietary calcium levels. Vitamin D₃ is necessary for the absorption and use of calcium, while PTH responds to low levels of ionized calcium by stimulating the release of calcium from bones and increasing the reabsorption of calcium by the kidneys. Parathyroid hormone (PTH) has a hypercalcemic effect in birds, causing rapid elevations in blood calcium levels. The hormone exerts precise control over calcium output through feedback mechanisms and primarily affects the kidney and bone, which are the key organs responsible for calcium management. Birds depend on dietary vitamin D₃ and exposure to UV-B radiation for the manufacture of vitamin D, which is essential for calcium metabolism. Insufficient intake of vitamin D from diet or lack of exposure to UV-B radiation can cause hypocalcemia, which in turn can result in calcium metabolism problems in birds. The avian calcium regulation system is a remarkably efficient homeostatic mechanism that can rapidly adapt to elevated calcium requirements, such as those occurring during egg production and the rapid growth of young birds.

Parathyroid hormone, vitamin D₃ metabolites, and calcitonin are crucial in the regulation of calcium in birds. Avian and mammalian calcium regulation differ, since birds exhibit swift skeletal responses to calcium stressors. The avian skeleton is an ideal subject for researching calcium control because it exhibits quick metabolic reactions. These findings

emphasize the complex mechanisms involved in maintaining calcium balance and physiological function in birds, particularly the role of vitamin D, PTH, and dietary variables in regulating calcium homeostasis. Jansen et al. (2020) provided comprehensive insights into the control mechanisms and maintenance of calcium homeostasis in birds. The publication presents major results about calcium homeostasis and regulatory mechanisms in birds. Calcium has a vital role in the development of eggshells and the maintenance of bone stability in female birds. Contemporary laying hybrids require a substantial amount of calcium for the process of eggshell calcification, requiring up to three grammes of calcium for each eggshell. Birds have the ability to extract calcium from their bones in order to fulfil the significant requirement for eggshell production. This process entails enhanced absorption in the intestines and heightened resorption in the bones. During sexual maturity, birds produce medullary bone as a result of the presence of estradiol-17 β . This easily changeable calcium supply may be rapidly refilled and acts as a vital source of calcium during the process of forming eggshells.

According to El-Sabrout et al. (2022), choosing chicken layer lines based on their ability to produce a large number of eggs efficiently may result in a decreased ability to handle calcium depletion. This can lead to the weakening of bones through demineralization and make them more prone to fractures, a condition known as osteoporosis. Phylogenetic origin can also influence differences in adaption responses. The study emphasizes the impact of genetics on the ability of hens to adjust to calcium deprivation. The phylogenetic origin and genetic traits of chicken lines influence their response to variations in calcium availability. The results highlight the complex mechanisms that regulate calcium balance in

birds, namely in laying hens. They also emphasize the significance of comprehending these processes in order to improve nutrition and wellbeing in poultry production.

2.10 Dietary Calcium and Gut Health in Guinea Fowl

The presence of calcium in the diet can impact the gastrointestinal health of Guinea fowl by altering the acidity level of the digestive system, the makeup of the microorganisms in the intestines, and the immunological reaction occurring in the stomach. The correlation between calcium intake and the gastrointestinal well-being of Guinea fowl has received significant interest in recent times. A study conducted in the US indicated that adults who primarily consume milk as their main source of nutrition require a significant intake of calcium (O'neil, Keast, Fulgoni, & Nicklas, 2012). It is essential to comprehend the significance of calcium in Guinea fowl nutrition, since research has demonstrated that diet can impact this essential mineral, which is vital for maintaining healthy bones (Bonjour, 2011). According to a study by D'Amelio and Sassi (2017), the immune system and gut bacteria play a crucial role in maintaining bone health. A potential correlation exists between gut health and calcium absorption, as prebiotics have been associated with the metabolism of minerals and bone (Whisner & Castillo, 2017). A study has examined the connection between the gut microbiota and mineral metabolism, highlighting the need of investigating the impact of dietary calcium on the gut microbiota in Guinea pigs (Skrypnik & Suliburska, 2018). Research has shown a correlation between vitamin D and cardiovascular health, as well as other minerals like as calcium. This suggests that dietary elements and the health of Guinea fowl may have an influence on each other (Latic & Erben, 2020).

2.11 Haematological Parameters as Indicators of Calcium Status in Birds

Haematological markers, including serum calcium concentration, parathyroid hormone levels, and alkaline phosphatase activity, can serve as indicators of calcium status in avian species. Multiple research has been undertaken to examine the haematological parameters as markers of calcium status in avian species. Ricardo de Matos (2008) conducted a study that offers comprehensive information on the calcium levels in birds, specifically focusing on the use of haematological markers as indicators. The following are the main discoveries about haematological measures as indications of calcium status in birds: It is important to carefully assess the measurement of blood calcium concentration, as normal levels of total calcium within the reference range for a particular species may not correctly indicate calcium problems. In the field of small animal clinical pathology, the calcium concentration can be corrected based on the total protein or albumin concentrations if these values are aberrant, using established methods. Several bird species have been studied to determine the correlation between plasma total calcium concentration and total protein and albumin concentrations, yielding varying outcomes.

The measurement of ionized calcium has been established as a crucial method for studying calcium problems in birds, particularly when there are aberrant levels of proteins in the blood. Ionized calcium levels remain unaffected by changes in protein binding, rendering them a more accurate indicator of an individual's calcium status. This is particularly true in situations involving aberrant protein concentration and imbalances in metabolites or electrolytes. These findings emphasize the intricacy of assessing the calcium status in avian species. Stanford (2006) conducted a study on birds and found that haematological

measures, such as total calcium levels, ionized calcium concentrations, and vitamin D metabolites, can be useful indicators of calcium status in birds. It is crucial to monitor the amounts of ionized calcium in the blood as it helps evaluate the active form of calcium that plays a role in several physiological processes such as bone metabolism, muscle function, and nerve transmission. Alterations in overall protein and albumin concentrations can impact the interaction of calcium in the bloodstream, hence influencing the overall assessment of calcium levels. Thus, directly evaluating the levels of ionized calcium offers a more precise indication of the bird's calcium condition. Measurement of vitamin D metabolites, such as 25-hydroxycholecalciferol, in the blood can be used to assess the vitamin D levels and its effects on calcium metabolism in birds.

