Bone quality and incidence of urolithiasis in male broiler breeders fed a male or female ration

by

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Discipline of Animal Science

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PREFACE

The research in this thesis was completed by the candidate while based in the Discipline of Animal and Poultry Science, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

The contents of this work have not been submitted to another university, and except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Signed: Dr. N C Tyler (*Supervisor*)

Date: 9|2|2024

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DECLARATION 2: PUBLICATION/PRESENTATIONS

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Signed: Miss. Sithembisiwe Dube (Candidate)

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ABSTRACT

Separate sex feeding is highly practiced in breeder farms as it gives a more targeted approach to nutrition, aligning the dietary requirements with the specific needs of male and female birds. Some breeder farmers give the female ration to male breeders as it saves having two feed silos per house and eliminates the chances of females receiving the wrong feed. However, the drawback is that the female ration contains high crude protein (CP), calcium (Ca), and phosphorous (P), which is more than recommended for male requirements. A total of 40 male broiler breeders fed a male-specific ration (MM), and 40 male broiler breeders fed a female ration (MF) at depletion were acquired from KwaZulu-Natal farms. Kidney weight (KW) and kidney lesion score (KLS) from euthanised male broiler breeders were examined as an indication of urolithiasis. Tibia bone weight (BW), bone thickness (BT), bone breaking strength (BBS), tibia ash% (TA%), organic matter (OM%) and Ca/P% were quantified to assess the effects of excess CP, Ca and P on bone quality. Data collected were subjected to a two-sample t-test at a 95% confidence interval using GenStat statistical analysis software, and principal component analysis (PCA) was performed using XLSTAT. The biplots from the PCA were used to predict correlation among the variables. The study results showed that the kidney weights of MF were significantly larger compared to MM ($P<0.001$). Kidney lesion scores were observed in 50% MF and were significantly higher than in MM ($P<0.001$). Significant differences in BW, BBS, TA%, and OM% were found between MM and MF. Bone thickness, and bone Ca%, P% and Ca/P% were not significantly different among the two groups. The findings suggested that high CP, Ca and P in female feed given to male broiler breeders can negatively affect kidney and bone quality. The results indicated a significant correlation between dietary composition, bone strength and the incidence of urolithiasis. The study concluded that the nutritional composition designed for female broiler breeders may not be entirely suitable for the physiological needs of male breeders, contributing to an increased susceptibility to urolithiasis. Also, high Ca can interfere with the absorption or retention of Ca and other minerals like P, resulting in low bone quality.

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TABLE OF CONTENTS

Contents

LIST OF TABLES

LIST OF FIGURES

Chapter 1: General introduction

1.1 Background

The broiler breeder industry is a subsector of the poultry industry, and in South Africa, there are approximately 126 commercial broiler breeder farms compared to about 523 broiler rearing farms (SAPA, 2022a). The poultry industry is still the most significant contributor to the agricultural sector in South Africa, with 17.1% of the total agricultural gross value and 39.1% of animal product gross value being produced from poultry production in 2020 (SAPA, 2022b). Broiler breeding farms are essential to ensure a consistent supply of high-quality broiler chickens for the meat industry (Nkukwana, 2018). Breeder rearing requires breeding management, nutrition, and animal welfare expertise to maintain healthy and productive breeding flocks.

Although the sector has grown, it still faces economic losses due to disease and conditions affecting birds. For example, South Africa, among other countries, has reported an outbreak of avian influence that has resulted in the culling of over two million birds in 2023 (SADALRRD, 2023). In addition, non-infectious conditions caused by inadequate nutrition have led to metabolic disorders that concern the industry (Crespo and Shivaprasad, 2013). For the last 70 years, metabolic diseases have been a constant concern in chicken production, aggravated by tremendous gains in genetic potential for growth (Crespo, 2020). In poultry, metabolic diseases have been associated with excess or deficient nutritional intake and rapid growth rates that cause increased workload on organs or systems and result in organ failure (Julian, 2005a, Zuidhof et al., 2017). The focus of this thesis will be on nutritional factors that may affect male broiler breeders' health and welfare.

Broiler breeders are a specific type of chicken that is a parent or grandparent stock of broiler chickens raised for meat production (Riber, 2020). While commercial broilers are raised for rapid growth and quick market readiness, breeders are selected for their reproductive potential. They are reared until sexual maturity before being used for breeding (Van der Klein et al., 2018) and are typically raised on specialised breeding farms. Aviagen Breeders, Cobb-Vantress and Hubbard currently dominate the global market for broiler breeding stock (de Jong and van Emous, 2017). A parent stock life span is typically between 60 and 65 weeks, depending on flock productivity and market conditions (CobbVantress, 2021).

During the growing stage (0-21 weeks), male broiler breeders are reared separately from females to control skeletal growth and body weight (de Jong and van Emous, 2017, Leeson and

Summers, 2010). After 21 weeks of age, the breeders are then placed together throughout production at a recommended female/male ratio of 8 to 9% in houses with slats and 9 to 10% without slats (CobbVantress, 2021). A separate-sex feeding system is used to ensure males are excluded from accessing female feed using exclusion grids that are usually 46-50 mm in width and 60-70 mm in height. Male feeders are raised higher, approximately 50-60cm above litter, depending on the feeder system, to restrict females from accessing male feed (CobbVantress, 2021). This also allows different feed allocations for males and females, but also a different feed.

1.2 Calcium and phosphorus

Dietary calcium (Ca) and phosphorus (P) requirements for male broiler breeders vary depending on weight, age, production phase or specific management practices (Driver et al., 2005, Ceylan et al., 2020). Male breeder Ca requirements during breeding are recommended at 0.7% and available phosphorus (aP) at 0.35% compared to 3.2-3.4% Ca and 0.36% aP for female rations (Aviagen, 2021). According to Cobb-Vantress (2018), the recommended Ca and aP required for males during breeding is 0.95 and 0.42, respectively, compared to 3-3.2% Ca and 0.38-0.42% P for female rations. Calcium absorption and retention are highly influenced by P (Sun et al., 2018a); therefore, in most diets, the ratio of Ca:P is essential because an inadequate supply of either limits the utilisation of both in the body. The optimal Ca:P ratio generally recommended in poultry diets is around 2:1 (Matuszewski et al., 2020).

1.3 Protein

During the feeding programmes of a breeder flock, dietary protein is controlled to ensure or promote fleshing and fat deposition reserves (Wisaquillo, 2022a). Dietary specifications vary in breeder diets, and typically, male broiler breeders require more protein (about 19%) during the early stages of development and less protein (12 to 13%) as they mature (Cobb-Vantress, 2018, Aviagen, 2021). When Tyler et al. (2021) investigated the effect of feeding excess crude protein and high calcium (High CP (14.7%): High Ca (3.04%)) to male broiler breeders, results showed that a high protein diet caused a low sperm concentration at 49 weeks of age. In another study, breeders who received a high crude protein diet had a 9% higher ammonia emission and a 9.2% higher ammonia concentration in litter than birds fed a low-crude protein diet (van Emous et al., 2019). The author concluded that reducing the dietary calcium of both male and female broiler breeders decreases ammonia emission and total N losses from litter and manure. The quality and amino acid balance in protein diets is essential for growth and reproduction performance (Oviedo-Rondón et al., 2021).

1.4 Urolithiasis

The urinary tract has a vital role in the excretion of waste products, and the system mainly includes the kidneys, ureter and cloaca (Goldstein, 2022). Chicken kidneys are located near the dorsal abdominal cavity and are responsible for blood filtering, removing waste products and excess ions to form urine. Kidneys also facilitate Ca regulation as they excrete excess through the urine to balance vital minerals in the body (David et al., 2023). Nutritional imbalances, infectious diseases and toxins have shown to damage the kidneys, resulting in kidney failure and damage (Bulbule et al., 2014). One of the conditions that affect the urinary system that causes kidney dysfunction or damage is urolithiasis (Crespo and Shivaprasad, 2013).

Wideman (2016) defined urolithiasis as an acquired and degenerative kidney disease characterised by uroliths in the urinary system and focal mineralisation of the kidney. Urolithiasis is a common condition in chickens, particularly in commercial breeders, although most reports have been on layer hens (Lopes et al., 2021a, Wideman Jr et al., 1985, Wideman, 2016). Urolithiasis reports have been associated with nutritional imbalances (high calcium, Ca:P imbalances and high protein diets), infectious diseases (infectious bronchitis virus and avian nephritis virus), and toxins (Moyle et al., 2011, Lopes et al., 2021b). However, the prevalence of urolithiasis in chickens may vary depending on several factors, including sex, breed, age, diet, and management practices.

1.5 Bone quality

Furthermore, dietary Ca and P have been highly associated with bone properties and qualities (Abdulla et al., 2017, Mansilla et al., 2020). Improper Ca and P ratios in the diet have led to the incidence of leg abnormalities or problems and poor performance in breeding flocks. High Ca/P in the diet can result in insoluble Ca/P molecules and can affect Ca reserves and structural bone formation (Moreki, 2005). Excess dietary Ca interferes with Ca absorption, availability of other nutrients like Vitamin D_3 and feed intake (Matuszewski et al., 2020). Chung et al. (2019) discovered that the bone-breaking strength of male broiler breeders was increased (336,59 N) when 0.5% monocalcium phosphate (MCP)was added to the commercial feed compared to the birds given commercial feed with dicalcium phosphate (DCP) (Ca 0.85% and P 0.42%) (300,06 N). After doubling the monocalcium phosphate on a third treatment, the breaking strength decreased (256,20 N). Although the Ca and P in the ash increased, the author concluded that adding 1% MCP may affect Ca absorption. Studies on nutritional issues and mortalities in male broiler breeders are still limited compared to female broiler breeders.

