A re-evaluation of calcium and phosphorous requirements for optimal performance and bone integrity of the Ross 308 broiler

By

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Declaration

I, the undersigned, declare that this thesis, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me or another individual for a degree at this or any other tertiary institution.

D, Linde

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Abstract

A re-evaluation of calcium and phosphorous requirements for optimal performance and bone integrity of the Ross 308 broiler

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Calcium and phosphorus are vitally important macro minerals and are the most abundant minerals found in the broiler, playing a vital role in bone development and mineralisation. Phosphorus also plays an important role in growth as it is prevalent within nucleic acids, nucleotides, phospholipids and phosphorylated proteins. Considering the fast growth rate of the broiler and the rapid production cycles that the modern broiler is exposed to, it should be essential to have the correct levels of Ca and P in the broiler diet. There is a likelihood that nutritionists have been over supplementing these minerals due to the lack of understanding of their absorption rates and bioavailability and how they interact with each other. As a result very large safety margins are applied due to fear of causing deficiencies. As phosphorus is the third most expensive ingredient in a broiler diet, it would also be of economic advantage if the inclusion levels are dropped without negatively influencing performance and health. New research suggests that the Ca and P levels in a broiler diet can be safely reduced compared to the levels that nutritionists have been supplementing up to now.

The main objective of this study was to determine if feeding lower levels of Ca and P to broilers throughout the rearing period compared to the current South African industry standard for Ca and P inclusion levels (308 Ross Broiler management manual, 2009) would affect body weight gain and performance as well as bone mineralisation. Other objectives of the study were to determine if reducing Ca and P levels in finisher feed would compromise bone integrity and also to determine the effect of the interaction between Ca and P at various inclusion levels on requirement levels in the broiler in terms of growth performance, bone mineralisation, phosphorous excretion and profitability of broiler production.

Six different treatments were tested; the only differences between the treatments were the Calcium (Ca) and Phosphorous (P) levels supplemented at the Pre-starter (PS), Starter (S), Grower (G), Finisher (F) and Post-finisher (PF) phases. The treatments were as follows: Treatment 1 also known as the Ross 308 guidelines (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%); Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of

0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%); Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). The dietary levels for Ca and P were chosen based on recommendations made by Dr R. Angel in consultation with AFGRI Animal Feeds (14 October 2015).

The dietary Ca and P levels used during the trial resulted in significant differences for the production parameters of the broilers that received the different treatments. The broilers that received Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), which had the lowest dietary Ca and P levels throughout the trial period had the poorest production efficiencies. For example, the body weight, feed conversion ratio (FCR) and performance efficiency factor (PEF), at 35 days of age were lower ($P < 0.05$) for treatment 4 and 6 than for those that received Ca and P according to the Ross 308 guidelines. The broilers from Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had significantly ($P < 0.05$) higher feed intake at 28 days of age, significantly ($P < 0.05$) lower growth rate at 28 days of age and significantly ($P < 0.05$) higher FCR at 35 days of age and significantly lower PEF at 35 days of age than those from Treatment 1 (Ross 308 guidelines). Mortality % was not affected by the different treatments, and no clear trend could be seen.

The amount of P excreted trended downwards with decreasing Ca and P levels included in the broiler diets. The broilers from Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) had a significantly ($P<0.05$) lower P excretion than those from Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) resulting in reduced wastage and pollution. A downward trend could also be seen for bone breaking strength and bone ash % with decreasing Ca and P supplementation. The broilers from

Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had significantly ($P<0.05$) lower bone breaking strength (kN) than those from Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%). There were no significant ($P > 0.05$) differences found between the bone breaking strength and bone ash % of the broilers from Treatment 1 (Ross 308 guidelines), Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), implying that the Ca and P can be reduced drastically before affecting the bone strength and integrity. The broilers from Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had significantly ($P<0.05$) lower bone ash % than those from Treatment 1 (Ross 308 guidelines), however, no other significant ($P>0.05$) differences were found.

Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) was not significantly ($P>0.05$) different to Treatment 1 (Ross 308 guidelines) for any of the recorded parameters. Therefore, Ca and P can safely be lowered at the Finisher phase without any detrimental effect on the production efficiency or the bone integrity and will in actuality decrease feeding costs and P pollution.

Keywords: calcium, phosphorus, bone ash, bone breaking strength, Ross, broilers

Contents

List of abbreviations

PS	Pre-starter
S	Starter
G	Grower
F	Finisher
PF	Post-Finisher
Ca	Calcium
P	Phosphorous
BWG	Body weight gain
BW	Bodyweight
FCR	Feed conversion ratio
PEF	Performance efficiency factor
RP	Retainable Phosphorous
RA	Dr Roselina Angel
H0	Null hypothesis
HA	Alternate Hypothesis
NPP	Non-phytate phosphorus
PP	Phytate phosphorus
AvlP	Available phosphorus
dP	Digestible Phosphorous
BBS	Bone breaking strength
DCP	Dicalcium phosphate
MCP	Monocalcium phosphate
MDCP	Mono-dicalcium phosphate

AA	Arbor Acres
M	Males
F	Females
rH	Relative humidity
NCB	New Castle Disease
IB	Infectious Bronchitis
FI	Feed intake
ANOVA	Analysis of Variance
GLM	General linear model

List of Tables and Figures

Chapter 1

General Introduction

Poultry is a vitally important protein source in South Africa as it is affordable to the average low and middle-class households. To stay competitive in the market, ways of decreasing production costs and increasing poultry efficiency should be actively researched. Research in mineral nutrition has moved towards reducing mineral supplementation and shows promise in reducing feed cost. In the past, it was assumed that only non-phytate phosphorous was available for absorption (Van der Klis & Versteegh, 1999). This incorrect assumption (Plumstead & Brake, 2007), together with the fear of failing to supply adequate phosphorous (P), resulted in nutritionists overfeeding P as a safety margin (Waldroup, 1999). A reduction in mineral supplementation also indirectly improves mineral retention (Rama Rao *et al.*, 2003) which reduces the excretion of unutilised minerals, thus making production more efficient and reducing environmental pollution, specifically when it comes to P excretion. Inorganic P, which is used to meet the P requirements in broiler diets, is the third most expensive feed ingredient after energy and amino acids (Angel *et al.*, 2002; Sacranie *et al.*, 2013). Therefore, lowering the supplementation of P in broiler feed will lower the feeding cost drastically.

Recent literature shows that the requirements of Ca and P for commercial broilers are lower than previously thought (Bronell *et al.*, 1990; Scheideler *et al.*, 1995; Rama Rao *et al.*, 1999). Since Ca and P are interdependent, it is important to examine Ca requirement levels at varying P levels and to find the optimum combination of dietary Ca and P levels for a broiler. However, this has not been practised in past research trials on mineral nutrition as Ca and P have been examined independent of one another. Very little research has been done on Ca requirement levels, whereas P requirements have only been studied at one or two levels of Ca making results inconclusive.

The objectives of this project were to determine if the lower recommendations for Ca and P requirements for broilers, as recommended by RA (2015, CR Angel, Pers. Comm. Department of Animal and Avian Sciences, University of Maryland, USA) from personal research and an overview of literature (Angel *et al.*, 2002; Cowieson *et al.*, 2006; Linares *et al.*, 2013; Wilkonson *et al.*, 2013), would result in improved body weight gain and performance as well as bone mineralisation relative to the South African industry standard (Ross 308 nutrition supplement, 2009); to determine if there is a possibility of reducing Ca and P levels in Finisher feed without compromising bone integrity; and lastly to determine the interaction between Ca and P levels on requirement levels in terms of effects on performance, bone mineralisation, phosphorus excretion and economics.

Hypothesis of the study

The null hypothesis (H_0) of this study is that including Ca and P during various phases of the rearing period (day 0-35; day 18-35; day 28-35) at lower concentrations compared to Ca and P levels currently recommended by the Ross 308 guidelines, will reduce broiler performance and bone integrity, whereas the alternative hypothesis (H_A) is that these lower levels of Ca and P will not have a negative effect on broiler performance and bone integrity.

Chapter 2

Literature Review

The objective of the review is to give an overall view of the effects of calcium and phosphorus on broiler production, the factors affecting the availability of these minerals and the requirements of them for optimal broiler nutrition.

2.1 Definitions of calcium and phosphorus

There has been much confusion in the animal nutrition industry regarding which P value to use when formulating broiler diets. This is due to different methods used to determine P concentrations as well as different definitions for expressing P. Following is a short summary of a variety of ways that Ca and P has been expressed in literature with their accompanying definitions.

Total phosphorus (P) is generally referred to as the phosphorus that encompasses any and all forms of phosphorus (Angel, 2011) or the analysed total P in the diet, including organic and inorganic phosphorus (Plumstead *et al.*, 2007).

Non-phytate phosphorus (NPP) is the most widely used term found in literature when determining P levels for broilers. This is due to the ease of measurement as it can be chemically determined. Non-phytate phosphorus represents a chemically defined entity calculated by subtracting the analysed P content of ingredients from their analysed phytate content (Angel & Applegate, 2001; Plumstead *et al.*, 2007). Similarly, Angel (2011) defined NPP as the phosphorus that is not bound to the phytic acid molecule. However, NPP cannot be assumed to be completely available, just as much as not all phytate phosphorus (PP) is unavailable to the monogastric animal, especially when using phytase enzyme in feed formulations.

Available phosphorus (AvlP) may be a more suitable term to use when discussing broiler requirements as it includes inorganic and organic P. Available phosphorus can be defined as the bioavailable P determined using a slope ratio assay and expressed relative to monocalcium phosphorous (Apke *et al.*, 1987; Soares, 1995; Plumstead *et al.*, 2007). However, this definition describes the term “Relative AvlP” as it sets the availability of a standard phosphorus containing product as 100 and compares other P products in relation to the standard. This definition is only useful when comparing products and cannot be used as an absolute value. Available P may be better described as the phosphorous that is absorbed from the diet into the animal (i.e. feed P minus P in the distal ileum). Although this definition seems logical, it is the same definition used to describe digestible P (dP) (Angel, 2011). Available phosphorus values are determined for each feed ingredient by conducting experiments using animal models (Van der Klis & Versteegh, 1996). However, as seen in literature, the availability of PP in feed ingredients is highly variable (Nelson *et al.*, 1976).

Retainable P (RP) is also a term that is often used in industry and refers to the P that stays in the body (feed P minus excreta P); therefore, includes the P from the urinary fraction. Some organisations have assumed that AvlP, NPP, and RP are interchangeable, which has been noted by Plumstead *et al.* (2007) to be distinctly different values due to the method of determination of these. This has resulted in an inconsistency in P levels recommended in the literature.

Table 2.1 Retainable Phosphorus (RP) coefficients of some common raw materials (van der Klis & Blok, 1997)

	Total phosphorus (g/kg)	Inositol phosphate (as % of total P)	Coefficient of P uptake (%P)
Maize	2.8	70	30
Soya oilcake	6.6	70	42
Sunflower oilcake	10.9	90	27
Wheat bran	9.3	85	27
Carcass meal	36.4	-	62
Fish meal	23.7	-	74
Monocalcium phosphate (MCP)	210	-	80

Research has been focused towards determining accurate digestible Ca and P values for various feed ingredients. Digestible P is described as feed P minus ileal P and therefore excludes P found in the urinary fraction. However, this will take place over a long time period and methodologies will have to be standardised to avoid confusion and inconsistencies (Angel, 2011).

Table 2.2 Relative values of different phosphorus terms (Plumstead & Brake, 2007)

Term	Value in a typical breeder diet (g/kg) and relative (%)
Total P (P)	6.3 (100)
Non-Phytate P (NPP)	3.8 (60)
Available P (AvlP)	4.0 (63.5)
Retained P (RP)	3.6 (57.0)

2.2 Evaluation methods of calcium and phosphorus availability in broilers

The evaluation of Ca and P availability fall into 3 larger categories, namely, qualitative, quantitative and *in vitro* tests.

2.2.1. Qualitative methods

Qualitative is defined by Henderson's Dictionary of Biology (2008) as: "concerned with only the nature of a property under investigation". The methodology is used to gain a greater understanding of underlying reasons and supports potential quantitative research. Qualitative methods are sought after by the commercial industry due to the direct effect it has on the industry. In studies that were concerned with P retention or digestibility, criteria such as bone breaking strength and bone ash and performance results, including body weight gains, feed conversion ratios and feed efficiency, feed intake and mortality rates were used as qualitative data (Proszkowiec-Weglarz & Angel, 2013; Li *et al.*, 2015). These results give insight into the effects of mineral supplementation and may be just as valuable as quantitative data such as in vitro and in vivo methods.

2.2.1.1 Bone breaking strength

Rowland *et al.* (1967) was the first to use bone breaking strength (BBS) as criteria for adequate calcium and phosphorus deposition in bone. It was found that there is a correlation coefficient of 0.98 between BBS and tibia ash. The authors concluded that both tibia ash and BBS were good criteria to measure supplemented calcium and phosphorus levels. Bone breaking strength has been used since then to evaluate P availability in broilers (Hayes *et al.*, 1979; Huyghebaert *et al.*, 1980; Ketels & De Groote, 1988; Burnell *et al.*, 1990; Chung & Baker, 1990; Orban and Roland, 1992; Coffey *et al.*, 1994; Ravindran *et al.*, 1995; Lima *et al.*, 1997; Fernandes *et al.*, 1999; Leske & Coon, 2002; Hemme *et al.*, 2005; Coon *et al.*, 2007). Bone breaking strength is a reliable indicator of the processing durability of the carcass (Lima *et al.*, 1997) and is, therefore, a vital criterion to measure for commercial purposes. A broiler carcass with a poor BBS will not withstand processing when slaughtered and will be discarded, therefore resulting in loss of income for the producer.

However, BBS was also found to have a poor sensitivity to phosphorus availability (Huyghebaert *et al.*, 1980; Ravindran *et al.*, 1995). When Skinner *et al.* (1992) removed limestone from the broiler diet during late phase feeding, a significant ($P < 0.05$) decline in BBS was noted. Conversely, when dicalcium phosphate (DCP) alone was removed from the late phase diet, it did not result in a significant decline in BBS. It was concluded that a sharp decline in Ca supplementation will result in a weaker bone; however, a complete removal of P supplementation will not affect the bone strength.

2.2.1.2 Bone ash

The tibia is the fastest growing bone in the body, and as such, rapidly shows a response to a P deficiency (McLean and Urist, 1961), making it a very valuable parameter to examine when researching Ca and P levels in broilers. Bone ash can be expressed in 3 different ways: milligrams (mg) ash per tibia (ash weight per volume of bone); ash percentage, which is the weight of ash (dry matter), divided by the weight of the defatted bone; and lastly ash percentage corrected for BW (mg ash divided by body weight in kilograms (kg)).