By integrating haematological data with clinical signs and food history, veterinarians and poultry farmers may evaluate the calcium status of birds and detect possible deficiencies or imbalances that could affect bird health and performance. It is crucial to regularly evaluate haematological markers connected with calcium metabolism in birds to promptly identify hypocalcemia or vitamin D deficits. This enables timely intervention and care to prevent related health problems. Haematological evaluations, in conjunction with dietary modifications and supplementation techniques, are essential for maintaining adequate calcium levels and promoting general health and production in avian species. The results emphasize the importance of haematological markers as valuable indicators of calcium levels in birds. This highlights the need for regular monitoring and suitable interventions to maintain normal calcium metabolism and overall well-being in avian populations.

Rao et al. (2003) primarily focused on identifying the ideal amounts of calcium for commercial broilers and White Leghorn layers. They did not particularly address haematological markers as indications of calcium status in birds. Nevertheless, there are certain overarching observations regarding haematological markers that are frequently employed as indications of calcium status in avian species. Measuring the content of calcium in the blood is a widely used approach to evaluate the calcium levels in birds. Low serum calcium levels may indicate a deficiency, whilst high levels could indicate an excessive consumption of calcium. Measuring serum phosphorus levels in addition to calcium levels can offer valuable information on the equilibrium of both elements inside the body. Discrepancies in the levels of calcium and phosphorus can have an impact on the health of bones and the general metabolism of minerals. Alkaline phosphatase is an enzyme involved in the process of bone mineralization. Fluctuations in serum alkaline phosphatase activity can suggest changes in bone metabolism and the utilization of calcium. Parathyroid hormone has a role in the regulation of calcium and phosphorus concentrations in the bloodstream. Deviation in PTH levels can suggest disruptions in calcium balance. Evaluating the bone mineral density, bone turnover indicators, and bone strength can offer significant insights on the calcium status and skeletal well-being of birds.

In a study conducted by Ansar et al. (2004), the researchers examined how the ratio of calcium and phosphorus affects the haematological parameters of birds. Notable discoveries include: Birds that were given a meal with a high ratio of calcium to phosphorus had noticeably higher levels of calcium in their blood. Birds that were given a

diet with a higher calcium ratio showed dramatically elevated levels of phosphorus in their blood serum, suggesting that a diet with a higher calcium ratio leads to hypophosphatemia. These findings indicate that imbalances in the dietary supply of calcium and phosphorus can cause alterations in the levels of these elements in the bloodstream. This highlights the significance of maintaining a proper ratio of calcium and phosphorus for the health of the birds.

Xing et al. (2019) conducted a study to examine the impact of various calcium sources and amounts on haematological markers in broiler chickens, which serve as indicators of calcium status. The results showed that the kind and amount of calcium did not have a significant influence on the blood parameters, indicating that these factors may not have a major effect on the birds' calcium levels as measured by blood parameters.

2.12 Influence of Calcium Levels on Carcass Characteristics

Calcium levels have an impact on various aspects of poultry carcass, including meat yield, muscle composition, and bone mineralization. Optimal calcium levels facilitate the growth of lean muscle and the formation of robust bones. Abdulla et al. (2017) conducted a study to examine how different levels of dietary calcium affect the physical properties of grill chickens' carcasses. The following are the main findings about the impact of calcium levels on the physical attributes of poultry carcasses. Birds that were given diets with 1.50 % calcium had a reduced body weight in comparison to those that were given diets with 1.00 % and 1.25 % calcium levels. Elevating the calcium concentration to 1.25 % enhanced bone quality irrespective of the oil source employed in the diet. Various calcium levels in

the diet did not have a significant impact on the proportions of commercially critical carcass sections, including dressing percentage, leg percentage, and breast percentage. These data indicate that the amount of calcium in the food can affect the weight of the body, the quality of the bones, and even the qualities of the carcass in grill chickens. Manipulating dietary calcium levels can be a viable approach to enhance growth performance and improve bone quality in poultry farming.

Driver et al. (2006) conducted a study to examine the impact of calcium levels on carcass characteristics in poultry. Their findings showed that feeding broiler chickens calcium- and phosphorus-deficient diets during the starter and grower-finisher phases had negative effects on the integrity of various bones during slaughter and processing. The calcium and phosphorus levels in the initial diet affected the durability of long bones, such as the tibia and femur, in resisting fractures. On the other hand, the food given during the later grower-finisher phase impacted the occurrence of broken clavicles. Strong correlations were seen between the proportion of tibia ash at 18 days and the occurrence of fractured tibias and femurs at 36 days, suggesting that early bone mineralization can serve as a predictor of bone strength during processing. Broilers that were given diets lacking in calcium and phosphorus without any additional supplements, showed inadequate bone mineralization, leg issues, and a high frequency of bone fractures following processing. The levels of ash in the tibia were found to be directly associated to the occurrence of fractures in both the tibia and femur bones, as well as the presence of bloody breast meat. This emphasizes the significance of maintaining appropriate levels of calcium and phosphorus for ensuring high-quality carcasses. These findings highlight the crucial significance of the quantities of

calcium and phosphorus in the diet in affecting the strength of bones, quality of carcasses, and outcomes of processing in chicken production.

2.13 Influence of Calcium Levels on Bone Quality in Poultry

Calcium obtained from the diet is essential for the formation and upkeep of strong and healthy bones in chickens. Sufficient calcium consumption guarantees optimal bone development, calcification, and resilience against fractures. The impact of calcium levels on bone quality in poultry has received considerable study in recent years. Xing et al. (2019) conducted a study to investigate the impact of various calcium levels on bone quality in poultry. The study revealed that dietary calcium levels had a substantial influence on the bone quality of broiler chickens. Elevated calcium levels were linked to enhanced bone mineralization and growth, whereas calcium insufficiency led to skeletal deformities, rickets, tibial dyschondroplasia, bone fractures, neurological weakness, and suboptimal feather condition in chicken.

Various calcium sources were also discovered to have an impact on bone mineralization and growth. For instance, calcium carbonate is found in both limestone and oyster shell. However, in limestone, it is inorganic and originates from calcite, whereas in oyster shell, it is organic and originates from marine sources. The variation in calcium supplies can impact the quality of eggshells, the process of bone mineralization, and the growth of poultry. The study also suggested that the particle size and solubility of calcium sources could affect bone health, as well as the effectiveness of calcium supplements in chickens. According to Reynolds and Christopher (2010), the impact of calcium levels on bone

quality in chicken is a crucial determinant of their reproductive success and overall health. These are the main conclusions derived from their research. The availability of calcium is essential for the process of egg formation. Prior to egg laying, birds require higher levels of dietary calcium, and the addition of calcium supplements can greatly influence their breeding effectiveness. When there is a shortage of calcium, the preservation of skeletal strength is prioritized over the needs for reproduction. Insufficient calcium availability can lead to birds stopping egg production before their bone strength is affected. Avian species experiencing calcium stress may produce eggs with atypical shells as a result of restricted calcium accessibility. These factors can ultimately result in the inability to reproduce, abandonment of nests, and failure to incubate eggs that are not viable. Examination of bone structure in laying birds at a microscopic level shows significant alterations in the types of bone cells and tissues. The bone composition of laying birds undergoes substantial variations over time after laying, which correspond to the fluctuations in calcium requirements during the process of egg production. Research has indicated that providing birds with calcium supplements can enhance their reproductive success, especially in regions where calcium resources are scarce. Nevertheless, in regions where birds acquire adequate calcium for the production of eggs without the need for additional supplements, the consequences may be insignificant. The findings emphasize the pivotal significance of calcium in avian reproductive success and underscore the necessity of comprehending and controlling the dietary calcium needs of breeding birds.