1.6 Problem statement

Urolithiasis is a condition that has been defined as a condition of layers and pullets. Recent studies show that it can also affect broiler chicks and male broiler breeders. Urinary tract stones can be caused by a number of circumstances, including microbiological infections, genetic predispositions, environmental stresses, and unbalanced diets. In addition to causing discomfort and suffering for afflicted birds, urolithiasis also increase mortality rates, lowers fertility, and reduces the efficiency of meat production. Moreover, a thorough knowledge of the particular risk factors linked to urolithiasis in male broiler breeders is lacking, which makes it difficult to establish focused preventative measures and treatment plans. The majority of current research focuses on urolithiasis in other poultry species, overlooking the physiological and management aspects of male broiler breeders.

The poultry industry in South Africa has recently suffered from the avian influenza outbreak that has led to the culling of infected chickens by incineration. The implementation of the Poultry Master Plan, through the South African Poultry Association (SAPA) and the government, is helping to improve the situation. However, the local supply is still insufficient to meet the shortfall with the reduction in imports. Although the number of producers may increase, it is also essential to maximise the productivity of existing broiler breeder flocks to supply an adequate number of broilers to both smallholder and commercial farmers. In many countries, the poultry business is a source of income at an individual, household, and national level, and poultry meat is a cheap protein source for human consumption. Therefore, addressing health and nutrition factors that may decrease production is of utmost importance.

1.7 Justification

The recommended mating ratio for broiler breeders is one male to 8-10 females (8% to 10% of the flock). For practical reasons, many South African producers feed the ration formulated specifically for females to males. It saves having two feed silos per house, deliveries of 2 different feeds and the possibility of females being fed the male ration mistakenly – which would cause a drop in production due to the lower CP content of male diets. However, the drawback is that the ration contains CP and Ca/P excess to requirements. This study will investigate the incidence of urolithiasis, which may go unnoticed but potentially contribute to reduced fertility due to pain and discomfort, impacting mating behaviour or even mortalities. In addition, Ca/P deviation from the requirements may affect bone quality by increasing lameness and other skeletal conditions, which can greatly affect mating behaviour. The information will help make decisions regarding male broiler breeder feeding. Addressing this knowledge gap is essential for improving the overall health and productivity of male broiler breeders. By investigating the incidence, aetiology, and risk factors associated with urolithiasis in male broiler breeders, researchers can identify key interventions to mitigate the prevalence of urinary tract stones effectively. Additionally, understanding the genetic predispositions and metabolic pathways involved in kidney stone/ urolith formation can facilitate the development of breeding strategies aimed at reducing susceptibility to urolithiasis.

1.8 Aims and objectives

The primary aim is to contribute to research that promotes increased poultry production and food security by addressing dietary factors that affect bone quality and incidences of uroliths.

1.9 Research question

i. What effect does feeding a female ration have on the incidence of urolithiasis in male broiler breeders?
ii. Is the bone quality of males fed a female ration different from that of males fed a diet formulated to requirements?

1.10 Specific objectives

i. To assess urolithiasis incidences between male broiler breeders given a specific male ration and those given a female ration.
ii. To assess the bone quality (breakage strength, thickness and mineral content of Ca and P) in male broiler breeders given a male ration and those given a female ratio.

1.11 Dissertation structure

Chapter 1: Introduction
This chapter offers a brief overview of the current trends in the poultry industry and the nutritional and health aspects that affect chickens. The research aims, objectives, problem statement, and justification of the study are also outlined in this chapter.

Chapter 2: Literature Review
This chapter overviews urolithiasis, bone properties and bone quality parameters influenced by dietary Ca and phosphorous. It highlights the background and causes of urolithiasis as well as the effects of feeding excess Ca/P, as stated in previous studies.

Chapter 3: Assessing incidences of urolithiasis and bone quality on male broiler breeders fed a female or male ration. The chapter reports on the effects of feeding a female ration to male broiler breeders compared to a specific male ration.

Chapter 4: General Conclusion

This chapter provides a general discussion of the experimental chapter, a conclusion and recommendations for future research.

Chapter 2: Literature review

2.1 Introduction

Avian urolithiasis is a metabolic condition related to kidney dysfunction or failure and may cause mortality and reduced productivity in poultry (El Sebaei et al., 2021, Hicham et al., 2011). Ahmed et al. (2003) described urolithiasis as a renal condition that causes kidney asymmetry or atrophy and urolith deposits in the ureter. The uroliths found in poultry are composed of compact concentrations of calcium-sodium urates (Crespo and Shivaprasad, 2013) formed due to high calcium levels and decreased hydrogen ions in urine. Uric acid is a nitrogenous waste product excreted by birds, and when the renal system fails to remove the waste, it causes hyperuricemia, and the insoluble matter precipitates to form urate deposits (Sandhyarani et al., 2021). Therefore, urolith blockage in the ureter and progressive kidney damage may cause sudden death in poultry.

Incidences of uroliths in the poultry industry have been reported since the 1960s (Shane et al., 1968). Urolithiasis incidences have been reported in domestic fowls (Lakkawar et al., 2018), pullets and laying hens (El Sebaei et al., 2021) and male broiler breeders (Moyle et al., 2011). Chickens are more susceptible to urolithiasis because they are uricotelic as they lack the enzyme uricase (Gangwar et al., 2015). Urolithiasis has been shown to have seasonal variations in chickens, with high incidences in summer and winter compared to autumn (Lakkawar et al., 2018).

Considering that male birds are as important as female birds and contribute 50% of the genetic traits, it is crucial to investigate this potential adverse effect of feeding a female diet on male breeders in South Africa. Although feeding male broiler breeders a female ration has shown to have no effect on fertility (Tyler et al., 2021), other health factors should also be assessed. The information will help breeder farmers to make informed decisions regarding male broiler breeder feeding systems and management. Incidences of urolithiasis usually go unnoticed but may contribute to reduced fertility due to pain and discomfort, potentially negatively impacting mating behaviour or even result in mortalities. Addressing health and nutrition factors affecting chickens is crucial as this will help maximise poultry production that will meet market demands.

2.2 Macroscopic and histological changes caused by urolithiasis

Avian urolithiasis is a distinct condition, and clinical signs noticed in birds, like depression or emaciation, can be tentative as they resemble other differential diagnoses. Therefore, a laboratory diagnosis is required to determine if the birds suffer from urolithiasis through macroscopic and histologic examinations. In most literature, urolithiasis is characterised by kidney atrophy, distended or dilated ureters, and varying sizes of uroliths/visceral urate deposits (Morishita and Porter Jr, 2021). These characteristics can be scored using a kidney lesion score that assists to determine the severity of the kidney damage. Wu et al. (2022) used a kidney lesion score of 0 to 3, with no lesions as 0, swollen and pale as 1, swollen with visible urates as 2, large swelling, pale and ureters distended with urates as 3. Other authors used a similar scoring with a few modifications (Karimi-Dehkordi et al., 2023, Sebaei et al., 2022). When Sebaei et al. (2022) were investigating the effects of a commercial diuretic on Hy-line chickens with induced urolithiasis, birds that were fed high protein (25%) and high calcium (5%) diets had similar post-mortem findings. Pale, swollen kidneys with whitish, chalky urate deposits and distended ureters were observed among those birds, including decreased kidney weight compared to birds fed a regular diet and diuretic supplements.

Post-mortem findings in layer birds during an outbreak of urolithiasis revealed that most birds (57.7%) had severe kidney atrophy and enlarged ureters due to uroliths (Hicham et al., 2011). Lakkawar et al. (2018) also observed distended ureters with semifluid to semisolid chalky white urates that gave a cord-like appearance in domestic male breeder chicks. The kidneys also exhibited varying degrees of degeneration. Kidney asymmetry was noticed in 44.4% of 64-week-old male broiler breeders, and the left kidney was larger (10.07 g) than the right kidney (9.26 g) 76.3% of the time.

2.3 Causes of urolithiasis

Published investigations of the causes of urolithiasis are mainly on the physiological state of affected layer hens and the environment in the layer houses (Crespo and Shivaprasad, 2013). However, research on other poultry revealed similar causes and post-mortem findings. In chickens, urolithiasis has been associated with multiple causes that can be infectious or non-infectious (Lopes et al., 2021a).

2.3.1 Infectious causes

The infectious causes of avian urolithiasis include avian nephritis and infectious bronchitis virus (IBV) (Bulbule et al., 2014). Avian nephritis and infectious bronchitis are highly contagious viral diseases (Saif et al., 2020, Timurkaan et al., 2022). Infectious bronchitis mainly affects the respiratory system, although some virus strains are nephropathogenic, leading to renal damage (Awad et al., 2014).

The nephritis virus affects the renal system and is characterised by visceral urates deposits (Bulbule et al., 2013). These viral diseases may affect the kidneys due to chronic viral invasion and lead to kidney or renal failure over time. Since young birds are particularly vulnerable to kidney damage from bronchitis, the initial infection may occur before renal damage and mortalities (Munuswamy et al., 2021). According to Timurkaan et al. (2022), broiler breeders and layers that exhibited signs of urolithiasis (swollen-pale kidneys and urates deposits) also tested positive for IBV. The study concluded that nephropathogenic IBV could cause incidences of urolithiasis in poultry.

2.3.2 Non-infectious causes

2.3.2.1 Excess dietary calcium

Non-infectious causes of urolithiasis are mainly associated with nutritional causes. These include excess dietary Ca, CP, Ca:P imbalances (high calcium, low phosphorus), P deficiency, increased sodium bicarbonate in feed, vitamin A and D_3 deficiency, and water deprivation (Sandhyarani et al., 2022). Calcium is one of the macro minerals essential in poultry diets. In broiler and layer diets, Ca levels and particle size increase with age to meet production levels and bone quality requirements (De Witt, 2006, David et al., 2021). In layers, giving pre-lay feed at an early stage can result in excess dietary Ca. During broiler rearing, diets containing limestone in coarse form may allow birds to pick more of these particles (Barshan et al., 2019), resulting in excessive Ca intake. Other managerial issues that may result in birds receiving excess Ca include accidental deliveries of production feed to rearing stocks, giving male chickens female feed and feed milling errors (Smith et al., 2000).