Tibia ash as a percentage of dry defatted bone weight has been used widely as response criterion for mineral deposition, due to its high sensitivity to dietary phosphorus availability (Nelson & Walker, 1964; Shastak *et al.*, 2012). Tibia Ash weight includes the weight of the bone and the amount of minerals present in the bone, therefore, reflecting the total amount of minerals contained in the bone. Ash weight is therefore linked to the weight and length of the bone, resulting in a correlation of mineral content with the size of the bone. Tibia ash weight may, therefore, be more accurate to determine the effect of mineral supplementation in the diet as it considers the variation in bone size and weight, whereas ash percentage may result in bones with large variations in weight and size with very similar ash percentage values (Skinner & Waldroup, 1995; Applegate & Lilburn, 2002; Shim *et al.*, 2012). For example, Shim *et al.* (2012) compared the bone development of slow-growing broilers as opposed to fast-growing broilers and found that they had similar bone ash percentage, even though the bones of the fast-growing broilers were much larger and heavier. Similarly, Shastak *et al.* (2012) found that the tibia ash percentage in 11-day old chicks were similar regardless of the level on NPP they received in their diets, even though, the ash weights were significantly ($P < 0.05$) higher in chicks fed 0.27% NPP compared to the chicks that received NPP levels of 0.19% in their diets. Li *et al.* (2015) found that ash weight better represented the amount of bone mineralisation compared to the most widely used criteria of ash percentage.

2.2.1.3 Performance results

Performance results such as growth rate, feed intake, feed efficiency and feed conversion ratio (FCR) are moderately sensitive response criteria when determining the bioavailability of macro and micro minerals (Ammerman, 1995). Performance, mostly growth response, together with bone ash has been used as a response criterion for phosphorus evaluation and bioavailability since the 1940's. Gillis *et al.* (1948) determined the relative bioavailability of P in chicks from different sources using body weight at 28 days as a response criterion, together with mortality and tibia ash.

Motzak *et al.* (1956) found an improved FCR with increased P supplementation. It has been stated in literature that growth rate is as sensitive as bone ash as a response criterion for P supplementation

(Vandepopuliere *et al.*, 1961; Jongbloed & Kemme, 2002). This may be true for broilers due to their low body mineral reserve and high growth rate. However, contradicting these results, Nelson & Walker (1964) found that growth rate was less sensitive than bone ash as a criterion for evaluating phosphates; they found that it was less accurate when the level of P supplemented met the chick's minimum requirement for P.

2.2.2. Quantitative methods

Quantitative methods include those in which a tangible value is determined which can be used by animal nutritionists to formulate as accurately as possible. These include *in vivo* and *in vitro* methods. *In vitro* method consist of laboratory analytical methods to test parameters such as solubility and aim to replicate biological systems (Sibbald 1982), whereas *in vivo* methods test actual digestibility, retention and bypass in a specific animal (De Groote & Huyghebaert, 1997).

2.2.2.1 *In vivo* phosphorus retention and digestibility

Phosphorus retention values and digestibility values for raw materials and mineral sources are needed to accurately formulate and to know what kind of performance to expect from the broilers being fed their diets. (Coon *et al.*, 2002).

Different methods exist for the determination of P retention: Complete excreta collection or the marker method in which the retention is calculated with the help of an indigestible marker such as chromic oxide (Edwards & Gills, 1959). Availability of minerals was determined by the retention of the minerals and corrected for endogenous faecal losses (Nwokolo *et al.*, 1976).

P availabilities were determined for common feedstuffs in 3-week old broilers under standardised conditions, known as balance trials. It consists of a 7-day period for the adaptation of the experimental diet and a 4-day balance period with restricted feeding and total excreta collection (Bourdillon *et al.*, 1990; Van der Klis & Versteegh, 1996; De Groote & Huyghebaert, 1997).

The marker method can vary in its methodology. Leske & Coon (2002) used a 5-day bioassay using an acid insoluble ash marker for retainable P determination. There was a 3-day acclimation period before the 48 hour excretion collection period after which the retainable P value was determined. Ileal digestibility (as developed for protein digestibility) is a favoured method as it avoids any interference due to the post-ileal microbial action. This method is also favourable as the contribution of the urine can be excluded (Rodehutscord, 2009). Pre-caecal or post-ileal digestibility of P has been determined in many inorganic feed phosphates and can be used alternatively to P availability, with the added advantage that P digestibility is less sensitive to the P level of the diet (Rodehutscord, 2009). The response of P pre-caecal digestibility to dietary P concentration is linear over a wider range of dietary P than the response in P retention (Rodehutscord *et al.*, 2012).

2.2.2.2. *In vitro* methods

In vitro Quantification of requirements includes methods done in a laboratory setting such as a solubility analysis of inorganic feed phosphates and calcium sources to estimate the bioavailability of the phosphates (Gueguen, 1999). *In vitro* methods are far quicker and cheaper than traditional *in vivo* methods, making *in vitro* methods very convenient to use. There have however been conflicting results when reporting the relationship between solubility and bioavailability of inorganic phosphates (Waldroup, 1999).

It would be assumed that high solubility of an inorganic phosphate would result in a high bioavailability. However, this has not been observed by Gillis *et al.* (1948). It was found that some highly soluble phosphates, namely, alpha, beta and gamma calcium phosphates were completely unavailable for absorption. Day *et al.* (1973) also found that solubility tests of inorganic phosphates done in acid had no correlation to bioavailability in poultry.

2.3. Functions of calcium and phosphorus in the broiler

Calcium and phosphorus are the most abundant minerals in the body. Within the body, 75-99% of Ca and 50-80% of P are stored in the skeleton as hydroxyapatite, therefore playing a vital role in bone development and mineralisation (Veum 2010; Proszkowiec-Weglarz & Angel, 2013). The remaining 1% of Ca is found in extracellular fluid, plasma, and within cells, and plays crucial roles in metabolism, blood clotting, enzyme activation, neuromuscular function, muscle contraction, cell adhesion, and intracellular signalling (Veum, 2010). The 20% of P that is not found in the skeleton is situated within nucleic acids, nucleotides, phospholipids and phosphorylated proteins. These structures play a vital role in growth, cellular and membrane function, energy metabolism and acid-base balance (Wardlaw *et al.*, 2002; Berndt *et al.*, 2007; Veum, 2010).

2.4 Factors affecting bioavailability of calcium and phosphorus

When doing research on biological systems and more specifically absorption of nutrients and the effects they have on the system, it is important to remember that there will always be a number of interactions affecting the rate at which it is absorbed (Angel, 2013). No two animals are the same, inconsistencies in the animals that are being fed; as well as the sources and quality of the minerals being fed will interact and affect the absorption of nutrients in the animal. The magnitudes of the effect it will have on the animal will depend on specific biological interactions (Kleyn, 2013).

2.4.1 Growth rate and feed intake

The growth rate of broilers has dramatically increased in the recent decades of broiler production (Havenstein *et al.*, 1994). In 1925, 112 days were required to achieve an average weight of approximately 1.13 kg. In 1980, 53 days were required to achieve an average weight of 1.78 kg. In 2011, 47 days were required to achieve an average broiler weight of 2.63 kg. This was similarly accompanied by a decrease in mortality, going from 18% in 1925, down to 5% in 1980 and finally reached an average of 3.8% in 2011.

2.4.2 Age and sex

With increasing age, the physiology of a broiler changes and thus the requirements of nutrients and minerals change, for example, as a broiler grows the energy requirement increases relative to their protein requirement (Kleyn, 2013). Therefore, broilers are fed in different phases to accommodate changing requirements from hatching to slaughter. Similarly, the Ca and P requirements will change according to age. The sequential pattern of growth has been described by Sir John Hammond (1984), which can be seen in Figure 2.1. A newly hatched broiler chick has a primary need for brain and organ development, secondly for mineral deposition in the bones for structural support, then with increasing age, muscle and fat deposition occurs. Literature has shown that P digestibility together with retention and the hydrolysis of phytin decreases significantly ($P<0.05$) with increasing age (Yan *et al.*, 2005). However, Edwards *et al*. (1989) showed contradictory results as the findings illustrated an increase in phytin P utilisation (from 19% at 7 days of age to 36% at 21 days of age). This, yet again, emphasised the fact that P retention and utilisation is dependent on many accompanying factors that may result in different responses from one trial to another (Angel *et al.*, 2002).

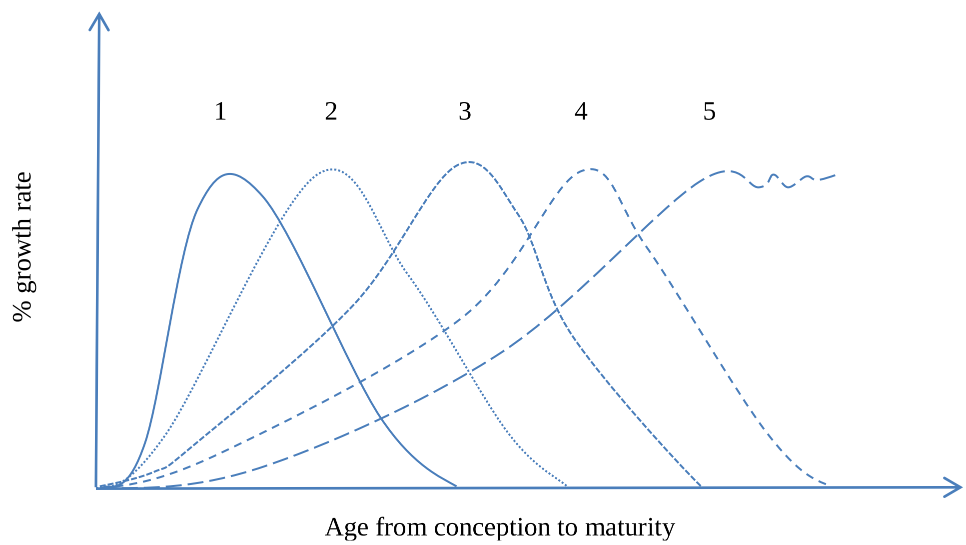


Figure 2.1 Sir John Hammond's analogy to describe the sequence of growth related to age. 1= Nervous tissue; 2= Bone; 3= Muscle; 4= Fat and 5= Feed intake (adapted from Hammond, 1984)

During the early stages of a chick's life (hatching to 18 days of age), there are periods of rapid bone mineralisation and formation, therefore requiring large mineral deposits. The challenge concerning the mineral supplementation of young chicks is that they require large amounts of P during rapid bone growth but has restricted feed intake due to the size of the chick (Williams *et al.*, 2000). During the rapidly growing phase of the bird, the fat content of the body increases and the bone growth slows down, therefore the P requirements decline as the bird ages (De Groote & Huyghebaert, 1997; Leske & Coon, 2002). Leske and Coon (2002) illustrated that the age of the bird has an effect on the requirement of minerals due to the physiological changes occurring at different ages.

2.4.3 Calcium and phosphorus sources

Phosphorus sources have developed over the years and include a variety of products on the market currently. These include monocalcium phosphate (MCP), dicalcium phosphate (DCP), mono-dicalcium phosphate (MDCP), defluorinated rock phosphate and monosodium phosphate. Broiler production serves an important part in the feed phosphate market as it accounts for approximately 50% of feed phosphates sold annually worldwide (Devereux *et al.*, 1994). Feed phosphates are an important contribution of available P in a broiler, supplying up to 60% of the NPP requirements of the broiler (Waldroup, 1999). If animal feed formulators are able to know the availability of P in the feed sources, they will be able to more accurately formulate to P requirements as a small difference in availability may have a significant effect on faecal P content. P availability has shown to vary among and between feed P sources (Waibel *et al.*, 1984; Van der Klis & Versteegh, 1999). Therefore, the use of an average P availability value for a P product may result in a considerable over- or under-estimation of the dietary P availability.

2.4.4 Calcium and phosphorus solubility

Solubility will vary within and between calcium and phosphorus sources. Ca sources that have a very high solubility (as can be found in fine limestone) can be detrimental to P absorption as Ca forms cation interactions with phytate phosphorus (Maenz & Classen, 1998; Cowieson *et al.*, 2011a) making it unavailable for absorption due to its insolubility (Angel *et al.*, 2002). Therefore, even if a high dosage of phytase is supplemented, it will not be able to act on the P. Due to these findings; it can be assumed that further lowering Ca supplementation would result in an improved effect of exogenous phytase and is the most realistic option for the South African commercial poultry industry. Literature showed that reducing Ca levels from 1% to 0.67% in combination with low NPP levels did not impair young bird performance (Létourneau-Montminy *et al.*, 2010; Powell *et al.*, 2011) and will have a positive effect on the bioavailability of the minerals.

Alternatively, supplying Ca sources with low solubility would also result in an improved effect on exogenous phytase as Ca would not dissociate to interact with phytate P (Hamdi *et al.*, 2015b). However, under the conditions faced in South Africa, in which most of the limestone is mined from one source and is of variable quality, it becomes difficult to regulate the quality of Ca source and to keep the quality and solubility constant.

2.4.5 Other nutrients in the diet

Mineral absorption may be complicated due to the many interactions between minerals as can be seen in Figure 2.2, which is a simplified diagram, only focusing on Ca and P interactions with other minerals and how they can potentially affect each other. Possible interactions include mutual antagonisms, which can reduce absorption of some minerals. Some interactions may cause the formation of insoluble precipitates, such as with phosphates, completely inhibiting mineral absorption (Cabell & Earle, 1965; Vohra *et al.*, 1965).

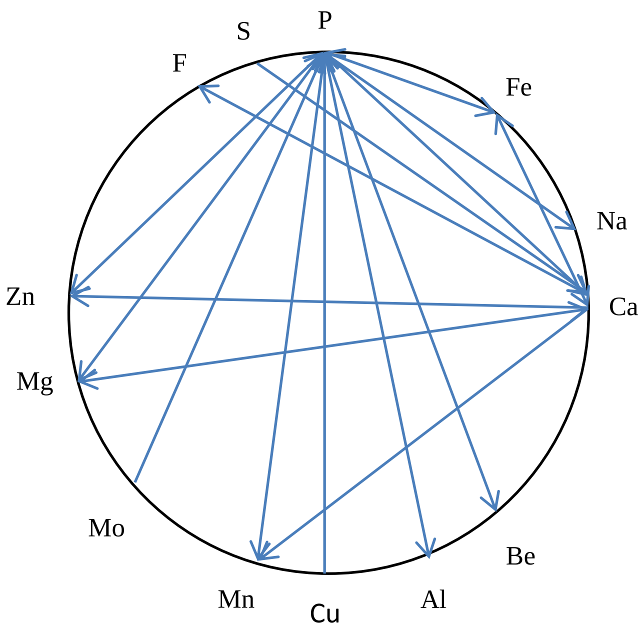


Figure 2.2 Potential interrelationships between minerals in intestinal lumen and metabolism, only considering the Ca and P interactions with other minerals (Adapted from Kleyn, 2013).

2.4.5.1 Calcium

Several minerals interact with Ca and should be considered when formulating diets for broilers (Kleyn, 2013). High Ca levels may interfere with the metabolism and availability of other minerals, including P, magnesium, manganese and zinc (NRC, 1994; Kleyn, 2013). More specifically, excess Ca is excreted as a Ca-P complex and thereby reducing P uptake, which may aggravate P deficiency for bone mineralisation (L´etourneau–Montminy *et al.*, 2008).