Gautier et al. (2017) conducted two trials to explore the impact of calcium levels on bone quality in poultry. The results indicate that the proportion of dietary calcium (Ca) to non-

phytate phosphorus (NPP) may have a greater impact than the individual levels of minerals when creating diets for chicken. Experiment 1 demonstrated that as the dietary Ca concentration increased while keeping the NPP content constant, there were consistent decreases in both overall growth performance and tibia ash. Furthermore, the observed retention of phosphorus (P) and calcium (Ca) decreased in birds that were given dietary treatments with calcium concentrations higher than 0.6 %. In Experiment 2, the apparent retention of P reduced for birds fed 0.8 % NPP when the Ca and NPP concentrations were varied to maintain a constant 2:1 ratio. However, P retention increased for birds fed 0.2 % NPP compared to other dietary treatments. Higher calcium concentrations led to a reduction in calcium apparent retention compared to birds fed 0.4 % calcium, regardless of the NPP concentration. The results emphasize the significance of the calcium-to-non-phytate phosphorus ratio in affecting the process of bone mineralization and the overall quality of bones in chicken. According to the research, maintaining a proper balance between dietary calcium (Ca) and non-phytate phosphorus (NPP) is crucial for achieving good bone mineralization and growth performance in grill chickens.

2.14 Influence of Calcium Levels on Lipid Profile

The lipid profile in poultry can be influenced by calcium levels, which impact the metabolism and storage of fats in the body. Sufficient calcium consumption can assist in controlling cholesterol levels and encouraging a beneficial lipid profile. Multiple studies have been undertaken to ascertain the impact of calcium levels on the lipid profile in chicken. The research conducted by Abdulla et al. (2015) examined the impact of varying doses of dietary calcium on the lipid profile of grill chicken breast muscle. The following

are the main discoveries about the impact of calcium levels on the lipid profile in poultry. The addition of palm oil (PO), soybean oil (SO), and linseed oil (LO) resulted in an elevation in the levels of oleic, linoleic, and α -linolenic acids, respectively, in the breast muscle of broiler chickens. The cholesterol concentrations of birds that were given palm oil (PO) were higher than those of birds that were given linseed oil (LO) and soybean oil (SO). Nevertheless, the levels of cholesterol in the fowl were within the standard limit. The polyunsaturated fatty acid (PUFA) to saturated fatty acid (SFA) ratio was elevated in the meat of broiler chickens that were fed with soybean oil (SO) and linseed oil (LO). This could perhaps account for the observed reduction in cholesterol levels. Modifying the content of polyunsaturated fatty acids (PUFAs) in poultry science while maintaining product quality is a difficulty. To enhance the n-3 PUFA content of meat and eggs, it is necessary to provide extra antioxidant protection. It has been proposed that using vegetable oils that are high in natural antioxidants is a useful method for managing the breakdown of fats after death and improving the nutritional value of human meals. The industry relies on methods that are both effective and safe, as well as cost-efficient, to control the storage stability of poultry meat. Introducing natural antioxidants into the diet can effectively manage the formation of lipid oxidation byproducts and offer nutritional options for health-conscious individuals (Ghosh et al., 2022). These findings emphasize the significance of considering the levels of calcium in the diet along with various oil sources to enhance the fatty acid composition, cholesterol levels, and total lipid profile in poultry meat.

In a similar vein, Alagawanya et al. (2021) examined the effects of different amounts of dietary calcium and phosphorus on the lipid profile, antioxidant capacity, and immune

parameters in growing Egyptian geese. Their findings shed light on the impact of calcium levels on the lipid profile of poultry. The following are the main discoveries on the influence of calcium levels on the lipid profile. Adding 0.85 % calcium to the diet resulted in a notable reduction in triglyceride levels compared to diets with lower calcium levels (0.70 %). The study found that incorporating 0.85 % calcium into the diet resulted in a notable reduction in total cholesterol levels, as compared to diets with lower calcium levels. The addition of 0.85 % calcium to the diet resulted in a considerable reduction in LDL-cholesterol levels. The study determined that the levels of HDL-cholesterol and VLDL-cholesterol were not significantly impacted by changes in dietary calcium intake. The results indicate that increased dietary calcium levels, particularly at a concentration of 0.85 %, can have a positive effect on the lipid profile of chicken. This leads to reduced levels of triglycerides, total cholesterol, and LDL-cholesterol.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location and Duration of the Study

The study was carried out in the Animal Science Department of the College of Agriculture Education at the Mampong-Ashanti Campus of the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development. The research period extended for about two months, from August 31st to October 19th, 2023. This location in the forest transitional zone provides an ideal environment for studying the effects of different quantities of dietary calcium on helmeted Guinea fowl, taking into account the area's climate and ecological features. The designated timeframe permits thorough data gathering and observation of the physiological reactions and carcass traits of the birds subjected to various calcium feeding regimens within this particular geographical setting.

The Mampong Municipality is categorized as one of the Municipal Assemblies located in the Ashanti Region of Ghana. The establishment of this designation occurred with the partition of the former Sekyere West District into two distinct entities: The Mampong Municipal and the Sekyere Central District. The division was formally established by the adoption of Legislative Instrument (L.I.) 1908. The municipal is bounded by the Sekyere South district to the south, Sekyere Central to the east, and Ejura Sekyedumase districts to the north. Mampong serves as the administrative hub of the Municipality. The place is positioned within the longitudes of 0005 W and 1030 W, and the latitudes of 6055 N and 7030 N.

The Municipality has an area of approximately 23.9 square kilometres. The Municipality has numerous notable towns, including Mampong, Krobo, Dadease, Asaam, Kofiase, and Adidwan.