2.3.2.2 Excess dietary protein

Similarly, excess dietary protein has been shown to increase the incidence of uroliths in chickens. Dietary protein is given in high quantities during growth when birds build muscle and mature. Therefore, unlike Ca, protein requirements in broilers decrease as the bird ages. El Sebaei et al. (2021) investigated the effects of induced urolithiasis caused by excess dietary Ca and protein. The investigation discovered that mortality was 16.7% when 79-day-old Hy line male chickens were fed high calcium (5%) from 44 days and mortality was 6.7% by the same age when fed a high crude protein (25%) from 44 days, compared to 0% mortality of chickens given standard rations of Ca (1%) and protein (20%). Furthermore, the kidney lesion score for the treatment groups was 3 (pale kidneys with urate deposits) compared to 0 (negative) in the control group, as illustrated in **Table *2.1***. Data from El Sebaei et al. (2021) concluded that urolithiasis signs were more pronounced in chickens that received a high calcium diet than a high protein diet.

Table 2.1: Kidney lesion score and mortality (%) in Hy line male breeder chickens at day 79 fed different treatments of Ca and Protein diet (El Sebaei et al., 2021)

	Male breeder chickens at day 79	
Diet groups	**Kidney lesion score**	**Mortality (%)**
Control (Ca (1%) and CP (20%))	0	0
High Ca (5%)	3	16,7
High CP (25%)	3	6,7

Kidney lesion scores: **0**: negative, **1**: congested kidneys; **2**: pale and swollen, **3**: pale kidney and urate deposits

2.3.2.3 Ca:P imbalances

Furthermore, the correct Ca:P ratio is essential in broiler diets, and imbalances (particularly high Ca and low P) have led to urolithiasis. The renal system maintains different physiological mechanisms in broilers when Ca and P are in a ratio of 2:1 (Matin et al., 2013). When Ca is in excess, P absorption is impaired, resulting in low aP. Phosphorus acts as a urinary acidifier (Sandhyarani et al., 2021) and helps prevent urolith formation, and low aP is associated with high urolith incidences in poultry.

Wideman (2016) discovered that 14% of 18-week-old pullets and 51-week-old hens had urolithiasis triggered by feeding a high calcium and low phosphorus (Ca 3,5%:P 0,4%) diet. In this study, the glomerular filtration rate on pullets fed this diet was lower (1,25 mL/Kg BW/min) compared to pullets on a normal calcium and phosphorous diet (1,42 (mL/Kg BW/min)). Also, urine pH was significantly higher (5,85) in pullets fed the high Ca and P diet and lower (5,52) on the standard diet. Uroliths were also found in 7,4% (10/136) male broiler breeders fed a commercial diet containing 16% protein, 3,25% calcium, and 0,4% phosphorus (Moyle et al., 2011). The findings indicated that broiler breeders develop kidney asymmetry and uroliths when fed a high-calcium diet.

2.3.2.4 Water deprivation

Water deprivation is an important factor contributing to avian urolithiasis incidences (Modesto et al., 2022). Birds can be deprived of water due to an inadequate number of nipples, nipple drinker blockage and extreme water temperature. Severe dehydration increases the water absorption rate, leading to a decrease in urine output. Urates may precipitate in renal tubules and ureters as uric acid output declines, potentially causing impaction and renal failure

(Rexhepi et al., 2015). During a case study by Rexhepi et al. (2015), uroliths in the ureter were found in pullets from two different farms that experienced high mortalities (> 17%). Water access was improved for 2 to 4 weeks, and suddenly, mortalities were reduced to normal (1.5-5%). The study suggested that dehydration contributes to the severity of urolithiasis, which could be significant if breeder farmers restrict water access to prevent excessive drinking and wet litter which is sometimes the case if birds are chronically hungry.

2.3.2.5 Excess sodium

Excess sodium is associated with kidney stone formation in birds because high sodium intake can make the urine more alkaline, and together with high levels of Ca, it can aid kidney stone formation. Sodium intoxication (hypernatremia) can cause stress on the kidneys as they have to work harder to eliminate the excess sodium (Julian, 2005b). Feed contaminated with mycotoxins and excess antibiotics may result in toxicity. These toxins may be nephrotoxic and could lead to kidney damage. Although toxins may cause alterations in kidney function, they may not necessarily cause uroliths but atrophy of the kidneys. Vitamin A and D3 deficiencies may contribute to incidences of urolithiasis when these vitamins are imbalanced or impaired due to excess Ca or other vitamins or minerals (Akinyemi and Adewole, 2021).

Although other factors cause/contribute to urolithiasis, dietary or nutrition factors significantly impact the performance of broiler breeders. Holtzhausen and Brannan (2013) discussed the importance of nutrition on broiler breeders and indicated that females and males require specific nutritional needs for maximum production.

2.4 Bone quality and its importance

Calcium and P play a crucial role in chickens' bone quality and welfare (Wei et al., 2021). Calcium is an essential mineral for the formation and maintenance of skeletal structures (Vannucci et al., 2018). In male broiler breeders, strong and healthy bones are crucial for supporting their body weight, reproductive activities, and movement. Sufficient dietary calcium intake helps maintain skeletal health, preventing issues such as lameness, fractures, and skeletal deformities (ABOKEDE, 2021) . Calcium also plays a role in the reproductive health of the chickens. Ideal calcium levels in male broiler breeders assist in the production and development of sperm cells and the contraction of muscles, including those involved in mating behaviour (Wisaquillo, 2022b). Calcium is also one of the essential minerals involved in hormonal regulation that assist in the proper function of the reproductive system, including the release of sperms (Tvrdá et al., 2013). Male broiler breeders indirectly contribute to the quality of eggs and offspring through their genes (Triques et al., 2019).

2.5 Bone quality parameters

Bone is a complex tissue composed of inorganic and organic matrices that provide support and mechanical strength (Güz, 2022). Bone ash fraction, bone mineral content, bone mineral density, and bone mechanical characteristics are frequently utilized as markers of bone mass and bone strength (Khan, 2022). Bone tissue rigidity results from the deposition of Ca and P during bone mineralization. Inorganic minerals comprise about 70% of the bone composition, while the remaining 30% consists of organic matter, mainly collagen (Sanchez-Rodriguez et al., 2019). Collagen contributes to tensile strength and plasticity, whereas inorganic minerals contribute to bone compressional strength and stiffness (Owuor et al., 2019).

2.5.1 Bone breaking strength

When 18-week-old pullets were subjected to 1%, 1,5% and 2% Ca, the bone strength was 269.78 N, 249.28 N and 268.15 N, respectively (Moreki et al., 2011). However, Li et al. (2020) discovered that Ca and P-deficient diets can significantly decrease bone-breaking strength and that breaking strength increases with age, as shown in **Table *2.2***. Therefore, research suggests that high or low Ca and P negatively affect bone quality parameters like bone-breaking strength. Additionally, tibia-breaking strength has been shown to be higher in fast-growing breeds than in slow-growing breeds. In a study, fast-growing broilers had a high breaking strength (278.7N) compared to slow-growing broilers (213.8N) at six weeks of age (Shim et al., 2012).

Table 2.2: Effects of Ca and P on bone-breaking strength of male broilers (Li et al., 2020)

Ca and NPP levels	Bone-breaking strength (kg) at 14 days	Bone breaking strength (kg) at 21 days
1% and 0.45%	10.5[a]	18.9[a]
1% and 0.23%	1.19[d]	3.08[c]
0.35% and 0.45%	4.99[b]	8.04[b]
0.35% and 0.23%	3.55[c]	8.87[b]

Ca: calcium; **NPP**: nonphytate phosphorus. a-d means within a row lacking a common superscript differ ($P < 0.05$)

2.5.2 Bone ash

Bone ash content is an indicator of bone strength and calcification (Hakami et al., 2022). The amount of ash in bone is proportional to its hardness or compressional strength (Shim et al., 2012) and directly correlates to the amount of dietary Ca and P. Chung et al. (2019) investigated different levels of Ca and P in male broiler chicks. The results indicated that tibia ash content increased with an increase in dietary Ca and P (0% MCP~56.37%, 0.5% MCP~56.44 and 1% MCP~56.56%).

2.5.3 Bone mineral content

Bone mineralization has an impact on bone strength, which allows the skeleton to withstand gravity or increased load and is mainly regulated by the activity of osteoblast and osteoclast (Shao et al., 2019). The degree of mineralization of the bone matrix determines bone strength in addition to the volume of bone tissue and the microarchitectural organization (Shim et al., 2012). High Ca and P diets have been shown to increase Ca and P mineral content in bones. Rama Rao et al. (2019) increased NPP (nonphytate phosphorus) in 72-week-old layer diet from 1.5-3.25%, and the Ca and P in the tibia bone increased with every increase in NPP. Also, Ca % in tibia bones of 60-week-old broiler breeder hens increased from 13.73% (2.5% calcium diet) to 14.52% (3.5% calcium diet) (Moreki et al., 2011). Although Ca% in bone increased when fed the 2.5% and 3.5% calcium diets, there was a decrease in bone Ca% from 14.33% (1.5% calcium diet) to 13.73% (2.5% calcium diet). Research shows that Ca and P dietary intake highly influence bone mineral content and can differ depending on age (Moreki et al., 2011).