2.4.5.2 Vitamin D

Vitamin D_3 has been found to have inconsistent effects on NPP requirements (Faridi *et al.*, 2015). It has been found that broiler chicks were less tolerant to vitamin D with high Ca levels in the diet (Edwards *et al.*, 2004; Faidi *et al.*, 2015). High Ca levels in the feed may cause a hypercalcaemic environment in the gastrointestinal tract and no P to associate with for bone mineralisation (Liem *et al.*, 2009). Responses to supplemented vitamin D are best when Ca and P levels are low. Vitamin D and phytase have a synergistic effect on tibia ash response. This may be due to tibia ash being a sensitive indicator of P utilisation in broilers (Faidi *et al.*, 2015). Literature has shown that increasing the level of supplemented vitamin D while decreasing the Ca level resulted in an improved utilisation of phytate, even in the absence of exogenous phytase supplementation (Edwards, 1992; Fisher, 1992). There are different metabolites of vitamin D and it is debated whether all the metabolites are of equal efficacy. This may explain the inconsistencies in responses seen in literature when vitamin D is supplemented (Fisher, 1992). Addition of vitamin D decreased the NPP required to maximise average daily gain and feed intake, however, showed no effect on the NPP required to maximise feed efficiency and tibia ash (Fisher, 1992).

2.4.5.3 Dietary energy and protein

An experiment has been done by Driver *et al.* (2005) looking at Ca requirements at different protein levels. This experiment showed a significant protein by Ca interaction observed for body weight gain and bone ash. This appeared to be due to the improved efficiency of Ca absorption at higher protein levels. Therefore, having higher protein levels in the feed will result in increased Ca availability. Ca and P interact with nitrogen absorption. When a high Ca supplementation is fed, it results in more P being used for bone mineralisation. That leaves less P available for muscle growth, therefore hindering growth rate and average daily gain (L, de Lange 25 February 2016).

2.4.5.4 Phytate

Phytate is the result of phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) found in a salt form, often bound to Ca, Mg and K and in some cases bound to proteins and starches, thus making them unavailable for absorption. The structural form can be seen in Figure 2.3 (Suttle, 2010). There is a significant interaction between Ca and phytate on Ca absorption. Qian *et al.* (1997) fed broilers various levels of Ca, vitamin D and phytase to evaluate the fractional retention of Ca. It was found that Ca retention fell as Ca intake increased, the absorptive mechanism was, therefore, down-regulated and Ca was excreted as insoluble Ca-phytate complexes. However, the addition of vitamin D and phytase alleviated this response, thus resulting in a higher fractional retention at the same dietary Ca intake.

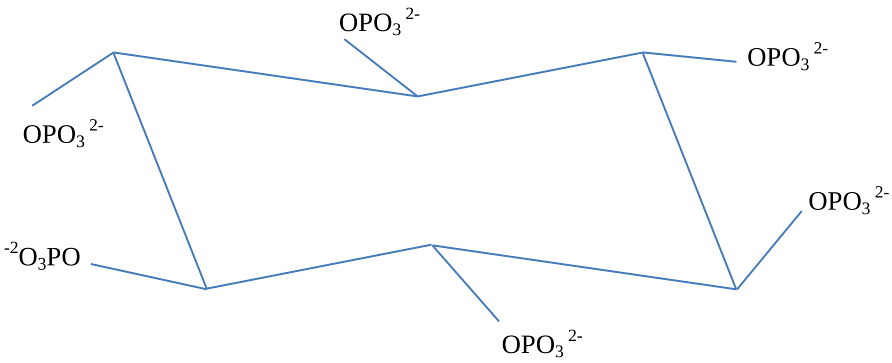


Figure 2.3 Structure of fully deprotonated form of phytic acid (Suttle, 2010)

The P found in most cereals and some oilcakes are largely present as phytate P which is unavailable to monogastric animals due to the lack of the phytase enzyme (Angel, 2011; Kleyn, 2013). Therefore, the addition of phytase enzyme reduces the negative interactions among nutrients in the digestive tract and has shown to improve trace mineral availability in monogastric animals; however, the presence of excess calcium may limit the action of this enzyme (Angel *et al.*, 2002b). The presence of additional phytase ("superdosing") also allows chicks to respond well to different Ca sources with varying levels of solubility (Hamdi *et al.*, 2015b).

2.4.5.5 Phytase

The target substrate of phytase is the plant form of phosphate. Phytase has three main functions: the release of phosphate, destruction of the anti-nutritional effects of phytic acid and the liberation of myo-inositol (Bedford, 2000). The use of phytase results in the animal to utilise plant P which would ordinarily be unavailable; thereby, reducing the need of supplementing with inorganic P sources, which ultimately results in a drastic decline in P pollution. P supplementation is the third most expensive feed ingredient (Angel *et al.*, 2002; Sacranie *et al.*, 2013), thus, using phytase in chicken feed would be economically beneficial as less P needs to be supplemented to achieve the same production efficiency (Bedford, 2000).

Over 66% of the P found in plant-based feed ingredients is not available for digestion as it is bound to phytic acid. This was thought to be due to low endogenous levels of phytase (Bedford, 2000; Woyengo & Nyachoti, 2011). Calcium forms insoluble complexes with phytate P, therefore, impeding phytase activity (Angel *et al.*, 2002). Consequently, reducing supplemented Ca also improves the activity of phytases and has an economic benefit in reducing dietary costs (Bedford, 2000). It is not only the level of Ca that affects the activity of phytase, but also the Ca to P ratio. The response to phytase becomes less notable when the Ca to P ratio increases (Qian *et al.*, 1997).

Phytase has been shown to enhance energy and amino acid digestibility, although the degree of improvement may vary between authors (Martin et al., 1998; Namkung & Leeson, 1999; Ravindran *et al.*, 1999). The improved energy and amino acid digestibility resulted in improved body weight gain with reduced feed intake (Ravindran *et al.*, 1999; Powell *et al.*, 2011). Exogenous phytase supplementation has shown to reduce the variation of P retention between cereals (Bedford, 2000). However, the exact improvement in P retention is highly variable and cannot be accurately quantified. This is due to the variation in response to different feed ingredients, the source of the phytase and age of the animal, making it difficult to predict. Phytase, while benefiting P retention, also improved the retention of Ca due to the strong interaction between Ca and P. Figure 2.4 shows the effect of phytase on Ca and P apparent ileal digestibility as described by Olukosi *et al.* (2007). A clear improvement in Ca and P retention can be seen with the addition of phytase in the broiler diet.

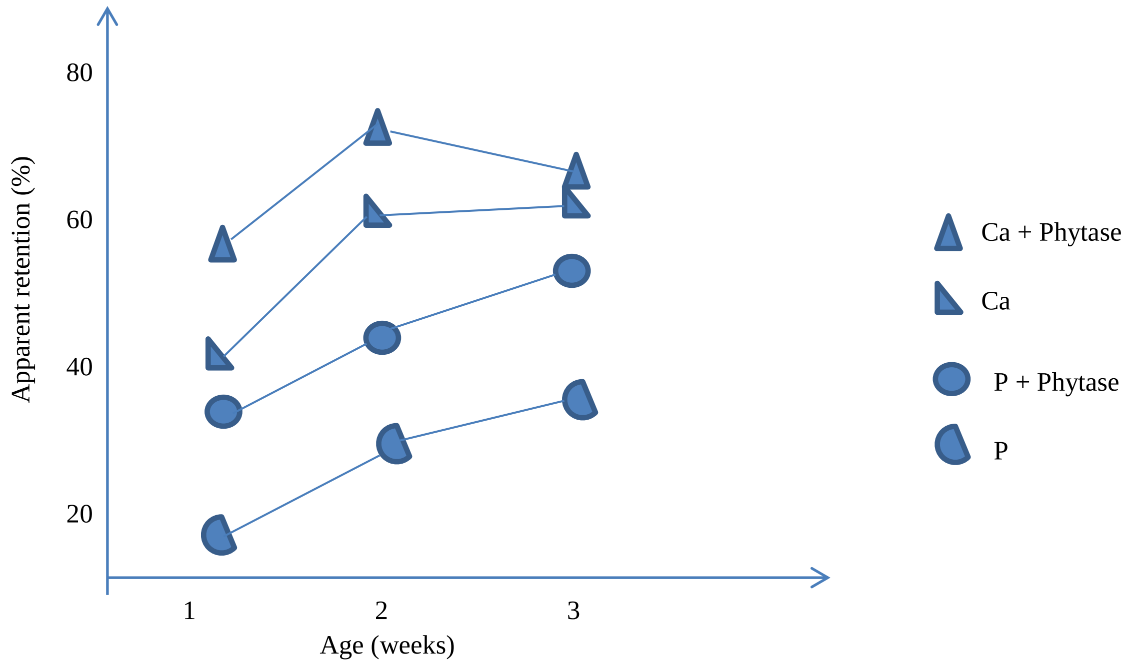


Figure 2.4 Effect of phytase on the apparent ileal retention of Ca and P during the first 3 weeks of a broiler's life (Olukosi *et al.*, 2007)

2.5 Calcium and phosphorous interactions

A significant interaction has been found between Ca and P for production parameters such as feed intake, body weight gain and feed conversion ratio, as well as bone development. Performance has been found to be poor when fed a diet high in Ca and low in available P. Similarly, performance has improved with increased AvP supplementation (Sacranie *et al.*, 2013; Wilkinson *et al.*, 2013). This is in agreement with the work done by Perney *et al.* (1993), which showed that feed intake of broilers was reduced at low levels of AvP and was significantly ($P < 0.05$) improved with higher levels of AvlP. This was thought to be due to the homeostatic regulation of Ca and P; feeding diets low in AvlP and high in Ca results in a decreased intake of the diet to restore the homeostasis. However, the mechanisms are not yet defined for this relationship. Plumstead *et al.* (2008) found that phytate P utilisation is negatively affected by Ca intake when feeding soybean meal as part of a broiler's diet.

Ca digestibility is the highest when Ca supplementation is high and AvlP is low (Cowieson *et al.*, 2006; Wilkinson *et al.*, 2013). Dietary Ca and AvlP were both found to influence apparent ileal digestibility of P. Wilkinson *et al.* (2013) concluded that the concentration of AvlP in a broiler diet is of more importance than the concentration of total Ca when performance parameters are observed.

Imbalances in Ca and P intake have been found to result in excess P excretion that has negative effects on the environment when poultry litter is applied as fertiliser, causing eutrophication and environmental pollution (Sharpley, 1995).

2.6 Broiler requirements for calcium and phosphorus

Calcium and phosphorus must be present in the broiler diet at sufficient amounts for proper enzymatic activity and bone development. The aim of poultry nutrition research is to determine the nutritional requirements of the birds and to meet these requirements in the most economical manner. These requirements change according to production phase which must be taken into consideration when formulating broiler diets (Van der Klis & Versteegh, 1999).

The mineral requirements are largely determined using the factorial approach in which the bird's requirement for maintenance and production are taken into account. The genotype, age, and level of performance of the bird are also considered (McDonald *et al.*,1995).

The NRC has compiled mineral requirements for broilers as illustrated in Table 2.3. P is represented as non-phytate phosphorus (NPP), which is often mistaken for available phosphorus (AvlP). NPP is wrongly assumed to be completely available and that all the phytate phosphorus is unavailable (Leske & Coon, 2002).

Table 2.3 Requirements for inorganic phosphorus (%) and calcium (%) for broilers from day old to 8 weeks of age (NRC, 1994).

Age (Weeks)	Inorganic phosphorus	Calcium
0-3	0.45	1.00
4-6	0.35	0.90
7-8	0.30	0.80

The research done for the determination of these requirements date from 1952 to 1983, making the applicability out-dated due to the leaps in genetic improvements of broilers as well as improvements in diet formulation as the requirements are better met for the rapidly growing commercial bird. Modern broiler strains are worlds different from older strains. Modern strains have faster growth rates and improved feed conversion ratios, efficiency of nutrient utilisation and bone structure (Dhandu & Angel, 2003). Therefore, new research needs to be done on the requirements of Ca and P for broilers.

Modern broilers have much faster growth rates and should, therefore, have higher requirements than older strains. On the other hand, they are more efficient at utilising nutrients, which lowers their requirements for Ca and P (Havenstein *et al*, 1994; Van der Klis & Versteegh, 1999; Havenstein *et al.*, 2002; Leske & Coon, 2002). Van der Klis & Versteegh (1999) determined that a young broiler requires between 2.35 and 3.68 g AvlP/kg, which would decrease with age due to the declined rate of bone growth. Leske & Coon (2002) did similar work and came to the conclusion that 3.9 g RP/kg would meet the requirement of young broilers.

In the later stages of production, the P supplementation can theoretically be drastically lowered due to the large feed intakes. As seen in Table 2.3, the current NRC recommendations for 4-6 weeks and 7-8 weeks of age are 0.35 and 0.30 % NPP, respectively. Dhandu & Angel (2003) determined a requirement of 0.20 and 0.16 for the same age groups, respectively. Their data is based on a broken line analysis of the bone ash. This data shows that the Ca and P requirements may be in actuality much lower than what the NRC recommended, which may be due to a better understanding of the field or the removal of excessive safety margins.

There have been innumerable amounts of intensive and extensive research done on Ca and P requirements for broilers, including research specifically on different breeds of broilers by the broiler breeder companies themselves, such as Aviagen (Ross 308, 2009), Cobb (Cobb 500, 2012) and Hubbard (Hubbard Flex) which is shown in Table 2.4. These three breeding companies seem to mostly agree with each other.

Table 2.4 A comparison of the calcium and phosphorus recommendations made by three primary breeding companies, Cobb, Aviagen, and Hubbard

	Cobb 500 (2012)	Ross 308 (2009)	Hubbard Flex
		Starter	
Ca (g/kg)	9	10.5	10
Available P (g/kg)	4.5	5	5
Ca:AvlP	2	2.1	2
		Grower	
Ca (g/kg)	8.4	9	9.5
Available P (g/kg)	4.2	4.5	4.5
Ca:AvlP	2	2	2.12
		Finisher	
Ca (g/kg)	7.6	8.5	8.5
Available P (g/kg)	3.2	4.2	4
Ca:AvlP	2	2.02	2.12

Recent research studied the requirements specifically for bone growth or for production (growth rate) purposes. However, due to the inconsistency in details provided in published literature and differences in methodologies used between studies, it becomes challenging to arrive at a fixed Ca and P requirement for broilers at different growth phases that apply to different breeds and under different management systems. Table 2.5 summarises research on Ca and NPP requirements for different breeds and different purposes, such as performance, body weight, tibia ash (skeletal structure) and bone breaking force.

Table 2.5 Summary of literature of calcium (Ca) and non-phytate phosphorus (NPP) requirements for broilers

Reference	**Criteria**	**Breed**	**Age (Days)**	**BW gain (g)**	**Ca (%)**	**nPP (%)**
Morgan & Todd, 1994	Performance	Ross X AA[1] M[2]	Hatch-21d	683	1	0.31
Waldroup *et al.*, 2000	Body weight	Cobb 500 M	Hatch-21d	620	1	0.32
Waldroup *et al.*, 2000	Tibia Ash	Cobb 500 M	Hatch-21d	678	1	0.39
Waldroup *et al.*, 2003	Performance	Cobb M	Hatch-21d	360	0.9	0.4
Huyghebeart, 1996	Performance	Ross, M	Hatch-21d	613	0.8	0.3
Ribeiro *et al.*, 2003	Performance, Tibia Ash	Cobb M/F	Hatch-15d	315	1	0.36
Brenes *et al.*, 2003	Performance, Tibia Ash	Cobb M/F	Hatch-21d	587	0.82	0.35
Waldroup *et al.*, 2003	Performance, Breaking force	Cobb	14-35 days	1512	0.8	0.3

[1] AA: Breed specified as AA (Arbor Acres)

[2] M, F: Sex Specified as M (males), F (females)

Angel (2011) presented a summary of the research pertaining to Ca and P requirements, which is shown in Table 2.5. Angel (2011) cited numerous research reports on the requirements of Ca and P for broilers, but hardly found any agreement between results when it comes to Ca and P requirements.