The Municipality experiences an average annual precipitation of 1,270 mm, characterized by the occurrence of two distinct rainy seasons. The main rainy season begins in March and concludes in August, while the secondary rainy season extends from September to November. The following months correspond to the duration of the Harmattan dry season. The average annual temperature is 27 °C, with monthly fluctuations in the average temperature ranging from 22 °C to 30 °C (Ghana Statistical Service, 2020).

3.2 Experimental Birds and Management

The investigation involved the use of 150 unsexed day-old Guinea keets. During the early brooding stage, the birds were given an unlimited amount ("ad libitum") of a typical beginning food and were able to continuously drink clean, fresh water. When the Guinea keets were around two weeks old (16 days), they were weighed and then randomly placed in 15-floor pens. Each pen had a size of 2.24 square meters. The pens were divided into 5 replicates per treatment, with each replicate containing 10 birds. In order to ensure sufficient nourishment and hydration, feeding and watering troughs were installed in every enclosure. This management arrangement guaranteed consistency and appropriate attention for the Guinea keets during the entire duration of the experiment.

3.3 Dietary Treatments and Experimental Design

The birds were assigned to three treatments with pure varying calcium concentrations (0.36 %, 0.6 %, and 0.9 %) and there were five replications for each treatment. The different experimental coops were categorized based on their dietary treatment and replication. The experiment consisted of three treatments: Treatment 1, Treatment 2, and Treatment 3. In T1, there were 10 birds per replicate with a calcium level of 0.36 %, resulting in a total of 50 birds for this treatment. In T2, there were also 10 birds per replicate, but with a calcium level of 0.6 %, resulting in a total of 50 birds. Finally, in T3, there were 10 birds per replicate with a calcium level of 0.9 %, resulting in a total of 50 birds for this treatment. The experiment employed a Completely Randomized Design (CRD). The diets were provided freely and without restriction for the whole 10-week course of the experiment. Throughout the investigation, the Guinea fowls were provided with the diets in a mashed condition. The composition of the experimental diets has been computed and is provided in Table 3.1.

Table 3.1: Composition and calculated analysis of experimental diets, %

Ingredient	Treatment 1	Treatment 2	Treatment 3
Maize	65	65	66
Soybean meal	15	15	14
Wheat bran	3	2.29	1
Fishmeal	16	16	16
Dicalcium phosphate	0	0.214	0.1
Salt	0.5	0.5	0.5
Oyster shell	0	0.5	1.3
Premix	0.5	0.5	0.5
Total	100	100	100
Nutrient Composition			
Crude Protein %	21.37	21.27	21.02
Calcium %	0.36	0.6	0.9
Available P	0.45	0.48	0.45
ME, Kcal/Kg	3015.5	3004.79	3010.3
Methionine	0.48	0.48	0.48
Lysine	1.39	1.39	1.38
Sodium	0.36	0.35	0.35
Chlorine	0.31	0.31	0.31

Vitamin A, 8,000,000 IU; Vitamin B1, 1300 mg; Vitamin B2, 2500 mg; Vitamin D3, 3000 IU; Vitamin E, 10, 000 IU; Vitamin K3, 1,500 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mg; Nicotinic Acid, 5,000 mg; Pantothenic Acid, 4000 mg; Choline Chloride, 8000 mg; Copper, 2,500 mg; Cobalt, 700 mg; Iron, 4,500 mg; Zinc, 55, 000 mg; Methionine, 50,000 mg; Lysine, 200,000 mg; Selenium (1%), 1,300 mg; Iodine, 2,000 mg; Manganese, 60, 000 mg; Antioxidant, 625 mg.

3.4 Parameters Measured

Parameters measured include: Growth performance, haematology, lipid profile, gastrointestinal pH, carcass characteristics and bone-breaking strength.

3.4.1 Growth Performance

Body weight, gain, and livability were calculated weekly. The body weight was determined by dividing the cumulative pen weight by the number of birds in the pen. The gain was calculated as the difference between the birds' body weight and their initial body weight. Livability was calculated by dividing the number of birds by the initial total number of birds and multiplying by a factor of 100. The pens were monitored for mortality twice daily and post-mortem examinations were conducted on the dead birds throughout the study period.

3.4.2 Haematological Analysis

A total of five birds from each treatment group were selected for the haematological investigation. A 25-gauge needle was utilized to extract 2ml of blood from the wing vein of each chosen Guinea fowl following the disinfection of the area by swabbing it with 70 percent alcohol. The needle was inserted into the vein in the same direction as the blood flow. The blood was placed in a vacuum-sealed tube with an anticoagulant and then transported to the laboratory for analysis.

The full blood count (FBC) was analyzed using a fully automated BC 5800 haematology equipment with five differentials, manufactured by Mindray in Germany, following the instructions provided by the manufacturer. The principles utilized in this study involved the impedance method for red blood cell (RBC) and platelet (PLT) counting, a cyanide-free reagent for haemoglobin testing, and a combination of flow cytometry (FCM), laser light scatter, and chemical dye method for white blood cell (WBC) differential analysis and

WBC counting. The haematological indices that were evaluated consisted of red blood cell (RBC) count, haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), and platelet distribution width (PDW). The leukocyte indices encompass the levels of white blood cells (WBC), as well as the percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

3.4.3 Lipid Profile

The hepatic enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and Gamma glutamyl transferase (γ GT), were measured using the Liver function test (LFT) on a Mindray BS 130 fully automated blood chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Germany), following the instructions provided by the manufacturer.

The enzymatic, metabolic, and blood electrolyte levels were evaluated by conducting a lipid profile test and analyzing the electrolytes using the Mindray BS 130 fully automated blood chemistry analyzer. The parameters tested included lipids (total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL)); and electrolytes: sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), chloride (Cl^-), magnesium (Mg), bicarbonate (HCO_3^-), and phosphorus (P). The metabolic parameters, NEFA and β HBA, were assessed using a colorimetric reaction

as per the manufacturer's instructions. Colorimetric Kits were used, and measurements were taken using a fully-automatic biochemistry analyzer (Kovacevic et al., 2016).

3.4.4 Gastrointestinal pH

Following the cervical head dislocation of 2 birds per replicate pens, they were immediately dissected. The pH levels of the crop, proventriculus, gizzard, duodenal, jejunal, ileal, and cecal segments were measured using a pH tester (Hanna instruments, UK). The pH tester was inserted directly into the digesta in the lumen of the proximal end of each segment of the same bird, while ensuring that the pH electrode did not touch the walls, as described by Zanu et al. (2020). After collecting readings for each bird, the probe was washed with distilled water. The average of the two readings per section of the tract was subsequently computed.