2.6 Effects of high dietary calcium on chickens

2.6.1 Bone and leg problems

Leg problems are among the most severe welfare issues in fast-growing chickens (Hartcher and Lum, 2020). To maintain rapid growth, the tibia increases circumferential and longitudinal growth at the expense of decreased mineralization and excessive porosity, which increases fracture risk (Thanabalan and Kiarie, 2021). Leg problems are associated with breakage, fractures, and a lack of strength of bones, caused by a decrease in bone mineral content, bone mineral density, and poor growth. Skeletal disorders caused by nutrition in commercial broilers include articular gout, rickets and tibial dyschondroplasia (Çapar Akyüz and Onbaşılar, 2020). The tibia bone is a primary target of skeletal disorders as it supports the body weight of birds and locomotion. In broilers, the inability to support body weight is usually a result of tibial dyschondroplasia (Thanabalan and Kiarie, 2021).

Chung et al. (2020) investigated the health performance of broilers when fed a standard commercial feed supplemented with 0.5% and 1% MCP. The authors discovered that leg weakness, recumbency, slay legs and lameness were observed in broilers with leg problems. The total number of broilers culled due to these leg problems was relatively highest (4 broilers) where chickens were fed with 1% of MCP compared to 0.5% MCP (no culls) and commercial fed only (2 broilers) (Chung et al., 2020). The study concluded that high1% MCP supplemented in the broiler diet may increase incidences of leg problems compared to 0.5% MCP. In another study, rickets and lameness in male Arbor Acres broilers were induced with a high Ca:P diet (1.11:0.18) (Xu et al., 2021). Lameness incidences were at 70% on day six of feeding a HC:P diet and reached 100% on the 9^{th} day of the trial due to high Ca:P feed.

2.6.2 Availability of other minerals

Calcium is a cation that can bind with other macro and micronutrients and precipitates with free saturated fatty acids, causing a decrease in energy digestibility. Excess Ca may lead to deficiencies of other nutrients like P, manganese, zinc, vitamin D_3, magnesium, copper, and boron (Upadhaya and Kim, 2020), most of which play a vital role in bone health, embryo development and reproduction. For example, magnesium is a cofactor in 80% of all cellular enzymes and is essential for converting vitamin D_3 to an active form and bone crystal formation. Vitamin D_3 is vital for efficient absorption and metabolism of Ca and P and, thus, normal bone calcification.

Zinc is a component in the matrix collagen thread production and may interfere with vitamin D's normal physiological action on Ca metabolism and its anabolic function on bone tissue when given in excess or deficient (Upadhaya and Kim, 2020). Al-Daraji and Amen (2011) discovered that Ca (7.83 mg/100ml) and P (4.27 mg/100ml) concentrations in blood plasma were higher in male broiler breeders given a diet with 0.5% zinc compared to a diet without zinc (6.98 mg/100ml Ca and 3.85 mg/100ml concentrations). In another study, when a standard Ca (0.9%) and P (0.45%) diet without zinc was fed to broiler chickens, tibia thickness (7.40 mm) was lower (P>0.05) compared to a standard Ca: P with 0.5% zinc (7.73 mm tibia thickness) (Askari et al., 2015). Literature suggests that adequate vitamins and minerals should be given to improve bone quality and reduce the chances of limiting the availability of other nutrients.

2.6.3 Growth performance

Excess calcium or P intake has been shown to reduce body weight (Askari et al., 2015). Although feed is strictly controlled in male broiler breeders, keeping them at recommended body weights is important to increase their growth performance.

2.6.4 Absorption, retention and excretion of Ca and P

Calcium and P availability for metabolism are determined by the rates of intestinal absorption, resorption, glomerular filtration, renal tubular reabsorption, and intestinal endogenous losses (Askari et al., 2015). Excess dietary P can reduce Ca availability in the intestinal lumen and result in PTH secretion, increasing bone resorption and affecting bone ash (Cetıngul et al., 2022). Furthermore, excess Ca reduces the solubility fractions and proportion of minerals, consequently reducing their absorption.

Sun et al. (2018b) investigated the excretion and retention of total Ca (TCa) and total P (TP) when broiler chickens were fed different levels of Ca and NPP at different stocking densities. At 0.9% TCa and 0.36% NPP diet, the excretion of TCa (0.82g/d) was more than a diet containing 0.7% TCa and 0.36%NPP (0.6g/d TCa excretion), thus high Ca increased Ca excretion. Moreover, the results indicated that Ca retention was reduced with a diet with high Ca (0.9%) and that feed intake influenced all factors.

2.7 Conclusion

Imbalances of Ca, P, and other essential minerals can potentially contribute to the development of urolithiasis and bone problems in poultry. These imbalances can have detrimental effects on the overall health and productivity of the flock, and this raises the concern over issues that may arise when male breeders are fed a female ration, higher in Ca, P and CP than requirements. Therefore, further research and investigation are needed to ensure welfare and efficiency of male broiler breeders in the industry.

Chapter 3: Assessing incidences of urolithiasis and bone quality in male broiler breeders fed a female or male ration

3.1 Introduction

Non-genetic factors, such as nutrition, significantly influence broiler breeder performance since genetic modifications have improved chicken breeds' performance and efficiency (Carneiro et al., 2019). In broiler breeders, feed intake is restricted to control body weight, breast muscle and improve performance (Zukiwsky et al., 2021). Separate sex feeding in breeder flocks is widely used in breeder nutritional management as it allows feeding different allocations to males and females as well as the use of dietary specifications for each (Brillard, 2004). However, some producers still feed a female feed to both males and females, albeit at different allocations (Silveira et al., 2014). Although the female ration is more costly, this is usually done for ease of management.

Factors that may impact the health and welfare of male breeders fed a female ration should be assessed as the female ration contains high CP, Ca, and phosphorous (P), which is more than recommended for male requirements (Tyler et al., 2021). Silveira et al. (2014) suggested that control of male body weight is better achieved when birds are fed according to requirements with a male specific diet.

Avian urolithiasis (kidney stones) is a condition that occurs when the kidneys are damaged due to various causes, namely infectious and non-infectious diseases, ingestion of toxins or a combination of factors (Rexhepi et al., 2015). Hicham et al. (2011) reported that urolithiasis caused mortalities in caged layer birds (0.7% per week), and egg drop was estimated at 12%. According to Moyle et al. (2011), only 55.6% of the male broiler breeders, fed a female ration (16% CP, 3.25% Ca and 0.4% non-phytate P) until 41 weeks of age showed bilateral symmetry kidneys (heavy: light kidney weight ratio) that indicated subclinical kidney damage. When urolithiasis was induced in male breeder chickens from 44 days to 79 days of age in El Sebaei et al. (2021) study, feed intake was significantly reduced and kidney lesions were observed during post mortem, due to a high Ca (5%) and CP (25%) diet compared to males given a normal diet (20% CP, 1% Ca).

Calcium and P imbalances also impact bone quality traits of male broiler breeders. Bone health, especially the legs, is important to provide support during mating and overall mobility and prevents skeletal disorders that may affect the welfare of birds (Waldenstedt, 2006).

The objective of the study was to assess the incidence of urolithiasis and bone quality traits between male broiler breeders fed a male and those fed a female ration. Therefore, the research aims to provide valuable insights into optimal nutritional strategies for male broiler breeders, focusing on the relationship between diet, urolithiasis incidences, and bone quality. By elucidating these interconnections, the findings of this study can potentially improve nutritional feeding regimens that enhance both the reproductive performance and overall well-being of male broiler breeders in the poultry industry.

3.2 Materials and methods

A contact list of farmers who rear male broiler breeders was acquired, with their permission, from Animal feed companies in KwaZulu Natal. The selection criteria included farmers who feed either a female or male ration to male broiler breeders. The request proceeded after getting approval from the University of KwaZulu Natal Animal Ethics Committee (AREC/00005297/2023) and the Humanities and Social Sciences Research Ethics Committee (HSSREC/00004774/2022). Information on nutrition, management, vaccination schedules, disease history and mortality percentages were acquired through a questionnaire from the selected farmers (Appendix A). Eighty male broiler breeders at depletion were euthanised at the farm using the cervical dislocation method (Khan et al., 2014). They were collected and transported to the University of KwaZulu-Natal Pietermaritzburg campus, Animal Science laboratory, where there were examined immediately. Carcass collection and transportation were done at different intervals depending on the agreement with farmers and when they depleted flocks.

3.2.1 Study area

Eighty male broiler breeder carcasses (20 from each flock) were acquired from farms around Howick area, KwaZulu-Natal, South Africa (**Figure *3.1***). Commercial producers with similar management were chosen to eliminate variation. Male broiler breeders collected from farms were all the Ross 308 breed. The farmers followed the vaccination programme given by state veterinarians, and all birds were vaccinated against Infectious bronchitis virus (IBV), commonly known to cause urolithiasis. None of the flocks used spiking as a management tool, and all flocks were grown according to the recommended target body weight.