Table 2.6 Summary of the findings of Angel (2011) showing the range of calcium and phosphorus requirements for broilers across literature (median shown in brackets)

Feeding Phase	**Ca %**	**Available P %**
Starter (1-21 days)	0.61-1.31 (1.0)	0.17-0.45 (0.35)
Grower (22-42 days)	0.69-0.91 (0.89)	0.15-0.45 (0.30)

Sacranie *et al.* (2013) conducted an experiment in which high and low Ca and digestible P levels were fed to broilers with and without the addition of phytase. It was found that the young broilers performed the best when fed a low Ca concentration with a standard digestible P concentration, whereas the high Ca levels resulted in poor performance results. These conclusions agree with the work done by Hopcroft *et al.* (2016) where it was found that broiler chicks still absorbed a large amount of Ca from the yolk sac during the first 3 days of life. The addition of Ca in the diets resulted in an over-abundance of Ca in the young chick and a decrease in bone mineralisation due to the antagonistic effect of Ca and P. Hopcroft *et al.* (2016) then concluded that Ca concentrations should be lowered for broiler diets. Walk *et al.* (2016) showed that there is a limit to the reduction of Ca concentrations in the feed. When the diets contained less than 0.60% Ca with a 0.30% AvlP, it resulted in a significantly ($P < 0.05$) lowered bone ash but also the highest Ca retention while maintaining adequate growth, however, not enough to increase bone ash. When Ca and P were fed at a 1:1 ratio of 0.50% Ca and 0.50% AvlP, Ca retention was not affected, but P retention declined significantly ($P < 0.05$), which showed that the bird was able to maintain an appropriate Ca and P balance in the body during periods of low Ca concentrations in the feed.

Chapter 3

Methods and Materials

3.1 Birds and housing

The trial was run at the test facilities at Daybreak Farms, Sundra, South Africa. Broilers were housed in a standard open-sided broiler house fitted with tunnel ventilation. Two thousand eight hundred and eighty (2880) vaccinated day-old Ross 308 chicks were purchased from Midway Chix. On arrival at the trial house, chicks were sexed using the feather sexing technique. A total of 60 chicks were placed per pen and were made up of 30 randomly selected male chicks and 30 randomly selected female chicks in one of 48 pens, which totalled in 2880 chicks placed. Each pen had an area of $3m^2$ and chicks were placed at a stocking density of 20 birds per m^2. The temperature profile that was followed from 2 days pre-placement to Day 35 is shown in Table 3.1; the lighting profile is shown in Table 3.2 and the vaccination program is shown in Table 3.3. The bedding consisted of pine shavings of approximately 10cm deep and extra pine shaving were added to the pen where needed throughout the trial. Each pen contained 2 auger feeders and 4 nipple drinkers.

The birds were monitored on a daily basis by the principal investigator and trial farm staff. There was farm personnel on the premises at all times throughout the trial to monitor the birds' comfort regarding heat, ventilation, feed and water supply and general health. Temperature and humidity loggers were installed in both houses at the beginning of the trial to ensure maximum comfort was maintained for the birds throughout the trial. The birds had *ad libitum* access to feed and water at all times.

Table 3.1 Temperature profile of the trial house from 2 days before placement to slaughtering at 35 days

Day	Target floor temperature (°C, 50 % rH[1])
1 day before placement to 2	35.5
3 to 5	34.5
6 to 8	33.5
9 to 11	29.7
12 to 14	27.2
15 to 17	26.2
18 to 20	25.0
21 to 23	24.0
24 to 35	23.0

[1]rH=Relative Humidity

Table 3.2 Lighting program of the trial house from placement of the Ross broiler chicks to slaughter at 35 days of age

Day	**Controller's set point**			
	Lights on	**Lights off**	**Hours of Daylight**	**Hours of Darkness**
1 to 3	00:00	23:00	23	1
4 to 8	00:00	21:00	21	3
9 to 11	05:00	22:00	17	7
12 to 15	05:00	20:00	15	9
16 to 33	05:00	19:00	14	10
34 to 35	02:00	22:00	20	4

Table 3.3 Vaccination program (New Castle Disease and Infectious Bronchitis) of the Ross 308 broilers during the trial

Age (days)	**Vaccination**	**Method**	**Trade name**	**Supplier**
Hatchery	NCB[1]	Spray	Avinew	Merial South Africa (Pty) Ltd
Hatchery	IB[1]	Spray	Bioral H120	Merial South Africa (Pty) Ltd
10-12 days	NCB	Water	TAbic VH	Phibro Animal Health
10-12 days	IB	Water	TAbic MB	Phibro Animal Health
16-18 days	NCB	Water	Avinew	Merial South Africa (Pty) Ltd

[1]NCB = New Castle Disease; IB = Infectious Bronchitis

3.2 Experimental design and treatments

A randomised block design was used in this trial. There were 6 treatments, each replicated 8 times. The house was divided into 8 blocks with a replicate per treatment in each block. Figure 3.1 depicts the layout of the pens and blocks within the house. The six treatment diets are shown in Table 3.4 and contained varying levels of dietary Calcium and Phosphrous in the different feeding phases and were determined using Ross 308 guidelines (Ross 308, 2009) and varying lowered Ca and P levels. The lower levels of Ca and P were chosen according to recommendations by Dr Roselina Angel (14 October 2015).

The feed was a soyabean-maize-based diet, formulated according to Ross 308 guidelines and was blended by AFGRI Animal Feeds at the Isando Plant (Isando, South Africa). The birds received *ad libitum* feed and water and were monitored daily to ensure water was running and feed was refilled as needed. A 5 phase feeding program was used, namely, Pre-starter (PS) fed as a an expanded crumble from placement of the day old chick to 10 days of age, Starter (S) also fed as an expanded crumble from 11 to 17 days of age, Grower (G) fed in pellet form from 18 to 27 days of age, Finisher (F) fed in pellet form from 28 to 30 days of age and lastly Post-finisher (PF) fed in pellet form from 31 to 35 days of age at which the broilers were slaughtered. The feed phases, feeding periods, expected feed intake (Ross 308, 2009) and feed allocation with a safety margin is shown in Table 3.5. The trial feeds were formulated separately on a least cost basis using Format (© Format International Limited, Woking, England. Version 1-May-1998/23.4) and were chemically analysed by Labworld (Pty) Ltd, a division of Philafrica as described in section 3.4.1. The raw material inclusions and calculated nutrient compositions can be found of each feeding phase in Table 3.6; 3.7; 3.8; 3.9 and 3.10 for the PS, S, G, F and PF diets, respectively. Phytase was added to all the treatments at a dosage of 1000 IU to the PS, S and G diets. The phytase levels had to be decreased to 650 IU for the F and PF diets to reach the levels that were defined for treatments 4 and 6.

Block 1	T[1]6[2] T1 T2 T5 T3 T4	T1 T5 T2 T4 T3 T6	**Block 5**
Block 2	T5 T2 T1 T3 T6 T4	T6 T4 T1 T5 T3 T2	**Block 6**
Block 3	T5 T1 T6 T3 T4 T2	T1 T3 T6 T4 T5 T2	**Block 7**
Block 4	T1 T5 T6 T2 T3 T4	T1 T4 T3 T6 T5 T2	**Block 8**

[1]T=Treatment [2]Number 1-6 = treatment number

Figure 3.1 Layout of the pens and blocks in the trial house with the random treatment allocations to each pen

Table 3.4 Calcium and phosphorus levels used in the trial treatments for Ross 308 broilers during the trial

	Pre-Starter		Starter		Grower		Finisher		Post Finisher	
Age,d[1]	0-10		11-17		18-27		28-30		31-35	
	Ca[2]	RP	Ca	RP	Ca	RP	Ca	RP	Ca	RP
Trt[3] 1	1.05	0.50	0.96	0.48	0.87	0.44	0.80	0.40	0.76	0.38
Trt 2	1.05	0.50	0.96	0.48	0.87	0.44	0.55	0.25	0.52	0.25
Trt 3	1.05	0.50	0.96	0.48	0.64	0.32	0.55	0.25	0.52	0.25
Trt 4	1.05	0.50	0.96	0.48	0.64	0.32	0.49	0.20	0.47	0.20
Trt 5	1.00	0.55	0.82	0.37	0.64	0.32	0.55	0.25	0.52	0.25
Trt 6	1.00	0.55	0.82	0.37	0.64	0.32	0.49	0.20	0.47	0.20

[1]d=day; 0=hatching

[2]Ca=Calcium; RP=retainable phosphorus

[3]Trt= Treatment

The only differences between the treatments were the different Ca and P levels. Treatment 1 consisted of the Ca and P levels described by the Ross 308 guidelines (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and was used as the positive control. Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%). Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%).Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). Treatment (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%). Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%).

Table 3.5 The feeding phases with feeding periods and expected intakes per bird (Ross 308, 2009).

Feed (5 phases)	Feeding period (days)	Feed intake (g/bird)	Feed allocation/pen (kg)
Pre-starter	10	450	27
Starter	7	633	38
Grower	10	1467	88
Finisher	3	633	38
Post-finisher	5	1000	60

Table 3.6 Raw material inclusion (%) and calculated nutrient composition for Pre-starter treatments

Raw material	Ross (Treatments 1-4)	LOW1 (Treatments 5, 6)
Maize yellow (fine)	55.6	55.6
Soya oilcake meal 46	32.6	32.6
Sunflower oilcake	5	5
Full fat germ	2.15	2.15
Synthetic lysine (Biolys®)	0.344	0.3434
Synthetic methionine (MetAMINO®)	0.275	0.275
Synthetic threonine (ThreAMINO®)	0.046	0.046
Amino acid additive (CreAmino®)	0.06	0.06
Limestone	1.7	1.45
Mono-dicalcium phosphate (Local)	1.25	1.5
Bicarbonate of soda	0.279	0.277
Fine salt	0.26	0.26
Broiler Premix 1 (Clinacox + Olaquindox)	0.27	0.27
Phytase (Axtra Phy 1000 TPT Broilers)	0.1	0.1
Feed enzyme (Axtra XAP)	0.05	0.05
Choline chloride liquid. Lm(75%)	0.045	0.045
Calculated nutrient composition (%)		
Dry matter	88.9	88.9
Crude protein	22.3	22.3
Crude fat	3.50	3.50
Crude fibre	3.76	3.76
Ash	6.34	6.34
Apparent metabolisable energy (MJ/kg)[1]	11.3	11.3
Total calcium[2]	**1.05**	**0.999**
Total phosphorus[2]	**0.693**	**0.747**
Retainable phosphorus	**0.503**	**0.545**
Available phosphorus	**0.499**	**0.550**

[1] AME for broiler chicks (CVB)

[2] 0.08% Ca made available by addition of phytase enzyme; 0.08% P made available by addition of phytase enzyme

Table 3.7 Raw material inclusion (%) and calculated nutrient composition for Starter trial feed

Raw material	Ross (Treatments 1, 2)	LOW1 (Treatments 3-6)
Maize yellow (fine)	50.8	50.25
Soya oilcake meal 46	22.2	21.7
Wheat bran	10.8	12.5
Sunflower oilcake	6	6
Gluten 60	4.15	4.38
Energy oil blend	1.57	1.52
Synthetic lysine (Biolys®)	0.47	0.476
Synthetic methionine (MetAMINO®)	0.222	0.217
Synthetic threonine (ThreAMINO®)	0.0545	0.0528
Amino acid additive (Creamino®)	0.06	0.06
Limestone	1.52	1.4
Mono-dicalcium phosphate (Local)	1.13	0.475
Bicarbonate of soda	0.3115	0.323
Fine salt	0.215	0.208
Broiler Premix 1 (Clinacox + Olaquindox)	0.27	0.27
Phytase (Axtra Phy 1000 TPT Broilers)	0.1	0.1
Feed enzyme (Axtra XAP)	0.05	0.05
Choline chloride liquid. Lm(75%)	0.045	0.045
Calculated nutrient composition (%)		
Dry matter	88.9	88.8
Protein	21.7	21.8
Crude fat	4.38	4.38
Crude fibre	4.18	4.28
Ash	6.04	5.35
Apparent metabolisable energy (MJ/kg)[1]	11.3	11.3
Total calcium[2]	**0.948**	**0.808**
Total phosphorus[2]	**0.700**	**0.571**
Retainable phosphorus	**0.480**	**0.372**
Available phosphorus	**0.505**	**0.380**

[1] AME for broiler chicks (CVB)

[2] 0.08% Ca made available by addition of phytase enzyme; 0.08% P made available by addition of phytase enzyme

Table 3.8 Raw material inclusion (%) and calculated nutrient composition for Grower trial feed

Raw material	Ross (Treatments 1, 2)	LOW1 (Treatments 3-6)
Maize yellow (fine)	60.1	58.6
Soya oilcake meal 46	24.2	24.1
Wheat bran	6.12	7.9
Sunflower oilcake	4	4
Full fat germ	2.6	2.58
Synthetic lysine (Biolys®)	0.343	0.341
Synthetic methionine (MetAMINO®)	0.244	0.242
Synthetic threonine (ThreAMINO®)	0.057	0.056
Amino acid additive (Creamino®)	0.06	0.06
Limestone	1.35	1.03
Mono-dicalcium phosphate (Local)	0	0.225
Fine salt	0.25	0.248
Bicarbonate of soda	0.127	0.128
Broiler Premix 1 (Clinacox + Olaquindox)	0.135	0.135
Broiler Premix 3 (Clinacox + Olaquindox)	0.135	0.135
Phytase (Axtra Phy 1000 TPT Broilers)	0.1	0.1
Feed enzyme (Axtra XAP)	0.05	0.05
Choline chloride liquid. Lm(75%)	0.04	0.04
Calculated nutrient composition (%)		
Dry matter	88.7	88.7
Protein	19.5	19.5
Crude fat	3.95	3.95
Crude fibre	3.87	3.96
Ash	4.39	4.39
Apparent metabolisable energy (MJ/kg)[1]	11.5	11.5
Total calcium[2]	**0.867**	**0.640**
Total phosphorus[2]	**0.618**	**0.477**
Retainable phosphorus	**0.440**	**0.321**
Available phosphorus	**0.446**	**0.310**

[1] AME for broiler chicks (CVB)

[2] 0.08% Ca made available by addition of phytase enzyme; 0.08% P made available by addition of phytase enzyme

Table 3.9 Raw material inclusion (%) and calculated nutrient composition for Finisher trial feed