3.4.5 Bone Breaking Strength

At day 34 and 77 after hatching, the femur and tibiae were obtained from the right leg of the selected birds used for measuring gut pH and carcass properties. These bone samples were analyzed to assess their weight, size, and breaking strength. The femur and tibia dimensions were calculated by measuring the length (in millimeters) from the proximal end to the distal end and the breadth (in millimeters) at the medial area using a digital caliper. To determine the breaking strength (BS), the flesh was removed from the femur and tibiae manually using a knife. The femur and tibiae obtained were subsequently tested using a universal texture analyzer (Inspekt table50-1, Hegewald & Peschke, Meß-Germany) equipped with a 50 KN load cell and a 3-point fixture bed. The testing was

conducted at a speed of 10 data points per second. The BenQ computer, equipped with a 24 Inch IPS monitor, operated the machine using Blue Hill 3 software.

3.4.6 Carcass Assessment

The liver, breast, thigh, heart, and empty gizzard were removed from the birds that were sampled. These organs were then weighed and their weights were expressed as a percentage of the overall body weight.

3.5 Statistical Analysis of Data

The data obtained from the experiment was analyzed using the General Linear Models (GLM) approach in Minitab 21.1. The Tukey's mean separation test was employed to compare the means of different treatments, with a significance level of $P < 0.05$.

CHAPTER FOUR

RESULTS

Table 4.1: Effects of different calcium dietary levels on growth performance of Guinea fowl at d 28

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Body weight, (g)	168.4	168.0	169.6	4.080	0.959
Gain, (g)	38.44	40.00	39.95	3.430	0.936
Livability, %	96.00	100.0	97.50	2.020	0.397

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

Table 4.2: Effects of different calcium dietary levels on growth performance of Guinea fowl at d 35

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Body weight, (g)	214.2	218.0	211.1	4.480	0.566
Gain, (g)	84.22	90.00	81.41	3.640	0.273
Livability, %	96.00	100.0	97.50	2.020	0.397

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

Table 4.3: Effects of different calcium dietary levels on growth performance of Guinea fowl at d 42

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Body weight, (g)	264.4	269.6	263.4	5.650	0.719
Gain, (g)	134.4	141.6	133.7	4.980	0.491
Livability, %	96.00	98.00	97.50	2.330	0.821

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

Table 4.4: Effects of different calcium dietary levels on growth performance of Guinea fowl at d 49

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Body weight, (g)	337.1	333.1	330.8	8.320	0.864
Gain, (g)	207.1	205.1	201.1	8.090	0.869
Livability, %	96.00	96.00	97.50	2.470	0.885

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

Table 4.5: Effects of different calcium dietary levels on growth performance of Guinea fowl at d 56

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Body weight, (g)	437.1	417.9	432.3	8.090	0.869
Gain, (g)	307.1	289.9	302.6	14.30	0.682
Livability, %	76.00	76.00	76.06	2.810	1.000

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

Table 4.6: Effects of different calcium dietary levels on growth performance of Guinea fowl at d 63

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Body weight, (g)	491.0	472.0	486.0	8.090	0.895
Gain, (g)	385.0	344.0	356.0	14.30	0.695
Livability, %	76.00	74.00	73.83	2.980	0.851

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

Table 4.7: Effects of different calcium dietary levels on growth performance of Guinea fowl at d 70

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Body weight, (g)	553.3	569.1	564.2	8.817	0.897
Gain, (g)	422.9	412.7	434.6	15.50	0.618
Livability, %	76.00	74.00	69.83	2.610	0.271

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

Table 4. 8: Effects of different calcium dietary levels on growth performance of Guinea fowl at d 77

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Body weight, (g)	644.7	650.2	650.6	15.30	0.955
Gain, (g)	514.7	522.2	520.9	15.90	0.938
Livability, %	76.00	74.00	69.83	2.610	0.271

Ca 0.36 %.; Ca 0.6 %; Ca, 0.9 %.; SEM, Standard error of mean

4.9: Effects of Different Calcium Dietary Levels on Growth Performance of Guinea Fowl from D 28 to D 77

The findings regarding the impact of different calcium levels on the growth performance of Guinea fowls from table 4.1 to table 4.8 suggest that there were no significant variations ($p > 0.05$) in growth performance from day 28 to day 77 across the entire duration of the experiment.

Table 4.10: Effects of different calcium dietary levels on the gastrointestinal pH of Guinea fowl at d 77

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Crop	4.901	5.216	4.989	0.314	0.770
Proventriculus	1.158	1.150	1.079	0.056	0.565
Gizzard	1.850	1.684	1.797	0.101	0.513
Duodenum	5.452	5.367	5.366	0.124	0.855
Ileum	6.658	6.642	6.881	0.106	0.244
Jejunum	5.511	5.580	5.673	0.083	0.407
Caecum	6.623	6.420	6.619	0.085	0.194

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

4.2: Effects of Different Calcium Dietary Levels on Gastrointestinal pH of Guinea Fowl at D 77

The data regarding the impact of experimental diets on the gastrointestinal pH of Guinea fowl suggest that there were no notable variations ($p > 0.05$) in the pH values of the gastrointestinal segments among the three experimental diets during the whole experimental period.

Table 4.11: Effects of different calcium dietary levels on carcass characteristics of Guinea fowl, % of BW at d 77

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Heart	3.800	3.500	4.100	0.289	0.370
Liver	11.40	10.20	12.00	0.935	0.410
Gizzard	20.00	17.30	18.20	1.20	0.306
Breast	29.70	27.10	24.80	2.23	0.331
Thigh	57.50	55.10	53.80	3.32	0.732

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

4.3: Effects of Different Calcium Dietary Levels on Carcass Characteristics of Guinea Fowl at D 77

The data on the effects of experimental diets on carcass characteristics of Guinea fowl indicate that there were no significant differences ($p > 0.05$) in the carcass characteristics among the three experimental diets throughout the experimental period.

Table 4.12: Effects of different calcium dietary levels on bone traits of Guinea fowl at d 77

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Tibial weight (g)	2.415	2.365	2.426	0.181	0.968
Femur weight (g)	2.170	2.060	2.170	0.169	0.869
Tibial length (mm)	62.61	61.91	62.08	1.76	0.959
Tibial width (mm)	3.693	3.586	3.724	0.179	0.851
Femur length (mm)	49.95	49.14	49.57	1.270	0.903
Femur width (mm)	4.054	3.946	3.706	0.157	0.311
Tibial BS (N)	63.90	63.75	63.60	8.490	1.000
Femur BS (N)	54.20	69.20	63.00	7.860	0.425

Ca 0.36 %; Ca 0.6 %; Ca 0.9 %; SEM, Standard error of mean

4.4: Effects of Different Calcium Dietary Levels on Bone Traits of Guinea Fowl at D 77

The data on the effects of experimental diets on bone traits of Guinea fowl indicate that there were no significant differences ($p > 0.05$) in bone traits among the three experimental diets throughout the experimental period.