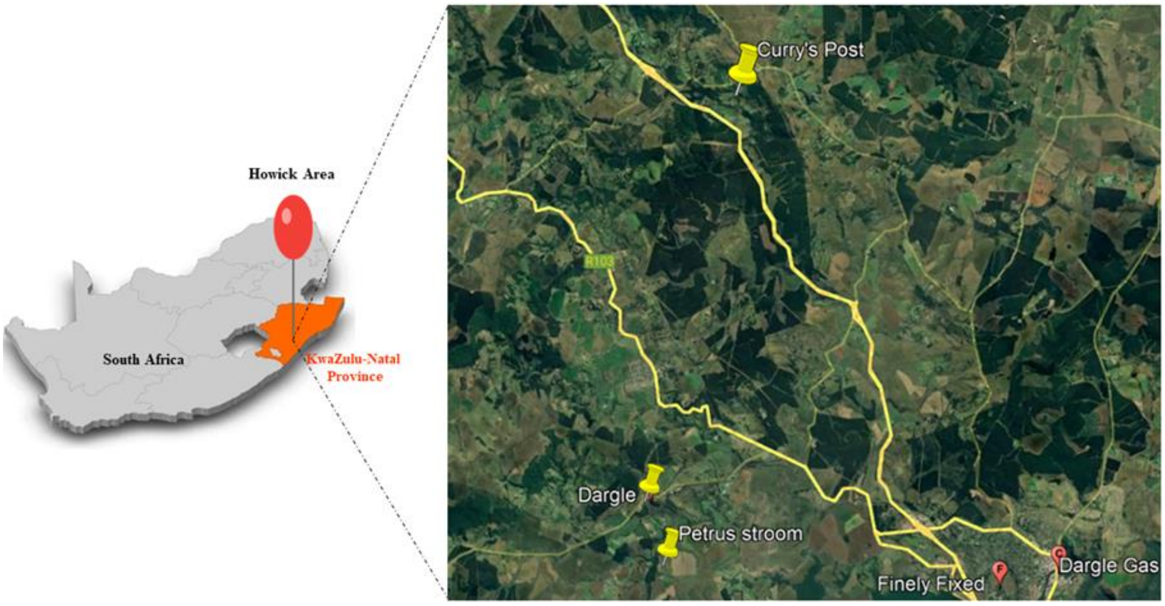

Figure 3.1: Map of South Africa showing the study area in KwaZulu-Natal province, Howick area

3.2.2 Experimental design

The study was a quasi-natural experimental design as it observed and evaluated pre-existing group treatments (male broiler breeders fed male and female diets). The experiment assessed the effects of feeding excess dietary Ca, P and CP (independent variables) to male broiler breeders on bone quality and kidney assessment (dependent variables).

3.2.3 Bird preparation

Birds were laid on their backs, and the skin on the thigh area was cut using a knife. The hip bone was gently dislocated from the hip socket, leaving the legs widely spread flat on both sides. The skin of the lower abdomen was slightly cut and hand-separated to expose the abdominal cavity. The visceral peritoneum was detached gently using fingers, and the dorsal visceral organs were pulled out, leaving the ventral visceral organs exposed (Figure 3.2). The kidneys were hand pulled with the help of a scalpel blade and examined. Before discarding the carcass, 10 left tibia bones from each flock were cut off the carcass and placed in sealable plastic bags and stored in a freezer at -7 °C until bone analysis.

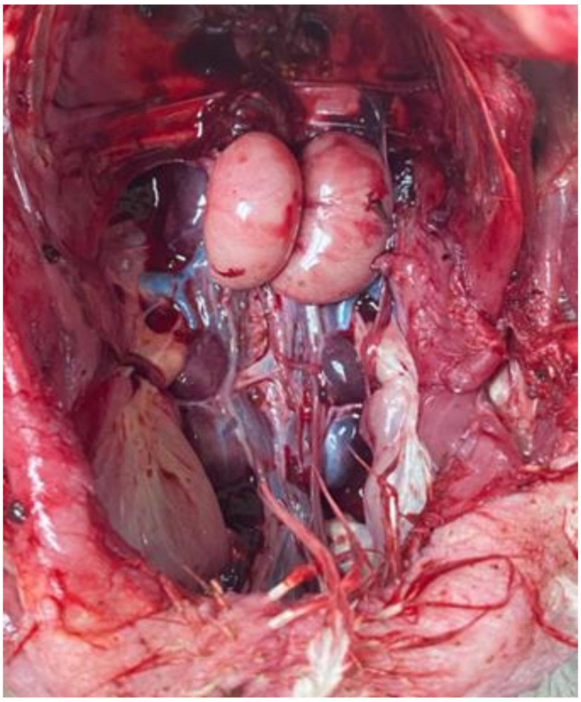

Figure 3.2: Image showing the exposed ventral visceral organs, including the kidneys, after a dissection process

3.2.4 Urinary tract analysis

After dissecting the birds, visual observations of the urinary system for abnormalities were performed (Moyle et al., 2011). Kidneys were weighed using a precision scale (UW4200H Shimadzu, Kyto, Japan). Kidney lesions were scored from 0-3 (0: no abnormalities; 1: pale and congested kidneys; 2: pale, swollen and congested kidneys; 3: pale, swollen and urate deposits) as El Sebaei et al. (2021) described.

3.2.5 Bone mechanical properties

Frozen tibia bones were left to thaw overnight at room temperature (25 °C) before the bone thickness and breaking strength assessment. A utility knife was used to cut through the meat, tendons and cartilage to free the bone.Excess meat tissues were scraped off using a scalpel blade and bones weighed using a precision scale (UW4200H Shimadzu, Kyto, Japan). The bone thickness was measured using a digital analogue micrometre gauge (Mitutoyo IP 65 coolant proof, 293-241-30). The center of each bone was placed between the anvil and spindle face of the micrometer, and the ratchet was twisted until the bone was firmly attached. The readings of each tibia bone were recorded. To assess the bone-breaking strength, Loadtech bone-breaking equipment (Series 104094, 200 kg loader) was used, as described by (Chung et al., 2019). The midpoint of the bones were places on the lower fixture of the machine and a lever was pumped to move the upper fixture with the load cell until bones broke, and measurements were recorded (**Error! Reference source not found.**). The fractured bone pieces from the breakage strength assessment were later used for the bone mineral analysis test.

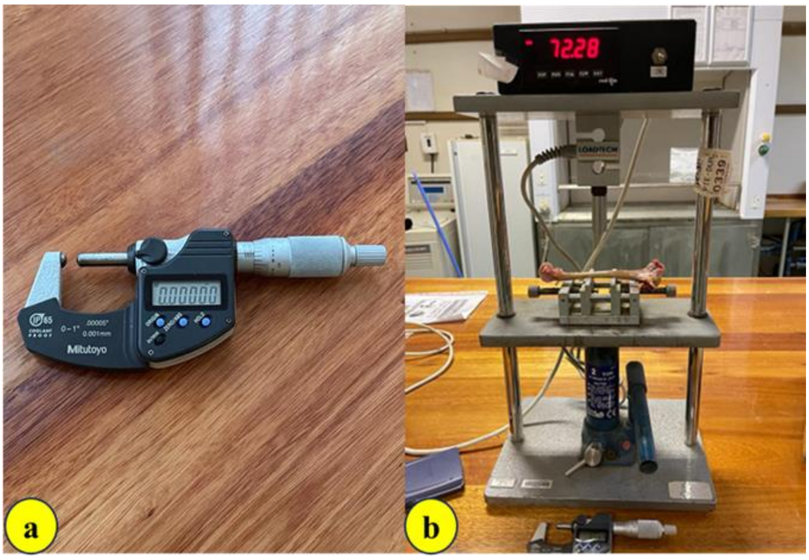

Figure 3.3: Equipment used to measure bone thickness and bone breaking strength respectively: (**a**) digital analogue micrometer; (**b**) Loadtech bone breaking equipment (Series 104094, 200 kg loader)

3.2.6 Bone mineral analysis

3.2.6.1 Sample preparation

Six tibia bone pieces (3 from MM and 3 from MF) were randomly chosen for bone mineral analysis. The bones were rinsed with high-pressure water to clean and remove bone marrow. The cleaned bones were oven-dried for 24 hours at 100 °C, as described by Kim et al. (2017). The crucible for each sample was weighed and mass was recorded. The samples were transferred to crucibles, weighed, and placed in a furnace for 24 hours at 650 °C. The samples were then cooled in a desiccator and final weight of the crucible and ash was measured (Equation *3.1*).

3.2.6.2 Ash content

Ash content wascalculated as described by (Chung et al., 2019) with a few modifications. The organic matter % was then determined from the ash content (Equation *3.2*).

Equation 3.1

$$\boldsymbol{Ash}\ (\%) = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where: $\mathbf{W_1}$ = mass of pre-dried crucible, $\mathbf{W_2}$ = mass of sample + crucible, $\mathbf{W_3}$ = mass of sample + crucible after ashing

Equation 3.2

$$\boldsymbol{Organic\ matter}\ (OM)\% = 100 - Ash\ (DM)\%$$

3.2.6.3 Bone mineral analysis

The ash content (about 1.5g) was further prepared for a mineral analysis test. 5 ml of HCl was added to each sample and placed in a hot water bath until the solution evaporated. 5 ml of HNO_3 was added to the samples and put in a hot water bath until boiling. The samples were cooled, thoroughly mixed and filtered through filter paper into a 25 ml volumetric flask. The filter water was rinsed with deionised warm water to fill the volume. The samples were then sent to the University of KwaZulu Natal Chemistry Laboratory for Ca and P mineral analysis. An Inductively Coupled Plasma (ICP) Optical Emission Spectrometer, Varian 720-ES Series was used to analyse the Ca and P% in bone.

3.3 Statistical analysis

Data collected were subjected to a two-sample *t*-test at 95% confidence interval using GenStat statistical analysis software (23rd Edition Version–23.1.0.651 VSN International Ltd, Hempstead, UK). The mean differences in KW, KSL, BW, BT, BBS, ASH%, OM%, Ca%, P% and Ca/P % ratio were compared between MF and MM. Principal component analysis (PCA) was performed on all variables using XLSTAT [XLSTAT 2023.5.1.1075] (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France, 2023). The loading factors derived from the PCA were used to identify variables with a strong relationship with a particular principal component. Principal component biplots were used to examine correlation analysis among observed variables (bone mechanical properties, kidney weight, kidney lesion scores, and tibia bone mineral content) using OriginPro 2024. All differences were considered significant at $P<0.05$. The KLS data was transformed using square root transformation (Equation 3.3) in order to run a *t* test (St-Pierre et al., 2018, Zhang and Wang, 2021, Bishara and Hittner, 2012, Mandizvo et al., 2016)

Equation 3.3

$$\sqrt{x+1}$$

Where: x = kidney lesion score

3.4 Results

A summary of the general information acquired from the questionnaire showed similar management practices among all flocks and similar nutrient composition of the feed in flocks fed the female ration and also between flocks fed the male ration (Table 3.1)

Table 3.1 A summary of the general management information acquired from the questionnaire.