Raw material	Ross (Treatments 1)	LOW1 (Treatments 2, 3, 5)	LOW2 (Treatments 4, 6)
Maize yellow (fine)	64.6	63.8	65.5
Soya oilcake meal 46	24.8	24.45	24.4
Sunflower oilcake	3	2.9	0
Full fat maize germ	2.8	2.65	2.43
Wheat bran	1.03	3.6	3.93
Gluten 60	0	0	1.43
Synthetic lysine (Biolys®)	0.333	0.332	0.348
Synthetic methionine (MetAMINO®)	0.246	0.242	0.235
Synthetic threonine (ThreAMINO®)	0.0585	0.056	0.0548
Amino acid additive (Creamino®)	0.06	0.06	0.06
Broiler Premix 3 (Clinacox + Olaquindox)	0.27	0.27	0.27
Limestone	1.28	0.925	0.85
Mono-dicalcium phosphate (Local)	1.05	0.22	0
Fine salt	0.26	0.258	0.255
Bicarbonate of soda	0.094	0.104	0.109
Phytase (Axtra Phy 1000 TPT Broilers)	0.065	0.065	0.065
Feed enzyme (Axtra XAP)	0.05	0.05	0.05
Choline chloride liquid. Lm(75%)	0.04	0.04	0.04
Calculated nutrient composition (%)			
Dry matter	88.9	88.7	88.6
Protein	18.9	19.0	19.1
Crude fat	4.00	4.00	4.01
Crude fibre	3.42	3.56	3.08
Ash	5.14	4.10	3.72
Apparent metabolisable energy (MJ/kg)[1]	11.9	11.9	12.1
Total calcium[2]	**0.796**	**0.552**	**0.487**
Total phosphorus[2]	**0.599**	**0.438**	**0.367**
Retainable phosphorus	**0.399**	**0.263**	**0.220**
Available phosphorus	**0.398**	**0.242**	**0.194**

[1] AME for broiler chicks (CVB)

[2] 0.08% Ca made available by addition of phytase enzyme; 0.08% P made available by addition of phytase enzyme

Table 3.10 Raw material inclusion (%) and calculated nutrient composition for Post Finisher trial feed

Raw material	Ross (Treatment 1)	LOW1 (Treatments 2, 3, 5)	LOW2 (Treatments 4, 6)
Maize yellow (fine)	67.9	67.6	67.5
Soya oilcake meal 46	25.8	24.25	24.55
Full fat germ	2.75	2.7	2.65
Sunflower oilcake	0	1.7	1.05
Wheat bran	0	1.38	2.15
Synthetic lysine (Biolys®)	0.298	0.321	0.314
Synthetic methionine (MetAMINO®)	0.24	0.236	0.237
Synthetic threonine (ThreAMINO®)	0.051	0.0533	0.0528
Amino acid additive (Creamino®)	0.06	0.06	0.06
Limestone	**1.23**	**0.85**	**0.85**
Fine salt	0.278	0.265	0.268
Mono-dicalcium phosphate (Local)	**0.98**	**0.22**	**0**
Bicarbonate of soda	0.0703	0.0947	0.0922
Broiler Finisher Premix (No Medication & Available Zinc)	0.1	0.1	0.1
Phytase (Axtra Phy 1000 TPT Broilers)	0.065	0.065	0.065
Feed enzyme (Axtra XAP)	0.05	0.05	0.05
Choline chloride liquid. Lm(75%)	0.04	0.04	0.04
Calculated nutrient composition (%)			
Dry matter	88.9	88.9	88.8
Protein	18.4	18.5	18.5
Crude fat	4.05	4.06	4.06
Crude fibre	2.89	3.25	3.19
Ash	4.88	3.88	3.68
Apparent metabolisable energy (MJ/kg)[1]	12.2	12.2	12.2
Total calcium[2]	**0.761**	**0.522**	**0.488**
Total phosphorus[2]	**0.552**	**0.410**	**0.363**
Retainable phosphorus	**0.380**	**0.255**	**0.218**
Available phosphorus	**0.375**	**0.229**	**0.188**

[1] AME for broiler chicks (CVB)

[2] 0.08% Ca made available by addition of phytase enzyme; 0.08% P made available by addition of phytase enzyme

3.4 Measurements

3.4.1. Chemical analysis of feed samples

The chemical analyses of the feed samples were done to verify that formulated and actual Ca and P were close. Representative samples were taken by collecting 100 g per 100 kg of feed that was produced. The individual 100g samples were then mixed per treatment and then split down to a 250 g sample. The representative sample of the 6 different treatments was collected and chemically analysed by Labworld (Pty) Ltd for total calcium and total phosphorus by segmented flow (AOAC 960.06) using a Skalar Auto Analyser, Model number 1050 (AOAC, 2000). The determination of phosphate utilised the reaction between phosphorus and molybdovanadate which formed a yellow complex in a buffered acidic medium at a pH of 1 to 0.5. The reaction was read at a wavelength of 420 nm. The automated procedure for the determination of Ca was based on the following reaction: the sample was mixed with an acid 8-hydroxyquinoline solution to complex magnesium. Calcium was complexed with a cresolphthalein complexone solution in an alkaline medium; this complex was measured at a wavelength of 580 nm. Table 3.11 shows the Ca and P results from the chemical analysis as well as the formulated levels of the different treatments.

Table 3.11 Formulated and analysed total calcium and total phosphorus concentrations in the final trial diets

		Calcium%		**P% (total P)**	
Phase	Treatment	Formulated	Analysed	Formulated	Analysed
Pre starter	1 - 4	1.05	0.96 ± 0.06	0.69	0.65 ± 0.02
Pre starter	5, 6	1.00	0.92 ± 0.02	0.75	0.73 ± 0.04
Starter	1, 2	0.96	0.89 ± 0.01	0.70	0.67 ± 0.01
Starter	3 – 6	0.82	0.81 ± 0.03	0.57	0.58 ± 0.01
Grower	1, 2	0.87	0.85 ± 0.02	0.62	0.63 ± 0.02
Grower	3 – 6	0.64	0.69 ± 0.04	0.48	0.61 ± 0.02
Finisher	1	0.80	0.78 ± 0.01	0.60	0.59 ± 0.01
Finisher	2, 3, 5	0.55	0.60 ± 0.01	0.44	0.51 ± 0.04
Finisher	4, 6	0.49	0.53 ± 0.02	0.37	0.41 ± 0.01
Post finisher	1	0.76	0.83 ± 0.01	0.55	0.49 ± 0.01
Post finisher	2, 3, 5	0.52	0.59 ±0.01	0.41	0.45 ± 0.03
Post finisher	4, 6	0.49	0.54 ± 0.01	0.36	0.43 ± 0.01

3.4.2 Performance measurements

3.4.2.1 Body weight (BW)

Broilers were weighed weekly to obtain average BW for each individual pen. All the birds in a pen were weighed collectively in a crate, which was tarred before every weighing, and the weight averaged by dividing by the number of birds in the pen. The day old chicks were weighed at placement and then again at 7, 14, 21, 28 and 35 days of age. The average body weight gain for the week was then divided by 7 to determine the average daily body weight gain.

3.4.2.2 Feed intake (FI)

Weekly feed intake was measured by weighing out a specific amount at the beginning of the phase and weighing the feed that was left over at the end of the week. The weekly weighing of feed intake occurred at the same time as the weighing of the birds. Cumulative feed intake was calculated by the summation of the weekly feed intakes.

3.4.2.3 Feed conversion ratio (FCR)

The cumulative FCR was calculated by dividing the cumulative FI by the total BW gained (g/bird) over the experimental period. Weekly FCR was calculated by dividing weekly FI by the weekly BW gained (g/bird day) and was corrected for mortality by adding the weights of the mortality during the week to the body weight gained during the week.

3.4.2.4 Performance efficiency factor (PEF)

The PEF value is a calculated figure incorporating all of the performance factors and is regarded as a good measure of overall performance.

PEF = (Liveability % x Mass (kg)/ Age in Days x FCR) x100

3.4.2.5 Mortalities

The trial house was inspected twice daily and any mortalities were removed and weights recorded.

3.5 Bone ash

At 10 days of age, four male chicks were selected according to the average pen weight. The weights of the birds were recorded and the selected birds were euthanised by cervical dislocation. The right legs were removed and placed into appropriately labelled sealable bags. The same was done at 35 days of age; however, both legs were removed as they were used for bone breaking strength in duplicate before bone ash was determined. All the flesh and tissue was removed from the bones leaving a clean tibia and ensuring that the cartilage cap was removed. Ash %, as well as mg ash per tibia, were determined on a dry defatted basis on individual bones (AOAC, 1990). After the defleshing of the tibias, they were dried in an oven overnight at 55^{o}C. The dried bones were then placed in individual muslin bags and placed into the large cylinder of the Soxhlet apparatus. The bowl heater was turned up to the highest point to allow the petroleum ether to boil. Once the petroleum ether reached boiling point, the temperature was turned down to prevent the petroleum ether from recycling too quickly. The petroleum ether was recycled for 12 hours which removed the fat from the bones. The bones were then removed and placed under an exhaust fan to dry and remove any excess ether. The bones were then weighed before being ashed for 12 hours in a muffle furnace at 600^{o}C. Ash measurements were averaged per pen and statistical analysis was done on pen means.

3.6 Bone breaking strength

The tibia bones that were removed at 35 days of age were used for bone breaking strength determination and were done in duplicate, meaning that both the left and the right tibia of each of the sampled birds were used and the totals averaged. A texture analyser (Lloyd Instron) was used to do a 3 point break test, with a support distance of 36 mm, using a 5 kN load cell descending at 4 mm per min.

Parameters that were measured included maximum load (kN), maximum machine extension (mm) and time that it took for the bones to break (Sec). From these parameters, Stress (kN/mm^2) could be calculated. Stress is a common parameter of measurement when examining bone breakage and can be calculated by dividing the load (kN) which the bone undertook by the transverse area at which the break occurred.

3.7 Phosphorus excretion

Litter samples were collected from 5 areas within each pen. Four samples were taken 1 m from each corner along the diagonal tangent of the pen and 1 area in the centre of the pen. The 5 samples from each pen were mixed and a composite sample was taken. The moisture content of the litter was analysed by drying samples overnight at 105°C. The phosphorus analysis was done at Nutrilab at the University of Pretoria (AOAC, 2000).

3.8. Statistical analysis

SAS (Statistical Analysis System, 2014) was used to do the statistical analysis on the data. An Analysis of Variance (ANOVA) was done with the general linear model (GLM) to determine significant ($P<0.05$) differences between treatments. Means, standard error, and significance of differences between means were determined by Tukey HSD test (Tukey, 1949) at the 95% confidence level. Treatment was considered a fixed effect and regressions were determined on pen averages of the measured parameters.

Chapter 4

Results

4.1 Performance data

4.1.1 Body weight

The average weekly body weights of the broilers during the 35 day trial period are shown in Table 4.1 and illustrated in Figure 4.1.

Day old to 21 days of age

There were no significant differences (P > 0.05) found in body weights between the broilers that received different treatments from day old to 21 days of age.

Day 28

At 28 days of age, the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) had the highest average body weight but was not significantly heavier (P > 0.05) than those that received Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%). The broilers that received Treatment 1, however, was significantly heavier (P < 0.05) than those that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%).

Day 35

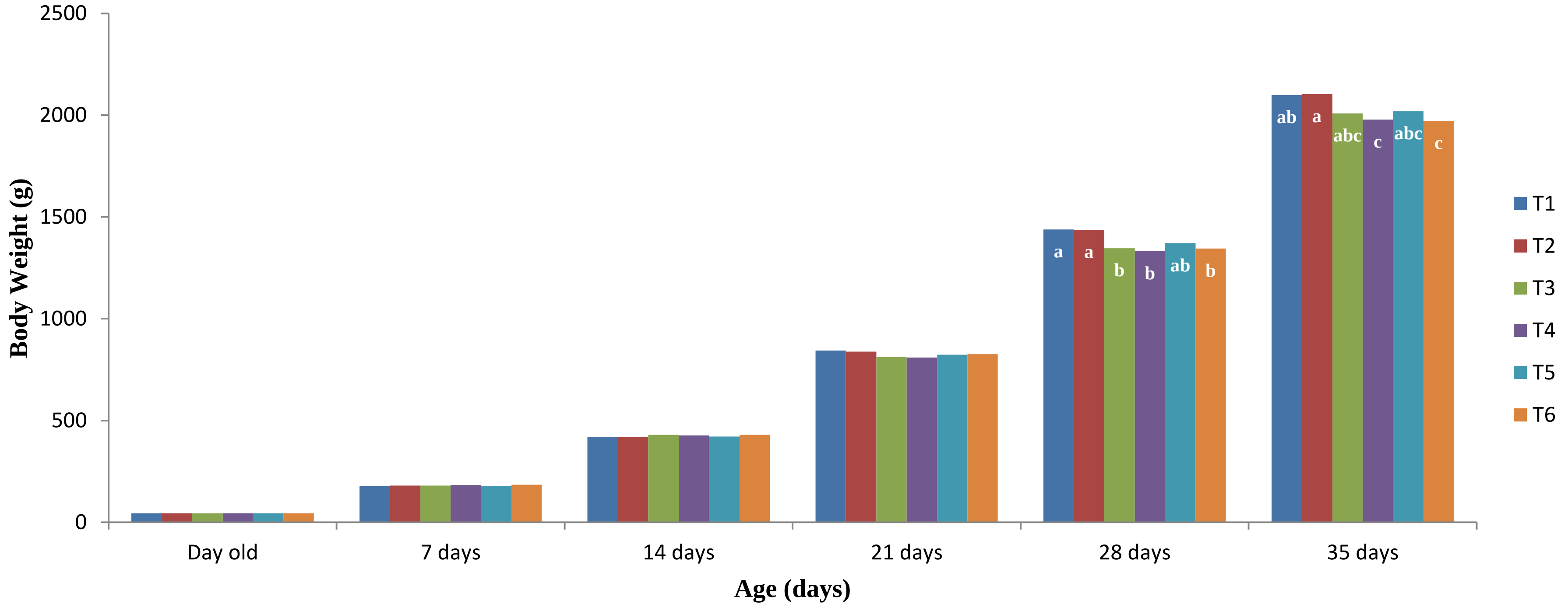
At 35 days of age, broilers that received Treatments 1 and those that received 2 were significantly heavier ($P \leq 0.05$) than those that received Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). No other significant differences (P > 0.05) were found between treatments for cumulative body weight gain.

Table 4.1 Weekly cumulative body weight gain (g) of Ross 308 broilers that received different levels of calcium and phosphorus

Treatment*	**Day old**	**7 days**	**14 days**	**21 days**	**28 days**	**35 days**
T1	43.2	176.9	420	843.6	1438^{a}	2098^{ab}
T2	43.2	180.0	417.5	837.9	1436^{a}	2103^{a}
T3	43.2	179.5	428.7	812.1	1347^{b}	2008^{abc}
T4	43.4	182.4	425.9	808.5	1332^{b}	1978^{c}
T5	43.2	178.4	421.2	823.1	1370^{ab}	2019^{abc}
T6	43.4	184.2	429.6	825.4	1345^{b}	1972^{c}
SEM	0.207	2.026	4.933	12.219	18.196	21.751

^{abc} Column means with the same superscripts are not significantly (P>0.05) different from each other.
T1: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

In summary, the different Ca and P levels did not affect the body weight before 28 days of age. At 28 days of age the broilers that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had lower body weights ($P \leq 0.05$) than those that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%). This trend carried on to 35 days of age as the broilers that received Treatment 4 and Treatment 6 had lower body weights ($P \leq 0.05$) than the other groups. At 35 days of age, the broilers that received Treatment 3 was significantly lower than those that received Treatment 1 and Treatment 2 which was similar to results found for 28 days of age and can be seen in figure 4.1.