Table 4.13: Effects of different calcium dietary levels on haematological parameters of Guinea fowl at d 77

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
WBC (10 ⁹ /L)	135.1	138.8	130.2	6.470	0.651
LYM (10 ⁹ /L)	103.5	104.8	102.5	3.110	0.872
MID (10 ⁹ /L)	13.86	15.22	13.66	2.170	0.859
GRA ((10 ⁹ /L))	17.78	18.79	14.46	4.880	0.810
LYM (%)	76.90	76.26	79.54	3.910	0.824
MID (%)	10.16	10.72	9.980	1.060	0.878
GRA (%)	12.94	13.02	10.48	2.920	0.787
RBC (10 ¹² /L)	2.300	2.502	2.348	0.140	0.582
HGB (g/dL)	12.62	11.80	13.72	1.380	0.627
HCT (%)	32.14	34.82	33.32	1.770	0.527
MCV (fL)	140.0	139.5	142.1	2.190	0.682
MCH (pg)	55.32	55.80	58.46	1.890	0.470
MCHC (g/dL)	39.48	39.98	41.18	1.070	0.532
RDW-SD (fL)	76.78	81.84	82.30	4.030	0.572
RDW-CV (%)	12.58	13.34	12.88	0.165	0.687
PLT (10 ⁹ /L)	8.400	8.000	7.800	1.240	0.941
MPV (fL)	5.600	5.580	5.540	0.209	0.979
PDW (%)	10.84	10.72	10.32	0.827	0.898
PCT (%)	0.005	0.004	0.004	0.001	0.826
P-LCR (%)	0.480	0.280	0.640	0.174	0.371

WBC (White Blood Cells), LYM (Lymphocytes), MID (Mid-sized Cells, including monocytes and some immature cells), GRA (Granulocytes), RBC (Red Blood Cells), HGB (Hemoglobin), HCT (Hematocrit), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), RDW-SD (Red Cell Distribution Width - Standard Deviation), RDW-CV (Red Cell Distribution Width - Coefficient of Variation), PLT (Platelets), MPV (Mean Platelet Volume), PDW (Platelet Distribution Width), PCT (Plateletcrit), P-LCR (Platelet Large Cell Ratio).

Ca 0.36 %; Ca 0.6 %; Ca 0.9 %; SEM, Standard error of mean; RBC, red blood cell; WBC, white blood cell; HGB, haemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration

4.5: Effects of Different Calcium Dietary Levels on Haematological Parameters of Guinea Fowl at D 77

The data regarding the impact of experimental diets on the haematological parameters of Guinea fowl suggest that there were no notable variations ($p > 0.05$) in these parameters across the three experimental diets during the entire duration of the experiment.

Table 4.14: Effects of different calcium dietary levels on lipid profile of Guinea fowl at d 77

Parameter	Ca, 0.36 %	Ca, 0.6 %	Ca, 0.9 %	SEM	P-Value
Total cholesterol (mmol/L)	5.470	5.012	5.876	0.430	0.393
Triglycerides (mmol/L)	1.252	1.362	1.494	0.171	0.618
HDL-cholesterol (mmol/L)	1.598	1.536	2.012	0.172	0.147
LDL-cholesterol (mmol/L)	3.302	2.858	3.182	0.394	0.719
VLDL-cholesterol (mmol/L)	0.570	0.618	0.680	0.078	0.618
Coronary risk (mmol/L)	3.440	3.300	3.100	0.332	0.772

Ca 0.36 %.; Ca 0.6 %; Ca 0.9 %.; SEM, Standard error of mean

4.6: Effects of Different Calcium Dietary Levels on Lipid Profile of Guinea Fowl at D 77

The data on the effect of experimental diets on the lipid profile of Guinea fowl indicate that there were no significant changes ($p > 0.05$) in these parameters across the three experimental diets over the whole study period.

CHAPTER FIVE

DISCUSSION

5.1 Effects of Experimental Diets on Growth Performance of Guinea Fowl

The results of this study revealed that varying dietary calcium levels had no significant effect ($p > 0.05$) on the growth performance of Guinea fowls from day 28 to day 77. This lack of significant variation suggests that Guinea fowls may tolerate a range of calcium levels without marked differences in body weight or weight gain over the observed period. These findings align with the observations of An et al. (2016) and Li et al. (2017), who reported that maintaining calcium within an optimal range did not significantly alter certain performance indicators in poultry. However, they contrast with studies by Talpur et al. (2012) and Gautier et al. (2017), which reported that varying calcium concentrations influenced growth performance, feed intake, and bone mineralization, particularly in broilers.

The disparity in findings across studies highlights the complex and species-specific nature of calcium utilization in poultry nutrition. While broilers may exhibit sensitivity to both deficient and excessive calcium intake—resulting in altered growth rates, carcass characteristics, and organ development (Xing et al., 2019). Guinea fowls in this study appeared less affected. Notably, improper calcium levels in chickens have been linked to skeletal deformities, reduced feed intake, and impaired nutrient absorption. Excessive calcium, for instance, may interfere with the uptake of other minerals such as phosphorus and magnesium, ultimately hindering growth (Gautier et al., 2017). Conversely,

insufficient calcium can impair bone development and productivity. Despite such potential impacts, the current findings suggest that Guinea fowls may possess a broader physiological tolerance for calcium fluctuations compared to broilers or layers. It is also possible that other dietary or environmental factors played a buffering role in minimizing the effects of varying calcium levels.

5.2 Effects of Calcium Levels on Gut pH

The data on the effect of different levels of dietary calcium (0.36 %, 0.6 %, and 0.9 %) on the gastrointestinal pH of Guinea fowl shows that there were no significant differences ($p > 0.05$) in the pH values across different parts of the digestive system among the three experimental diets during the entire experiment. Within the range of values studied, there is no substantial impact of calcium levels on the gastrointestinal pH of Guinea fowl.

Existing evidence indicates that dietary calcium has the potential to impact gastrointestinal health by altering the acidity levels of the digestive system, the makeup of intestinal microbes, and the immunological response in the gut. A study conducted by Whisner & Castillo (2017) suggests that prebiotics, which promote the growth of beneficial bacteria in the stomach, have the ability to affect mineral metabolism and bone health. This corroborates the notion that calcium's function is intricately linked to the gut microbiota. Skrypnik and Suliburska (2018) highlight the importance of studying the impact of dietary calcium on gut microbiota, as this connection can have an influence on mineral metabolism as a whole.