Flock	**A**	**B**	**C**	**D**
Carcasses examined (N)	20	20	20	20
Age of depletion (weeks)	60	62	65	63
Initial mating ratio	10.5	9.5	10	10
Final mating ratio	7.8	7.6	8	±9
Female mortality (%)	4.56	6.61	9	±6
Male mortality (%)	29.06	24.5	20	±26
M/F ration	Male	Male	Female	Female
CP (%)	12	12	13	13
Ca (%)	0.73	0.7	4	4
TP (%)	0.61	0.45	0.5	0.5
Ca:P	1.2:1	1.6:1	8:1	8:1

N; number, Flocks from farm A, B, C and D

3.4.1 Postmortem findings, kidney weights and lesion scores

Gross lesions associated with urolithiasis were observed in 50% of male broiler breeders fed a female ration. The affected kidneys were pale, congested and swollen, with deposits of whitish-chalky urate (**Error! Reference source not found.**a). Male broiler breeders fed a male ration (92.5%) exhibited no gross lesions associated with urolithiasis (**Error! Reference source not found.**b**)**. In this study, kidney weights were significantly larger from MF compared to MM ($P<0.05$) (Table 3.3). Kidney lesion transformed scores showed a significant difference ($P<0.001$) between MF and MM (Table 3.3).

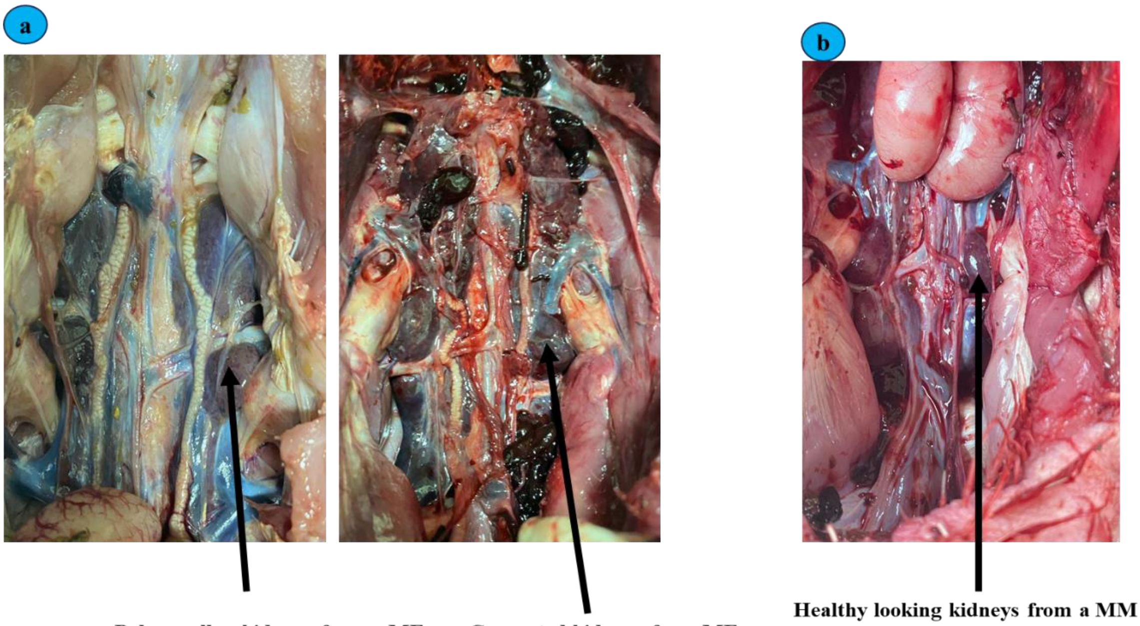

Figure 3.4: Image showing kidneys from (a) two of the MF and (b) one MM

Table 3.2 Kidney lesion score % from male broiler breeders fed a female and male ration

	Kidney lesion score %			
	0	1	2	3
MM	92.5	7.50	0	0
MF	50	35	10	5

(0: negative; 1: pale and congested kidneys; 2: pale, swollen and congested kidneys; 3: pale, swollen and urate deposits) MM (male broiler breeder feed a male ration), MF (male broiler breeders fed a female ration)

Table 3.3: Two sample *t*-test, showing mean kidney weight and kidney lesion transformed scores of male broiler breeders fed a male-specific ration (MM) and female ration (MF)

Variable	Flock	*M*	*SD*	*T*	*P*<
Kidney weight (g)	MM	8.581	1.144	5.61	0.001
	MF	11.307	2.852		
Kidney lesion score	MM	1.031	0.111	4.6	0.001
	MF	1.268	0.307		

M: mean; ***SD***: standard deviation; ***t***: test statistic; ***P***: probability, MM: male broiler breeders fed a female ration, MF: male broiler breeders fed a female ration

3.4.2 Bone mechanical properties

The mean differences between MF (High-Ca/P) and MM (Recommended-Ca/P) in tibia bone weight, thickness and breaking strength are presented in **Error! Reference source not found.**. Tibia bone weight was significantly higher in MM compared to MF (P<0.05). Bone thickness showed no significant difference between the two groups (P>0.05). Male broiler breeders fed a male ration had a significantly higher bone -breaking strength (P<0.05) than MF.

Table 3.4 Two sample t-test showing tibia bone weight, thickness, and breaking strength of male broiler breeders on a male diet and female diet

Variable	Flock	*M*	*SD*	*T*	*P*
Bone weight (kg)	MM	0.053	0.011	-2.62	0.014
	MF	0.045	0.006		
Bone thickness (mm)	MM	9.026	0.202	-0.33	0.746
	MF	8.950	0.113		
Bone breaking strength (N)	MM	626	4.368	-2.23	0.033
	MF	515	2.573		

M: mean; ***SD***: standard deviation; ***t***: test statistic; ***P***: probability, MM: male broiler breeders fed a female ration, MF: male broiler breeders fed a female ration

3.4.3 Tibia bone ash, organic matter, Ca and P content analysis

The ash analysis showed that bones from MF had low ash content (P<0.05). Conversely, the organic matter % was significantly higher in bone from MF (P<0.05). No significant difference was observed in Ca% and P% in tibia bones from MF and MM. The Ca/P ratio derived from the Ca% and P % also resulted in no significant difference (Table 3.5).

Table 3.5: Two sample t-test showing tibia bone ash%, organic matter%, Ca%, TP% and Ca:TP % content from MM and MF

Variable	Flock	*M*	*SD*	*T*	*P*
Tibia bone ash %	MM	69.20	0.1531	-3.10	0.036
	MF	68.51	0.3550		
Organic matter %	MM	30.80	0.1531	3.10	0.036
	MF	31.49	0.3550		
Ca%	MM	0.7646	0.03664	0.39	0.719
	MF	0.7789	0.05277		
P%	MM	0.4419	0.1281	0.04	0.974
	MF	0.4449	0.0760		
Ca:P % ratio	MM	1.845	0.6004	-0.19	0.856
	MF	1.774	0.2188		

M: mean; ***SD***: standard deviation; ***t***: test statistic; ***P***: probability, MM: male broiler breeders fed a female ration, MF: male broiler breeders fed a female ration

3.4.4 Principal component analysis (PCA) for assessed variables

Table 3.6 shows the PCA with factor loadings, eigenvalues, and percent variance for the evaluated variables. In MF, principal component (PC) 1 accounted for 64.09% of the total variation and had a strong positive correlation with KLS, BW, BT, OM and Ca/P. PC2 positively correlated with KLS and BT, contributing to 29.4% of the total variation. No strong positive correlation was observed in PC3. In MM, PC1 accounted for 61.7% of the total variation and was positively correlated with TA and P. Bone weight, BT and Ca% were positively correlated with PC2, accounting for 31.8% of the total variation. There was no strong correlation on PC3 for MM.

Table 3.6: Summary of factor loadings, eigenvalue, percent and cumulative variation for kidney analysis, tibia bone mechanical properties, and tibia bone mineral analysis assessed between MM and MF

	Male-fed female diet (MF)			**Male-fed male diet (MM)**		
Variable	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
KW	0.809	-0.573	-0.136	-0.970	-0.237	0.046
KLS	-0.285	0.958	0.003	0.411	-0.849	0.332
BW	0.901	0.395	-0.179	0.157	0.865	0.477
BT	0.268	0.956	0.117	0.562	0.814	0.146
BBS	-0.734	-0.102	0.672	0.765	-0.455	0.455
TA	-0.937	0.290	-0.192	0.981	-0.138	-0.135
OM	0.937	-0.290	0.192	-0.981	0.138	0.135
Ca	-0.779	-0.622	-0.073	0.544	0.829	-0.132
P	-0.965	-0.220	-0.146	0.986	-0.098	-0.138
Ca/P	0.978	-0.105	0.180	-0.968	0.216	0.128
Eigenvalue	6.409	2.942	0.648	6.166	3.177	0.657
Variability (%)	64.092	29.423	6.484	61.662	31.765	6.573
Cumulative (%)	64.092	93.516	100	61.662	93.427	100

KW: kidney weight; **KLS**: kidney lesion score; **BW**: bone weight; **BT**: bone thickness; **BBS**: bone strength; **TA**: tibia bone ash; **OM**: organic matter; **Ca**: calcium; **P**: phosphorous

The PC biplots based on PCA analysis were used to analyse the relationship among observed variables in male broiler breeders fed a male diet (Figure 3.5a) and male broiler breeders fed a female diet (Figure 3.5b). The angle between two vectors indicates the correlation between the corresponding variables. When two vectors point in the same direction or close to each other, they are positively correlated. Conversely, if they point in opposite directions, they are negatively correlated. When the angle is close to 90°, the variables are not correlated.