[abc] Column means with the same superscripts are not significantly different (P > 0.05) from each other.

* **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.1 Weekly cumulative body weight gain (g) of Ross 308 broilers that received different levels of calcium and phosphorus

4.1.2 Body weight gain (g/day)

Day 0 to day 14

No significant differences (P > 0.05) were found for the daily growth rates (g/bird/day) between the broilers that received different treatments during the first 2 weeks of the experiment as seen in Table 4.2.

Day 15 to 21

During the third week, the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) had the highest average daily growth rate and grew at a significantly faster rate (P ≤ 0.05) than those that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). No other significant (P > 0.05) differences were found between treatment means.

Day 22 to 28

Figure 4.2 shows that during the fourth week from 22 to 28 days of age that the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and 2 gained body weight at a faster rate (P > 0.05) than those that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). The broilers that received Treatment 1 also had a higher average gain (P ≤ 0.05) than that of Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%). No other significant (P > 0.05) differences were found between treatment means.

Day 29 to 35

The broilers that received Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) had the highest average daily growth rate during the last week, however, no significant (P > 0.05) differences were found between treatment means.

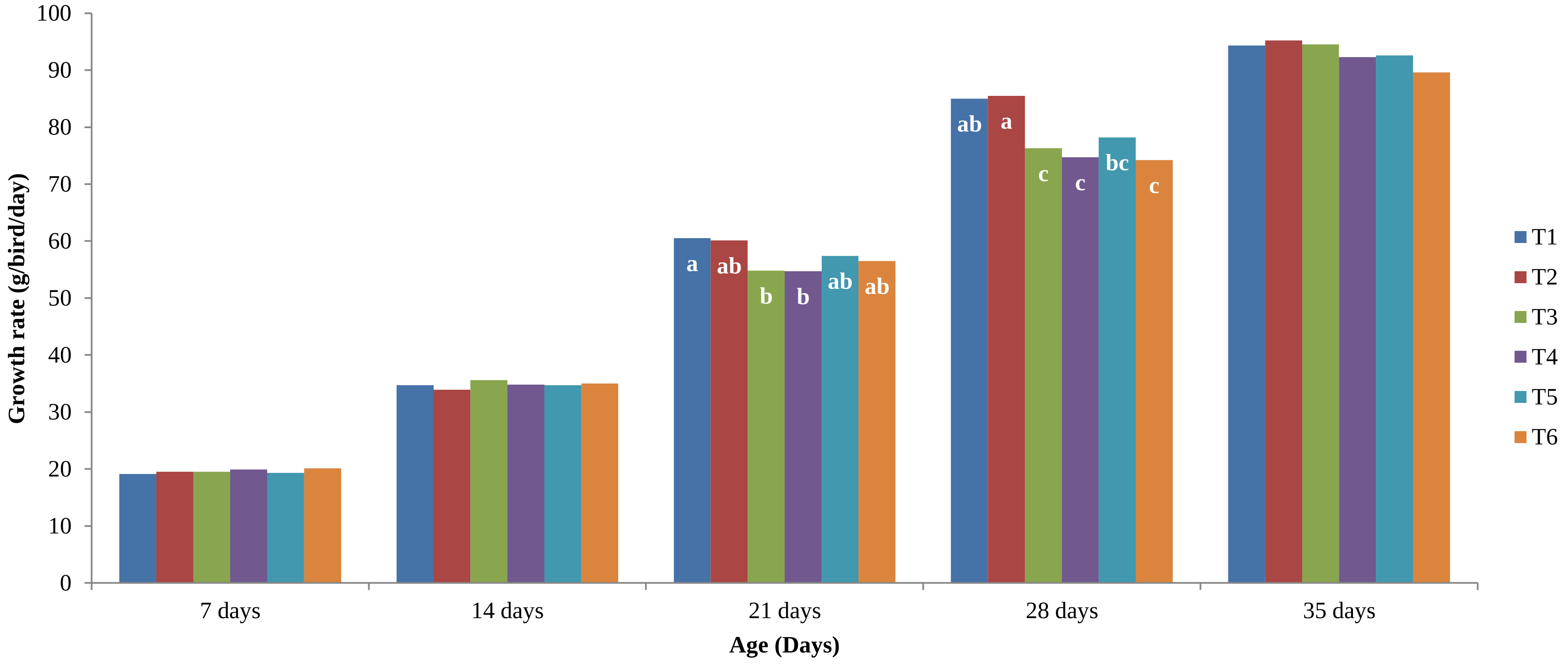
Table 4.2 Average daily gain over week periods (g/bird/day) of Ross 308 broilers that received different levels of calcium and phosphorus

Treatment	**Days of Age**				
	0-7 days	**8-14 days**	**15-21 days**	**22-28 days**	**29-35 days**
T1	19.1	34.7	60.5^{a}	85.0^{ab}	94.3
T2	19.5	33.9	60.1^{ab}	85.5^{a}	95.2
T3	19.5	35.6	54.8^{b}	76.3^{c}	94.5
T4	19.9	34.8	54.7^{b}	74.7^{c}	92.3
T5	19.3	34.7	57.4^{ab}	78.2^{bc}	92.6
T6	20.1	35	56.5^{ab}	74.2^{c}	89.6
SEM	0.289	0.721	1.342	1.627	1.926

[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other.

T1: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

In summary, the average daily growth rate was only affected during the third and the fourth week of rearing. During the third week of growth, the broilers that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had a slower rate of gain, showing significant differences (P<0.05) with Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%). During the fourth week of rearing, the broilers that received Treatment 1 and 2 had significantly faster growth rates (P<0.05) than Treatment 3, 4 and 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%).



[abc] Column means with the same superscripts are not significantly (P > 0.05) different from each other.

T1: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.2 Daily growth rates (g/bird/day) of Ross 308 broilers that received different levels of calcium and phosphorus

4.1.3 Cumulative feed intake

Day 0 to day 14

There were no significant (P > 0.05) differences found between treatment means for cumulative FI (g/bird) during the first 2 weeks of growth.

Day 0 to 21

At the third week of growth, broilers from Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) showed signs of lower feed intake (P ≤ 0.05) than those that received Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). No other significant differences (P > 0.05) were found between treatment means.

Day 0 to 28

At the end of the fourth week, the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) had the lowest feed intake, which was significantly lower (P ≤ 0.05) than all the other treatment means, except that of Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%). The broilers that received Treatment 2 was not significantly lower (P > 0.05) than those that received Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), however was significantly (P<0.05) lower than those that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). No other significant differences (P > 0.05) were found between treatment means.

Day 0 to 35

At the end of the growth period, the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF:

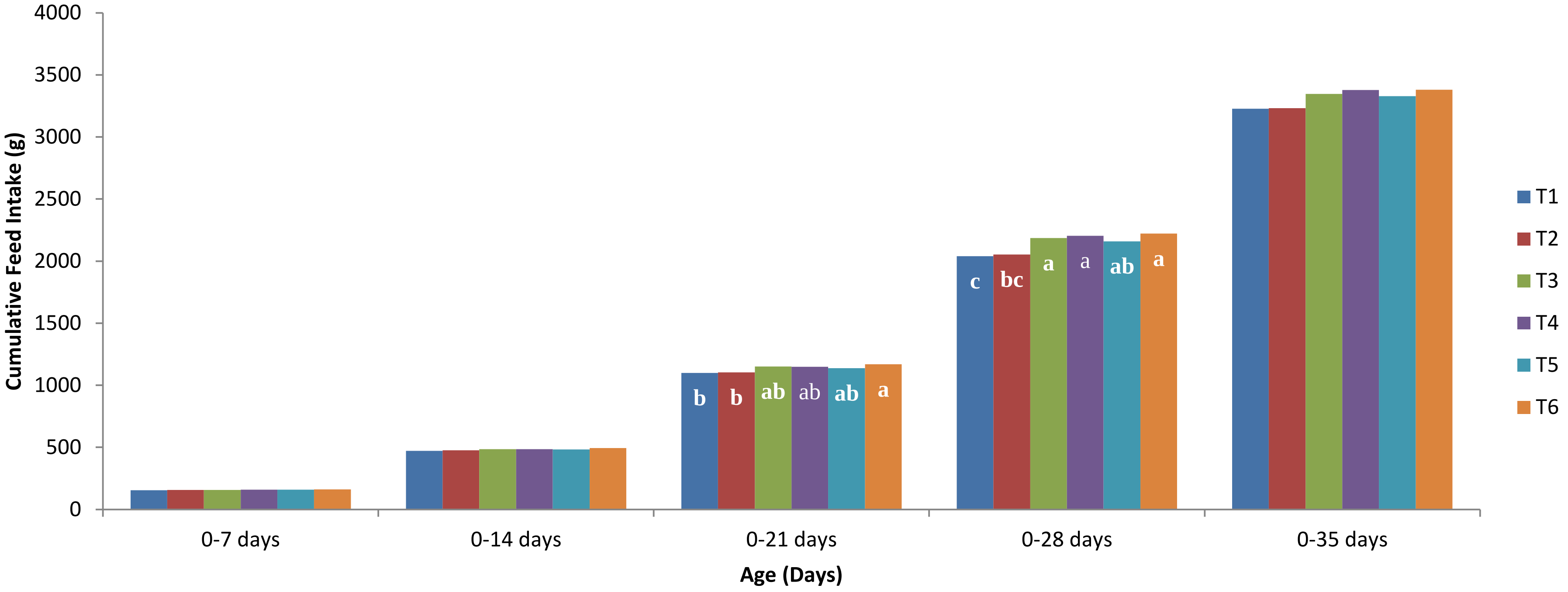
Ca of 0.76% and P of 0.38%) had the lowest feed intake, however, there were no significant ($P > 0.05$) differences found between treatment means.

In summary, the cumulative feed intake between treatment means only began to show significant differences ($P \leq 0.05$) at day 21 in the cycle. The cumulative feed intake results of broilers that received different levels of calcium and phosphorus are shown in Table 4.3 and presented in Figure 4.3. At 21 days of age, the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) was significantly lower ($P \leq 0.05$) than those of Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). At 28 days of age, broilers from Treatment 1 and 2 had significantly lower ($P \leq 0.05$) feed intakes than broilers from Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6. Additionally, the broilers from Treatment 1 had significantly lower ($P \leq 0.05$) feed intakes than broilers from Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%). However, no significant differences ($P > 0.05$) were noted between treatment means for cumulative feed intake over the full 35 day period.

Table 4.3 The cumulative feed intake (g/bird) of Ross 308 broilers that received different levels of calcium and phosphorus

Treatment	Days of Age				
	0-7 days	0-14 days	0-21 days	0-28 days	0-35 days
T1	155	472	1099[b]	2040[c]	3227
T2	158	477	1103[b]	2053[bc]	3231
T3	158	485	1152[ab]	2186[a]	3346
T4	159	486	1148[ab]	2205[a]	3377
T5	159	483	1137[ab]	2160[ab]	3328
T6	162	494	1169[a]	2221[a]	3381
SEM	1.6	5.1	12.9	27.2	33.2

[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other. **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%



[abc] Column means with the same superscripts are not significantly (P<0.05) different from each other.

T1 - Ross 308 guidelines; **T2** - Ross 308 guidelines from PS to G phase and LOW1 from F to PF. **T3** - Ross 308 guidelines from PS to S and LOW1 Ca and P from G to PF. **T4** - Ross 308 guidelines from PS to S and LOW1 Ca and P for G and LOW2 Ca and P for F and PF. **T5** - LOW1 Ca and P from PS to PF. **T6** - LOW1 Ca and P from PS to G and LOW2 Ca and P for F and PF.

Figure 4.3 Cumulative feed intake (g/bird) of Ross 308 broilers that received different levels of calcium and phosphorus

4.1.4 Cumulative feed conversion ratio

Day 0 to day 14

During the first 2 weeks of growth, there were no significant ($P > 0.05$) differences found between treatment means for cumulative feed conversion ratio. This is shown in Table 4.4 and depicted in Figure 4.4.

Day 0 to 21

At 21 days of age, the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) had the lowest feed conversion ratio which was significantly lower ($P \leq 0.05$) than that of Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). The cumulative FCR of Treatment 2 was significantly lower ($P \leq 0.05$) than Treatment, Treatment 4 and Treatment 6. No other significant differences ($P > 0.05$) were found between treatment means for cumulative FCR at the end of 3 weeks of growth.

Day 0 to 28

At 28 days of age the cumulative FCR of broilers from Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) were again significantly lower ($P \leq 0.05$) than that of Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). No other significant differences ($P > 0.05$) were found between the treatment means for cumulative FCR at the end of the fourth week of growth.

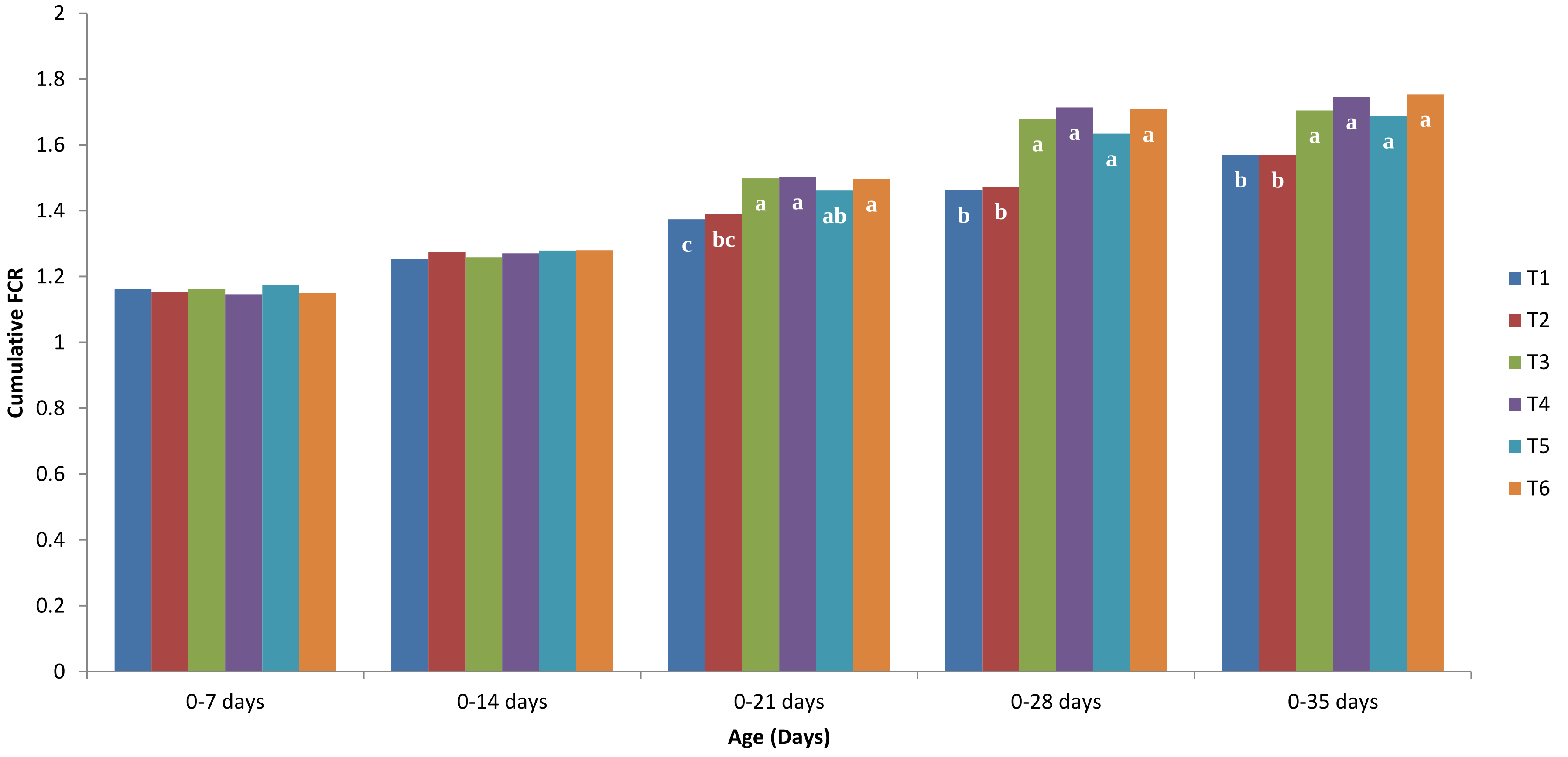
Day 0 to 35

At 35 days of age, the cumulative FCR for broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) were significantly lower ($P \leq 0.05$) than that of Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). No other significant differences ($P > 0.05$) were found between the treatment means for cumulative FCR at the end of the full growth period.