Research conducted by Bonjour (2011) and D'Amelio & Sassi (2017) highlights the essential significance of calcium in preserving bone health, as well as its possible indirect impacts on the immune system and gut flora. There is a connection between the health of the gut and the absorption of calcium. This implies that by improving the circumstances in the gastrointestinal system, calcium uptake can be increased, which in turn can strengthen the strength of bones. The research conducted by O'neil et al. (2012) focuses on the significance of calcium in the diet of humans, emphasizing its role in sustaining bone health. This finding is relevant to Guinea fowl, as calcium required in their diet to support their bone health. Latic and Erben (2020) explored the wider consequences of dietary constituents, such as vitamin D and probiotics, on health, implicitly endorsing the importance of a well-rounded diet that includes sufficient calcium.

The potential favourable effects of probiotics and prebiotics present in yoghurt and fruits, as seen by Fernandez and Marette (2017), indicate that these dietary elements may also have a good impact on the gastrointestinal health of Guinea fowl. However, this specific aspect was not specifically investigated in the current study. Although the experimental data showed that changing calcium levels does not have a substantial effect on the gastrointestinal pH of Guinea fowl, the wider body of research emphasizes the intricate and essential role of calcium in gut health and bone metabolism. The correlation between dietary calcium and gut microbiota, immunological response, and overall health has been well shown. Thus, although the pH findings are particular, it is crucial to prioritize sufficient calcium intake for the overall welfare of Guinea fowl, since it promotes gastrointestinal and bone health.

5.3 Effects of Calcium Levels on Carcass Characteristics

The results on the effects of experimental diets with varying calcium levels (0.36 %, 0.6 %, and 0.9 %) on the carcass characteristics of Guinea fowl indicate no significant differences ($p > 0.05$) throughout the experimental period. This finding suggests that within the tested calcium range, there is no notable impact on the carcass characteristics of Guinea fowl.

In comparison, literature indicates that calcium levels can significantly influence various aspects of poultry carcass, including meat yield, muscle composition, and bone mineralization. Optimal calcium levels are essential for the growth of lean muscle and the formation of robust bones. Abdulla et al. (2017) examined the effects of different dietary calcium levels on the physical properties of grill chickens' carcasses. Their study found that birds fed diets with 1.50 % calcium had reduced body weight compared to those on diets with 1.00 % and 1.25 % calcium levels. Increasing calcium to 1.25 % improved bone quality regardless of the oil source in the diet, though different calcium levels did not significantly affect commercially critical carcass sections like dressing percentage, leg percentage, and breast percentage. These results of Abdulla et al. (2017) suggest that dietary calcium can influence body weight, bone quality, and some physical carcass characteristics in grill chickens, highlighting the potential for manipulating dietary calcium to enhance growth performance and bone quality in poultry.

Driver et al. (2006) also investigated the impact of calcium levels on poultry carcass characteristics. Their findings showed that calcium- and phosphorus-deficient diets during the starter and grower-finisher phases negatively affected bone integrity during slaughter

and processing. The calcium and phosphorus levels in the initial diet significantly influenced the strength of long bones, such as the tibia and femur, making them more resistant to fractures. The grower-finisher phase diet impacted the incidence of broken clavicles. Strong correlations were observed between the proportion of tibia ash at 18 days and the occurrence of fractured tibias and femurs at 36 days, indicating that early bone mineralization is predictive of bone strength during processing. Broilers on calcium- and phosphorus-deficient diets without additional supplements showed inadequate bone mineralization, leg problems, and a high frequency of bone fractures post-processing. The tibia ash content was directly related to the occurrence of fractures in both tibia and femur bones and the presence of bloody breast meat, underscoring the importance of adequate calcium and phosphorus levels for high-quality carcasses.

5.4 Effects of Varying Dietary Calcium Levels on the Bone Traits of Helmeted Guinea Fowl

The findings suggest that Guinea fowls were able to maintain normal bone development regardless of the calcium level in their diet, as there were no significant differences ($p > 0.05$) in bone characteristics across the different dietary treatments. This indicates a possible broad tolerance for calcium levels in terms of bone health within the tested range. While this contrasts with results from some poultry studies where calcium levels significantly affected bone strength and mineralization, it may reflect species-specific differences or suggest that the calcium levels used in this study were all within a suitable range for Guinea fowl bone development.

On the other hand, literature emphasizes the crucial significance of dietary calcium in the creation and upkeep of robust and sound bones in chickens. Adequate calcium consumption guarantees proper bone growth, mineralization, and resilience against fractures. In their study, Xing et al. (2019) discovered a significant correlation between dietary calcium levels and bone quality in broiler chickens. They observed that higher levels of calcium in the diet improved bone mineralization and growth. Conversely, a deficiency in calcium resulted in skeletal deformities, rickets, tibial dyschondroplasia, bone fractures, neurological weakness, and poor feather condition. The study also observed that the origin of calcium, whether inorganic from calcite or organic from marine sources such as oyster shells, has an impact on the process of bone mineralization and growth. In addition, the size of particles and the ability to dissolve calcium sources have an impact on bone health and the effectiveness of calcium supplements in hens.

According to Reynolds and Christopher (2010), calcium levels play a vital role in the reproductive success and overall health of birds. Their study shown that adequate calcium availability is crucial for the process of egg creation, necessitating a higher intake of dietary calcium prior to the initiation of egg laying. Supplementing with calcium can greatly improve breeding success, whereas a lack of calcium can result in decreased egg production, weakened skeletal structure, and reproductive failures. It is crucial to comprehend and regulate the nutritional calcium requirements of breeding birds in order to guarantee reproductive success and preserve skeletal well-being.

In their study, Gautier et al. (2017) investigated the influence of calcium levels on the quality of bones in poultry using two separate trials. Their findings suggested that the proportion of calcium in the diet compared to non-phytate phosphorus (NPP) may have a greater impact than the specific amounts of these minerals. Elevating the levels of calcium in the diet, while maintaining a constant level of non-phytate phosphorus (NPP), resulted in a decline in growth performance and tibia ash, as well as a decrease in the retention of phosphorus and calcium. These results indicate that maintaining a suitable equilibrium between calcium and NPP is crucial for achieving good bone mineralization and growth performance in chicken. The study emphasized the importance of maintaining an appropriate calcium to net primary productivity (Ca: NPP) ratio for optimal bone health in hens.

In general, the experimental data on Guinea fowl did not reveal any significant variations in bone characteristics when calcium levels were altered. However, existing literature emphasizes the significance of sufficient calcium consumption and its equilibrium with phosphorus for maintaining good bone quality and overall health in poultry. These results indicate that while Guinea fowl may not show noticeable variations in bone characteristics within the calcium range that was studied, it is crucial to maintain proper amounts of calcium and phosphorus for optimal bone growth and overall health in poultry.