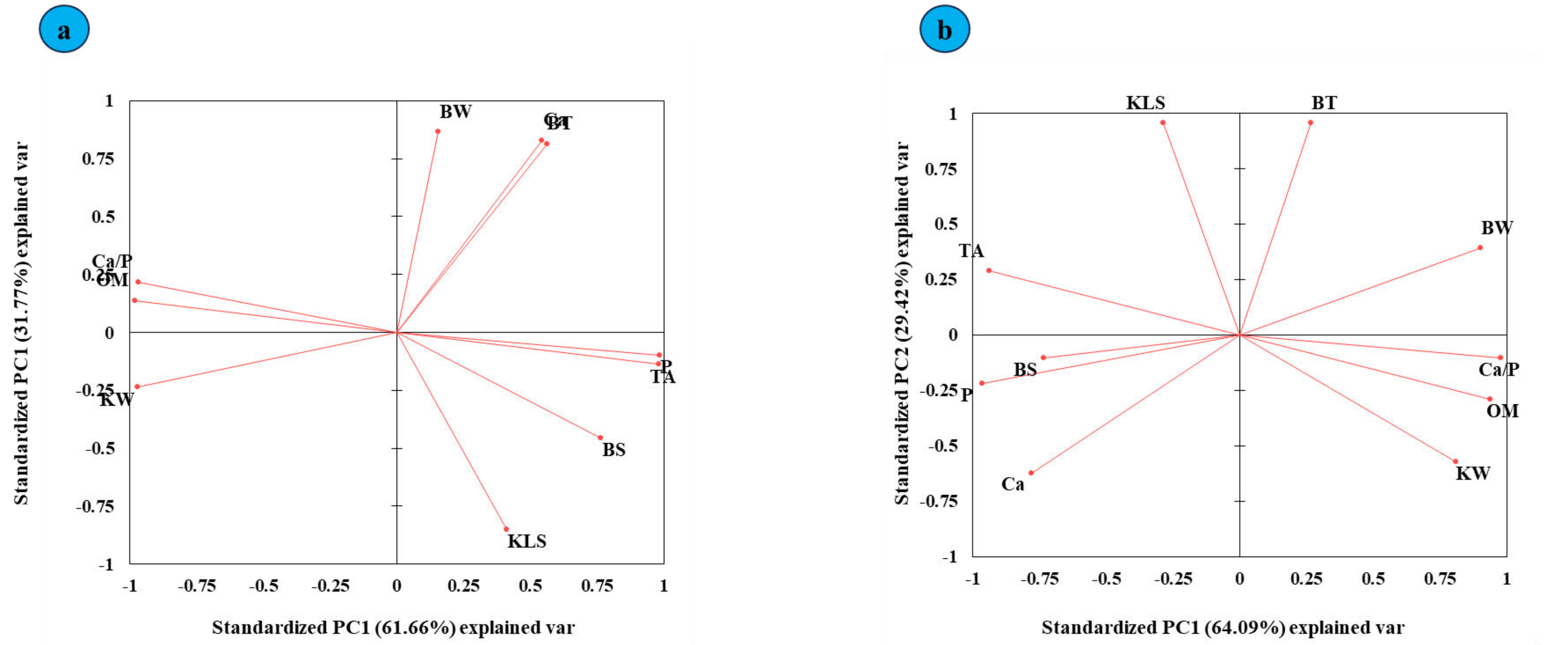

Figure 3.5: Principal component (PC) biplot of PC 1 vs PC 2 demonstrating the relationships among bone mechanical properties, mineral content, kidney weight and kidney lesion scores in (**a**) male broiler breeders fed male diet (MM), (**b**) male broiler breeders fed female diet (MF); **KW**: kidney weight; **KLS**: kidney lesion score; **BW**: bone weight; **BT**: bone thickness; **BBS**: bone breaking strength; **TA%**: tibia bone ash; **OM%**: organic matter; **Ca%**: calcium; **P%**: phosphorous, **Ca/P%**

3.4.5 *Pearson correlation analysis*

Pearson's correlation coefficients showing relationships among variables assessed for kidney assessments and bone quality traits in MM and MF are summarised in Figure 3.6. In MM significant and positive correlations were observed between KW and OM (r=0.9), Ca% and P% (r=0.9), Ca/P% and OM% (r=0.98) (Figure 3.6a). In male broiler breeders fed a female diet, significant and positive correlations were observed between KW and OM (r = 0.96), KW and Ca/P% (r=0.89). The Ca% was positively correlated with BT (r = 0.93) (Figure 3.6a).

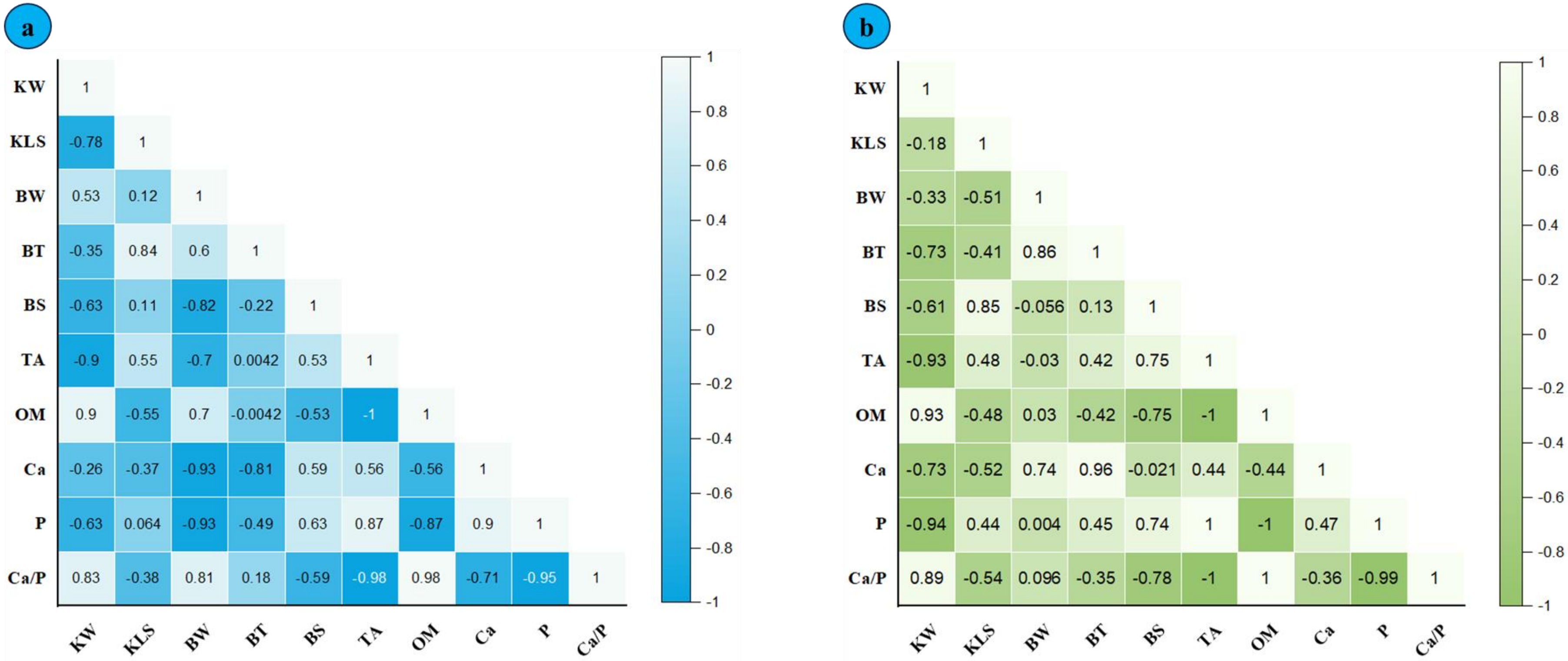

Figure 3.6: Colour palette showing Pearson's correlation coefficients for evaluated variables in (**a**) male breeders fed male diet (MM) and (**b**) male breeders fed female diet (MF); **KW**: kidney weight; **KLS**: kidney lesion score; **BW**: bone weight; **BT**: bone thickness; **BBS**: bone breaking strength; **TA%**: tibia bone ash; **OM%**: organic matter; **Ca%**: calcium; **P%**: phosphorous, **Ca/P%**

3.5 Discussion

Urolithiasis in poultry is not considered a single disease entity but is a manifestation of several renal dysfunctions resulting in renal damage or failure. Most studies report excess dietary Ca or P as a major cause among the infectious and non-infectious aetiologies of urolithiasis (Julian, 2005a, Lopes et al., 2021a, Guo et al., 2008). Incidences of urolithiasis in male breeders fed a female ration may occur because the female feed exceeds male recommended nutritional requirements (Moyle et al. (2011). According to Aviagen (2021), the recommended dietary CP, Ca and P are 12% 0.7% and 0.35% (breeder stage), respectively where as in this study, MF were fed a high CP (13%), Ca (4%) and TP (0.5%) .

The kidney weights of MF were heavier ($P<0.05$) than MM (g) suggesting that the kidneys were inflamed or swollen. Most renal diseases, including urolithiasis, have been shown to cause inflammation of the kidneys due to the workload on organs in an attempt to maintain renal function (Julian, 2005a). Pale, swollen and congested kidneys were observed in some birds . Hicham et al. (2011) reported the same postmortem finding when 19–35-week-old layers were assessed for urolithiasis. In Moyle et al. (2011) study, 7.4% of male broiler breeders showed visible uroliths and concluded that urolithiasis is present in male broiler breeders. Furthermore, the author concluded that female breeder rations (protein 16%, Ca 3.25%, non-phytate 0.4%) possibly cause urolithiasis.