Table 4.4 Cumulative feed conversion ratio (FCR) of Ross 308 broilers that received different levels of calcium and phosphorus

Treatment	Days of Age				
	0-7 days	**0-14 days**	**0-21 days**	**0-28 days**	**0-35 days**
T1	1.16	1.25	1.37^{c}	1.46^{b}	1.57^{b}
T2	1.15	1.27	1.39^{bc}	1.47^{b}	1.57^{b}
T3	1.16	1.26	1.50^{a}	1.68^{a}	1.70^{a}
T4	1.15	1.27	1.50^{a}	1.71^{a}	1.75^{a}
T5	1.18	1.28	1.46^{ab}	1.63^{a}	1.69^{a}
T6	1.15	1.28	1.50^{a}	1.71^{a}	1.75^{a}
SEM	0.01	0.01	0.01	0.03	0.02

^{abc} Column means with the same superscripts are not significantly ($P>0.05$) different from each other. **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%



[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other.

T1: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.4 Cumulative feed conversion ratio (FCR) of Ross 308 broilers that received different levels of calcium and phosphorus

In summary, significant ($P<0.05$) differences were noted from 21 days of age for cumulative FCR. The broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) showed significantly lower ($P \leq 0.05$) cumulative FCR values than that of Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%).

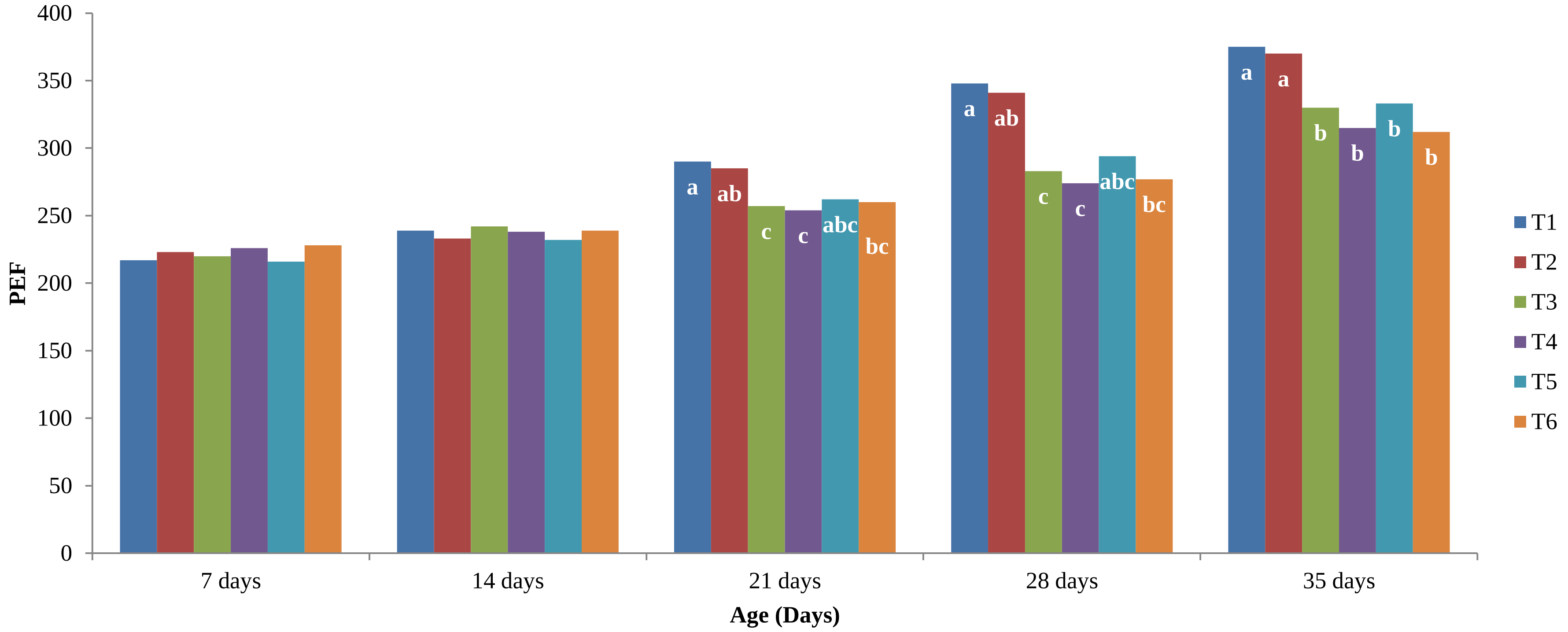
4.1.5 Performance efficiency factor (PEF)

No significant differences ($P > 0.05$) were found between treatment means at 7 and 14 days of age, as shown in Table 4.5. At 21 and 28 days of age, the PEF values for broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) and Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) were significantly higher ($P \leq 0.05$) than the PEF values for those of Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). The broilers that received Treatment 1 had a significantly higher ($P \leq 0.05$) PEF value than Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). As presented in Figure 4.5, no other significant ($P>0.05$) differences were found between treatment means at 21 and 28 days of age. At 35 days of age, the PEF values of the broilers that received Treatment 1 and Treatment 2 were significantly higher ($P \leq 0.05$) than those of Treatment 3, Treatment 4, Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6.

Table 4.5 Performance efficiency factors (PEF) of Ross 308 broilers that received different levels of calcium and phosphorus throughout the trial

Treatment	Days of age				
	7 days	**14 days**	**21 days**	**28 days**	**35 days**
T1	217	239	290[a]	348[a]	375[a]
T2	223	233	285[ab]	341[ab]	370[a]
T3	220	242	257[c]	283[c]	330[b]
T4	226	238	254[c]	274[c]	315[b]
T5	216	232	262[abc]	294[abc]	333[b]
T6	228	239	260[bc]	277[bc]	312[b]
SEM	4.63	4.38	6.60	7.94	6.82

[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other. **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%



[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other.

T1: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.5 Performance efficiency factors of Ross 308 broilers that received different levels of calcium and phosphorus

4.1.6 Cumulative mortality

As shown in Table 4.6, the cumulative mortality % for the broilers that received different levels of Ca and P did not differ significantly (P > 0.05) at any age period in the cycle. It can however be noted that Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) had the highest mortality % at 14, 21 and 28 days of age and that Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) had the highest mortality % at 35 days of age.

Table 4.6 Cumulative mortality (%) of Ross 308 broilers that received different levels of calcium and phosphorus

Treatment	**Days of age**				
	0-7 days	**0-14 days**	**0-21 days**	**0-28 days**	**0-35 days**
T1	0.21	0.21	0.83	1.04	1.67
T2	0.21	0.42	1.04	2.08	3.33
T3	0.42	0.42	0.63	1.46	2.08
T4	0.63	0.83	1.04	1.67	2.71
T5	0.42	1.46	2.5	2.71	3.13
T6	0.42	0.42	1.04	1.88	3.13
SEM	0.32	0.41	0.53	0.66	0.80

abc Column means with the same superscripts are not significantly (P>0.05) different from each other.

T1: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

4.2 Phosphorus excretion

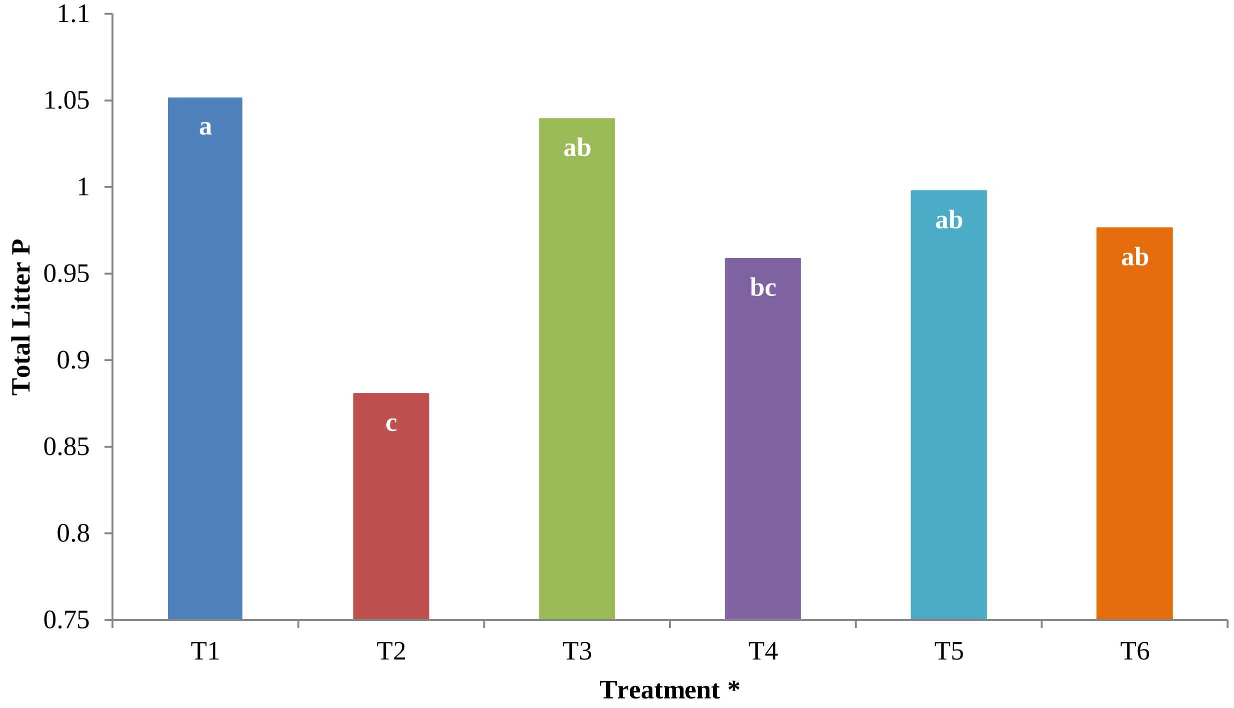
The treatment means for total P excreted at 35 days of age is shown in Table 4.7 and presented in Figure 4.7. The broilers that received Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) had the lowest total phosphorus % found in the litter and was significantly lower ($P \leq 0.05$) than that of Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%), Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82%

and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). The broilers that received Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) was significantly lower ($P \leq 0.05$) than those of Treatment 1. No other significant differences ($P > 0.05$) were found between treatment means.

Table 4.7 Total P (%) in the litter of Ross 308 broilers at 35 days of age that have received different levels of calcium and phosphorus throughout the trial

	Treatment						
	1	**2**	**3**	**4**	**5**	**6**	**SEM**
Total P (%)	1.05^{a}	0.88^{c}	1.04^{ab}	0.96^{bc}	0.99^{ab}	0.98^{ab}	0.02

^{abc} Column means with the same superscripts are not significantly (P>0.05) different from each other. **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%



[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other.

* **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.7 Total P (%) found in the litter at 35 days of age of Ross 308 broilers that have received different levels of calcium and phosphorus throughout the trial

4.3 Bone breaking strength

The bone breaking strength results, together with the time that it took to break the bone, the machine extension (mm) and the stress that the bone underwent during the breaking process are shown in Table 4.8. Figure 4.8 shows the distribution of the load (kN) that the bones could withstand before breaking. The broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) could withstand the highest load (kN) before breaking and was significantly higher ($P \leq 0.05$) than those of Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). The broilers that received Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96%

and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) was also significantly higher ($P \leq 0.05$) than Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) for bone breaking strength. No other significant differences ($P > 0.05$) were found between treatment means for bone breaking strength. The treatment means did not differ significantly ($P > 0.05$) for both the time it took to break a bone and the machine extension, in other words, the distance the compressive arm had to travel to cause the bone to fracture.

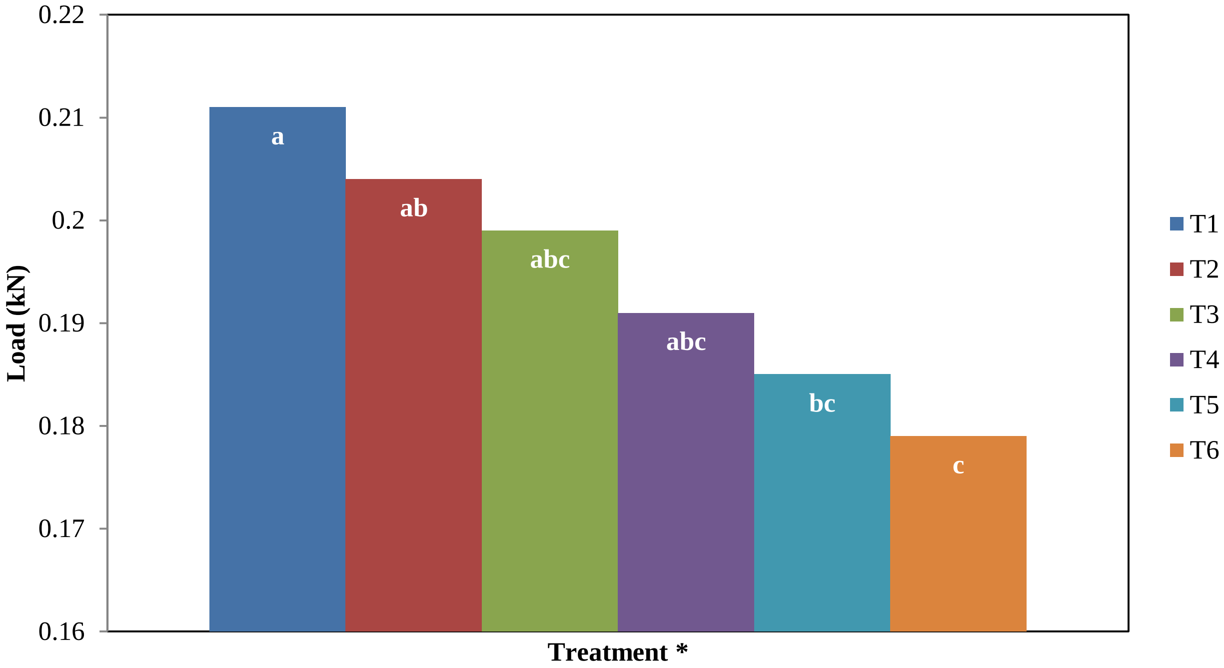
Table 4.8 Bone breaking strength results in Kilo Newton (kN), Time (seconds) in which it took for the bone to break, the machine extension (mm) and the stress of the fracture of the tibias from 35 days old Ross 308 broilers that received different levels of calcium and phosphorus

Treatment *	**Time (sec)**	**Load (kN)**	**Machine Extension (mm)**	**Stress (kN/mm^2)**
1	108.2	0.211^{a}	7.204	0.0082^{a}
2	107.4	0.204^{ab}	7.155	0.0078^{ab}
3	109.1	0.199^{abc}	7.268	0.0078^{ab}
4	123.3	0.191^{abc}	8.222	0.0073^{abc}
5	111.6	0.185^{bc}	7.432	0.0072^{bc}
6	124.8	0.179^{c}	8.338	0.0067^{c}
SEM	4.94	0.01	0.33	0.0003

^{abc} Column means with the same superscripts are not significantly (P>0.05) different from each other.