5.5 Effects of Varying Dietary Calcium Levels on the Hematological Parameters of Helmeted Guinea Fowl

The findings about the effects of experimental diets containing different levels of calcium (0.36 %, 0.6 %, and 0.9 %) on the blood-related parameters of Guinea fowl showed that there were no significant differences ($p > 0.05$) in these parameters among the three diets for the entire trial. Within the range of calcium levels studied, the haematological parameters of Guinea fowl remained steady, suggesting that shifting dietary calcium levels do not have a major influence.

On the other hand, the literature emphasizes the significance of haematological markers, such as blood calcium concentration, parathyroid hormone levels, and alkaline phosphatase activity, in avian species as indicators of calcium status. Ricardo de Matos (2008) highlighted the importance of doing a thorough evaluation of blood calcium levels, since he observed that readings within the normal range may not correctly reflect calcium-related issues. Ionized calcium, which is not influenced by alterations in protein binding, offers a more precise assessment of an individual's calcium level, especially in situations involving aberrant protein levels or imbalances in electrolytes.

Stanford (2006) emphasized the importance of using haematological measurements, such as total and ionized calcium concentrations and vitamin D metabolites, to evaluate the calcium status in birds. Monitoring ionized calcium is essential for assessing the active calcium involved in bone metabolism, muscle function, and nerve transmission. Changes in protein and albumin levels can influence the way calcium interacts in the blood, which

can have an impact on the overall assessment of calcium levels. Assessing vitamin D metabolites can offer valuable information on calcium metabolism, assisting in the assessment of calcium levels and informing dietary modifications and supplementation.

In their study, Gautier et al. (2017) showed that the proportion of calcium in the diet compared to non-phytate phosphorus (NPP) may have a greater impact than the specific quantities of these minerals. Their research revealed that higher quantities of calcium in the diet, while keeping the net protein production (NPP) constant, resulted in reduced growth performance and tibia ash. This emphasizes the significance of maintaining a balanced ratio of calcium to NPP for optimal bone health and overall growth performance. In their study, Alagawany et al. (2021) investigated the effects of different dietary calcium and phosphorus levels on the hematological parameters of growing Egyptian geese. They observed that geese fed diets with a higher calcium level of 0.85 % had significantly increased plasma calcium levels but decreased plasma phosphorus levels, an indication of hypophosphatemia. These findings emphasize the importance of maintaining an appropriate equilibrium of calcium and phosphorus to guarantee the well-being of birds.

In a study conducted by Xing et al. (2019), it was observed that manipulating the kind and quantity of calcium did not have a significant effect on haematological indicators in broiler chickens. This implies that these factors may not have a substantial impact on calcium levels as determined through blood parameters. This is consistent with the empirical evidence on Guinea fowl, suggesting that certain levels of dietary calcium do not have an impact on haematological markers.

In general, results on Guinea fowl did not reveal any significant differences in haematological parameters when exposed to varied calcium levels. However, existing literature highlights the significance of haematological markers in evaluating calcium status. Consistent surveillance of these indicators, in conjunction with suitable nutritional control, is essential for preserving calcium equilibrium and guaranteeing the general well-being and efficiency of avian species.

5.6 Effects of Varying Dietary Calcium Levels on the Lipid Profile of Helmeted Guinea Fowl

The results on the impact of experimental meals with different calcium levels (0.36 %, 0.6 %, and 0.9 %) on the lipid profile of Guinea fowl show that there were no significant changes ($p > 0.05$) in these parameters across the three experimental diets for the whole study period. Within the spectrum of dietary calcium levels studied, the lipid profile of Guinea fowl remained consistent, showing no noticeable effect from the various dietary treatments.

On the other hand, the literature emphasizes the impact of calcium levels on the lipid profile in poultry, providing insight into the complex connection between dietary calcium intake and lipid metabolism. The studies conducted by Abdulla et al. (2015) and Alagawanya et al. (2020) offer useful insights into the impact of different dosages of dietary calcium on the lipid profile of chicken.

Abdulla et al. (2015) investigated the influence of varying doses of dietary calcium on the lipid profile of grilled chicken breast muscle. Their investigation unveiled that the use of various oils, such as palm oil, soybean oil, and linseed oil, resulted in alterations in the fatty acid content in the breast muscle of grill chickens. Furthermore, there were changes in cholesterol levels, with birds who consumed palm oil showing elevated cholesterol concentrations in comparison to those that consumed linseed oil and soybean oil. The study also observed changes in the ratio of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs), which may impact cholesterol levels and the overall lipid profile.

In a similar vein, Alagawanya et al. (2021) examined the impact of varying doses of dietary calcium on the lipid profile, antioxidant capacity, and immunological markers in developing Egyptian geese. Their research showed that increasing the amount of calcium in the diet, specifically at a concentration of 0.85 %, led to notable decreases in triglyceride, total cholesterol, and LDL-cholesterol levels. The results emphasize the possibility of higher dietary calcium consumption to positively impact the lipid profile of chickens, resulting in improved cardiovascular health outcomes.

In summary, the results on Guinea fowl did not show any significant alterations in the lipid profile when calcium levels were varied. However, existing literature emphasizes the need to take into account dietary calcium intake along with other factors, such as oil sources, to maximize the fatty acid composition, cholesterol levels, and overall lipid profile in poultry meat. Additional research could investigate alternative dietary interventions or

combinations in order to gain a deeper understanding of and improve the lipid profile of Guinea fowl, leading to enhanced health and nutritional benefits.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

- The varying levels of dietary calcium (0.36 %, 0.6 %, and 0.9 %) had no significant effect on the growth performance of Guinea fowls.
- The varying levels of dietary calcium (0.36 %, 0.6 %, and 0.9 %) had no significant effect on the gastrointestinal pH and carcass characteristics of Guinea fowls.
- The study found no significant variations in the bone characteristics of guinea fowls across the different calcium levels (0.36 %, 0.6 %, and 0.9 %) tested.
- The hematological parameters of Guinea fowls remained unaffected by the varying levels of dietary calcium (0.36 %, 0.6 %, and 0.9 %).
- The lipid profile of Guinea fowls did not show any significant changes in response to the different dietary calcium levels (0.36 %, 0.6 %, and 0.9 %).

6.2 Recommendations

- Ensure Guinea fowls receive sufficient calcium to support overall health, despite no significant effects observed in the study parameters.
- Future research should include various nutrients and supplements along with calcium to potentially improve Guinea fowl health and performance.

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