Kidney lesion scores ranged from 0 to 3 for MF, and 0 to 1 for MM . El Sebaei et al. (2021) reported similar results: kidney lesion score for birds fed a high Ca (5%) and high CP (25%) diet was 3 (pale kidneys with urate deposits), while no kidney lesions were observed in birds fed a normal diet (CP 20%, Ca 1%). Kidneys are one of the most resilient organs that can function or survive at minimum capacity (Kellum et al., 2021), this suggests birds can show no clinical signs until at an acute stage of kidney damage that results in death. Hicham et al. (2011) discussed treatment methods for urolithiasis and stated there was a reduction in mortality when birds had excess to plenty of water. Proper hydration supports kidney function and prevents urate or stone formation associated with high Ca levels (Sandhyarani et al., 2021).

Calcium is a macro mineral abundant in most diets; therefore, can significantly impact the formation of calcium phytate complexes. Recommended dietary requirements for breeders have been shown to have a positive effect compared to providing excessive or deficient nutrition. Male broiler breeders fed a male diet had a significantly high ($P<0.05$) BW, BBS, and TA% compared to MF , suggesting that HCa/P (8:1) can negatively affect bone quality, despite no differences in size or mineral composition. Gautier et al. (2017) found similar results that high Ca/P (5.33:1) significantly decreased bone breaking strength (7.978 N) and tibia ash (33.9%), while recommended Ca/P (2:1) had a high breaking strength (13.061 N) and tibia ash (40.8%). Excessive dietary Ca may decrease the availability of other minerals, slow down digesta transit time, hinder absorption, and reduce nutrient retention (Khan and Iqbal, 2016), possibly because of the formation of insoluble complexes in the intestinal lumen. Similarly, Fallah et al. (2018) reported that bone ash and bone weight were significantly lower with high Ca (1.3%) concentrations than with low Ca (0.5%) concentrations. Bone strength is determined by a combination of bone characteristics, including ash content and mineral density, which are highly influenced by feed form, particle size, and other nutrients like vitamin D and zinc (Torres and Korver, 2018). Although the tibia bone breaking strength was significantly higher in MM (given low dietary Ca), Rousseau et al. (2012) discovered that Phytase increased bone breaking strength in broilers fed the highest Ca diets (Ca(0.77%) and phytase(500units/kg); $P < 0.05$).

The Ca% and P% in the tibia bone of MF were not different to that in the bone of MM. Most studies have shown that high Ca/P increases the Ca and P% in bones, but this probably depends on the levels in the diet in relation to requirements. Chung et al. (2019) reported that the bone-breaking strength was significantly lower (256.2 N) when 1% MCP was added in feed compared to 0.5% MCP (336.59 N) in broilers, suggesting that increases Ca may weaken bones when fed excess to requirements. Excess Ca intake may trigger the parathyroid gland to produce calcitonin, inhibiting renal Ca reabsorption and increasing Ca excretion via the urine (Zhu et al., 2019).

The positive correlations between kidney weight and Ca/P ratio (r = 0.95) in MF may indicate the physiological roles of the kidney in regulating Ca and P homeostasis. Kidneys play a crucial role in maintaining mineral homeostasis. If the Ca to P ratio in the diet is not optimal, the kidneys change in size and function to regulate these minerals effectively (David et al., 2023).

Calcium is an essential element that plays a crucial role in bone mineralization. Bones acquire their strength and density through Ca, ensuring their ability to support the body. Bello et al. (2020) reported a positive correlation between higher dietary Ca and bone mineralization in increasing bone thickness. This aligns with this study findings, which showed a positive correlation (r = 0.93) between bone thickness and Ca levels in MF . Similarly, (Valable et al., 2018) found a positive correlation between bone mineral content and tibia ash. In this study, the interaction between Ca and P (significant correlation (r = 0.9)) illustrated their influence and dependence on each other, as Yang et al. (2020) speculated. The relationship coexists since high dietary Ca can inhibit P uptake in the gut, and P can reduce the absorption of Ca (Proszkowiec-Weglarz and Angel, 2013).

3.6 Conclusion

The study's findings indicated that there may be potential negative effects on the kidneys and bone quality of male broiler breeders, when fed with female feed that has high levels of CP, Ca, and P. Although these effects are not severe or harmful, they should still be considered as they may affect the welfare of birds and impact on general wellbeing and mating behaviour or libido. The study also revealed that elevated Ca levels in the feed can potentially disrupt the absorption or retention of Ca and other essential minerals such as P, leading to a decline in bone quality, which may also not appear as a pathogenesis but can make the birds more prone to leg issues which could potentially affect mating behaviour.

Chapter 4: Summary and conclusions

4.1 General discussion

The literature shows that there are various causes of urolithiasis, one of which is when Ca and P are fed excess to requirements or at the incorrect ratio as high Ca/P diets limit the availability of other minerals (Akter et al., 2016, Hamdi et al., 2015, Li et al., 2017) This has been shown to predispose birds to urolithiasis and leg issues. The aim of this study was to determine if producers feeding male broiler breeders a female ration has negative consequences on kidney function and bone quality.

When minerals are fed excess to requirements, it puts strain on the excretory systems, and can predispose birds to urolithiasis. Although birds can function well despite reduced kidney function, it is possible that wellbeing and behaviour (including mating behaviour) may be negatively affected. This study showed that males fed a female diet had reduced kidney health and significantly more males fed a female ration showed signs of predisposition to urolithiasis.

The outcome of this study showed that male broiler breeders fed a female diet had lower bone-breaking strength, thought to be due to Ca and P intake excess to requirements. The female diet is also higher in CP, and this potentially exacerbates this effect.

It is possible that high Ca may have limited the availability of other minerals. Calcium and P work with other minerals to give bones its strength, mineral density, mineral content, elasticity, and structure. For example, copper and iron are essential for collagen crosslinking, which gives bone its breaking strength, flexibility and elasticity (Güz et al., 2019). Güz et al. (2019) discovered that even when Ca and P levels were adequate (but in excess), bone strength was reduced due to a lack of accessibility and availability of Cu and Fe.

4.2 Conclusion

Feeding male breeders a female feed, which has excess CP, Ca and P to requirements and a higher Ca/P ratio has significantly adverse effects on bone density and strength as well as kidney weight and lesion score. The results of this study indicate a significant correlation between dietary composition, bone quality and the incidence of urolithiasis. Urolithiasis is a multifaceted condition involving various factors, and this research emphasises the role of nutrition as a potential contributor. The findings suggest that feeding males a female ration may have distinct impacts on urinary tract health, potentially influencing the development of urolithiasis in male broiler breeders. Although urolithiasis may not be manifested as a patholodyof diseases or conditions that cause high mortalities, the potential to reduce production exists, which may be exacerbated with any other form of stress encountered such as heat stress. Producers need to weigh up this potential limitation with the ease of practical management in feeding a female ration.

4.3 Limitations

The study was not carried out in a controlled experimental setting and although an effort was made to ensure as little variation as possible in the farms birds were sourced, the differences in management and environment could have contributed to variation between birds. Hatchability records throughout the production cycle would also have been useful to assess any differences in fertility, although there are the confounding effects of female fertility to consider.

4.4 Recommendations

Based on the findings of this study, several recommendations can be made for the poultry industry and future research. A recommendation to broiler breeder producers would be to consider feeding a specific male ration, despite the practical considerations. Urolithiasis can also be investigated and compared in different breeds since only Ross 308 was used in this study. Additionally, to determine urolithiasis incidences in South Africa, the necropsy examination of the kidneys should be assessed to investigate kidney atrophy or degeneration.

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APPENDIX A

Bone quality and incidence of uroliths in the urinary tract of male broiler breeders fed male or female rations

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Questionnaire

INFORMATION

This questionnaire is for research purposes on the topic mentioned above and for completing a master's thesis as required by the University of Kwa-Zulu Natal in South Africa.

INSTRUCTIONS

Please answer the questions below by filling in the blank spaces provided with the correct required information or marking the correct answer in the box. Your co-operation will be greatly appreciated. Thank You!

Please note that the information provided will be treated with confidentiality.

PARTICIPANT CONSENT

I, ……………………………………………………………………………………………, agree to take part in the project. I understand that my responses to this questionnaire will be treated with confidentiality. I understand that I will not receive any compensation for participating in this research project.

Signature: ………………………………………………………………………….

Date: …………………………………………………………………………………

QUESTIONS

What is your flock size?

Which breed do you use?

Which feed company do you buy from?

Which feed type do you use for male broiler breeders?

Specific male diet ☐ Female diet ☐

Have you observed any kidney stones/ urolithiasis cases in any production cycle?

Yes ☐ No ☐

What are the nutrient specifications indicated on the feed label for male broiler breeders of this flock?

Female feed (early breeder)

Nutrient	**Percentage (%) or g/kg**
Crude protein	
Lysine	
Methionine	
Moisture	
Calcium	
Fat	
Fibre	
Phosphorus	
Calcium: phosphorus	

Female feed (late breeder)

Nutrient	Percentage (%) or g/kg
Crude protein	
Lysine	
Methionine	
Moisture	
Calcium	
Fat	
Fibre	
Phosphorus	
Calcium: phosphorus	

Male feed (week 22-depletion)

Nutrient	Percentage (%) or g/kg
Crude protein	
Lysine	
Methionine	
Moisture	
Calcium	
Fat	
Fibre	
Phosphorus	
Calcium: phosphorus	

The following questions are related to the flock from which the sample of birds will be bought/donated.

What is/was your Male: Female mating ratio in the flock at the beginning of production?

What is/was your Male: Female mating ratio in the flock at the end of production?

Did you spike/replace males during production?

Yes ☐ **No** ☐

If yes, how many males did you replace?

Do you keep production records?

Yes ☐ No ☐

If yes, Did the male broiler breeders grow according to the growth curve target for this flock? (If possible, please show the growth curve during our visit).

What has been your mortality rate for this flock (up to date)?

Total ☐ Female ☐ Male ☐

Did the flock/is the flock having any problems with fertility?

Yes ☐ No ☐

Have you observed any diseases or conditions of concern in the male broiler breeders \during rearing and production?

Do you follow a routine vaccination programme specified by a Veterinary Doctor in your area?

End**********************************