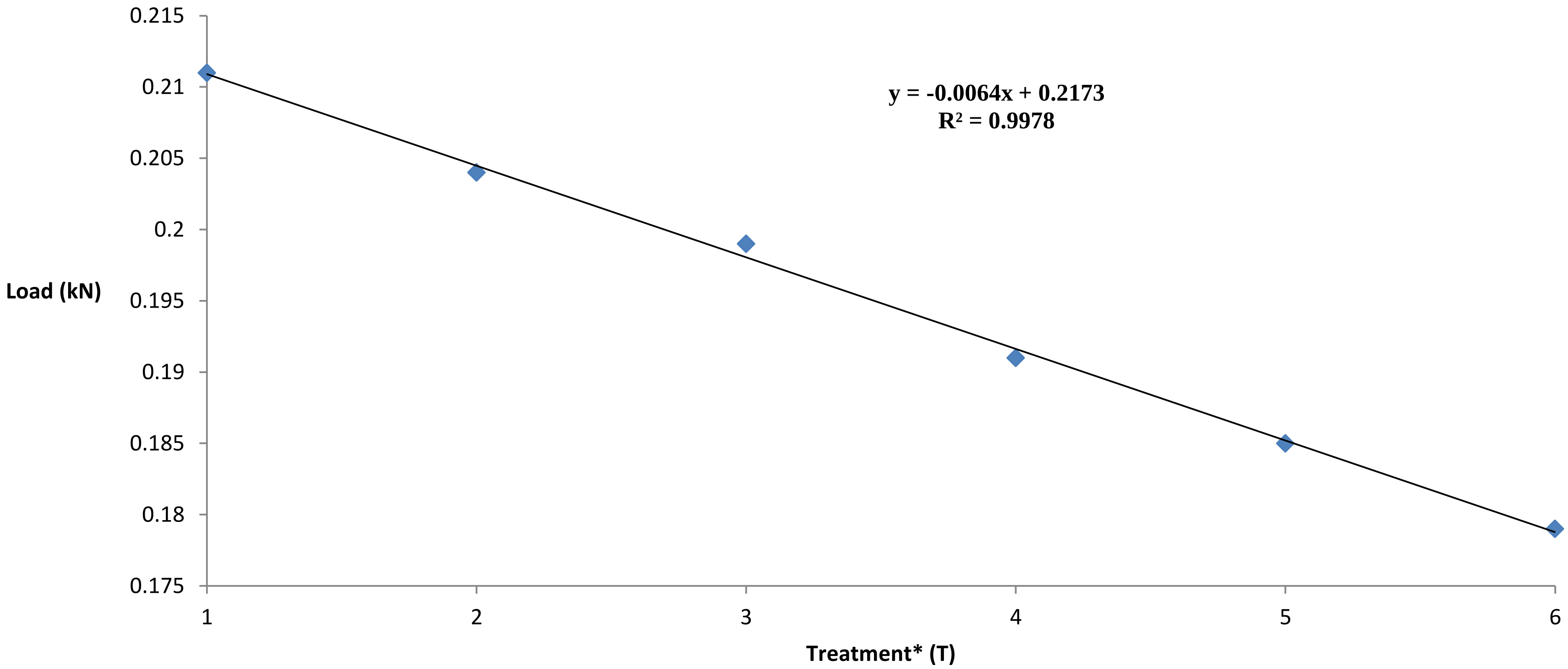
*** T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

4.3.1 Maximum load (kN)



[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other. *** T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.8 Bone breaking strength results in Kilo Newton (kN) of tibias taken from 35 days old Ross 308 broilers that have received different levels of calcium and phosphorus



* **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

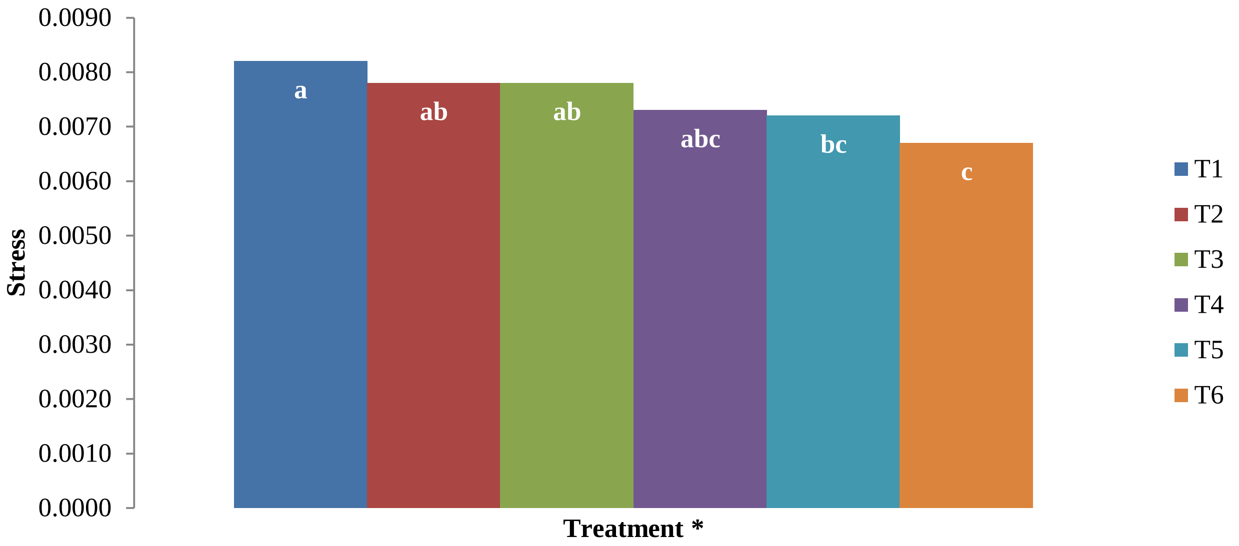
Figure 4.9 Correlation of bone breaking strength results in Kilo Newton (kN) of tibias taken from 35 days old Ross 308 broilers that have received different levels of calcium and phosphorus throughout that period

The correlation between the treatment and the maximum load as well as the equation of the line is shown in Figure 4.9. The correlation coefficient is strong at 0.9978 and the relationship is linear showing a very clear relationship.

4.3.2 Stress

Figure 4.10 shows the treatment means for the stress that the bones underwent. The stress results follow the same trend as the maximum load results.

The bones of the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) could withstand the highest stress before breaking and was significantly higher ($P \leq 0.05$) than that of Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). The bones of the broilers that received Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) could also withstand significantly higher ($P \leq 0.05$) stress than that of Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). No other significant differences ($P > 0.05$) were found between treatment means.



[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other.
*** T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.10 The stress of the fracture of the tibias taken from 35 days old Ross 308 broilers that have received different levels of calcium and phosphorus

4.4 Tibia ash

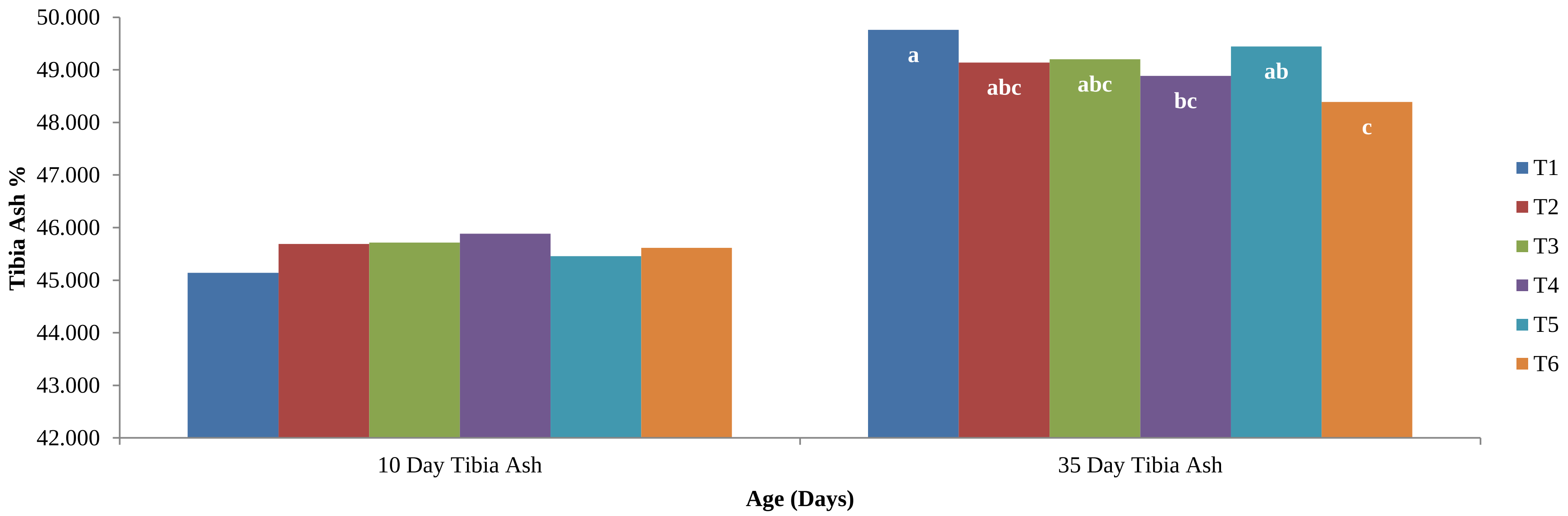
Table 4.9 shows the 10 and 35-day tibia ash % results as well as the mg of ash per tibia. Figure 4.11 shows the distribution of tibia ash % at 10 and 35 days of age. At 10 days of age, there were no significant differences ($P > 0.05$) found between the treatment means for both tibias ash % and mg/tibia. However, at 35 days of age, the tibia ash % for broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) was found to be significantly higher ($P \leq 0.05$) than that of Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). Additionally, the tibia ash % for broilers that received Treatment 5 was found to be significantly higher ($P \leq 0.05$) than that of Treatment 6. No other significant differences ($P > 0.05$) were found between the treatment means for tibia ash %.

Table 4.9 Tibia ash % and tibia ash (mg/tibia) for Ross 308 broilers at 10 and 35 days of age that have received different levels of calcium and phosphorus throughout that period

Treatment *	10 Day Tibia Ash		35 Day Tibia Ash	
	%	**(mg/tibia)**	**%**	**(mg/tibia)**
1	45.14	0.22	49.76[a]	2.27[a]
2	45.69	0.23	49.14[abc]	2.21[ab]
3	45.72	0.23	49.20[abc]	2.16[ab]
4	45.88	0.24	48.88[bc]	2.05[b]
5	45.46	0.23	49.45[ab]	2.10[ab]
6	45.62	0.24	48.39[c]	2.04[b]
Mean	45.58	0.23	49.14	2.14
SD	0.80	0.01	0.46	0.12
Prob>F	0.6194	0.1767	<0.0001	0.0042
SEM	0.31	0.0045	0.17	0.044
CV	1.75	5.17	0.94	5.61

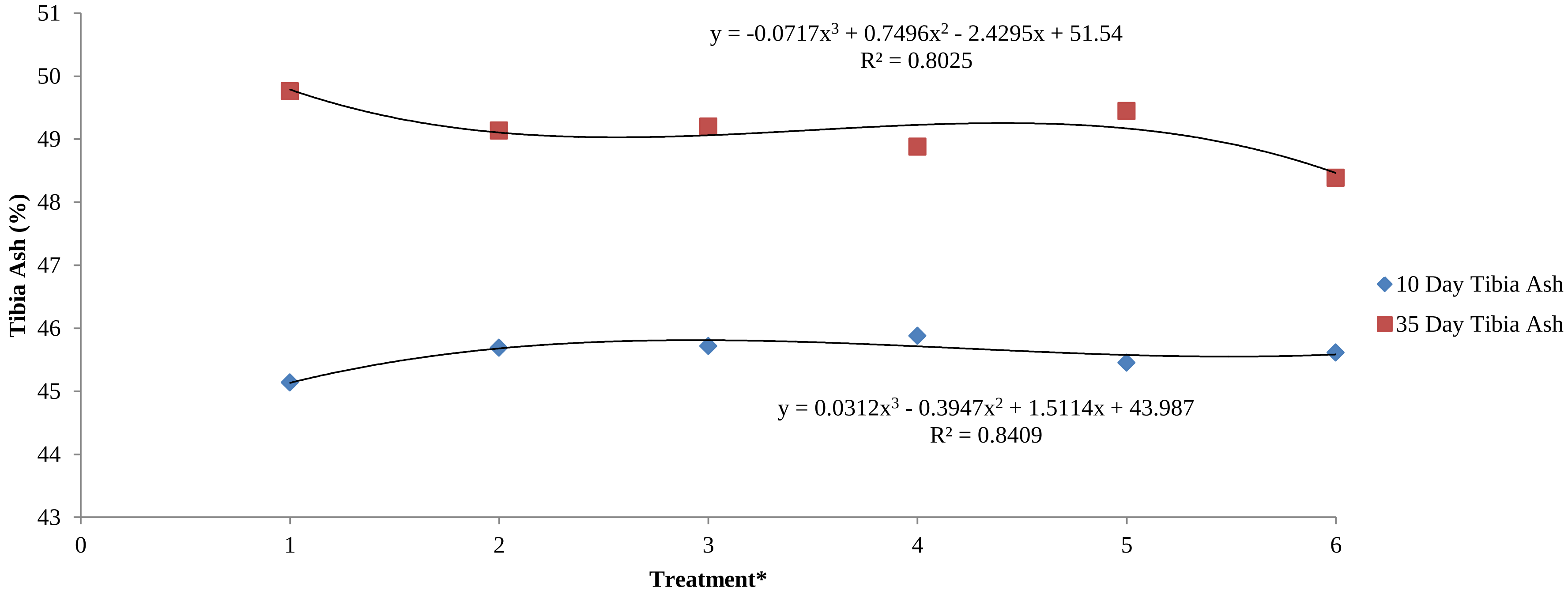
[abc] Column means with the same superscripts are not significantly (P>0.05) different from each other.

*** T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%



abc Column means with the same superscripts are not significantly (P>0.05) different from each other. **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.11 Tibia ash % at 10 and 35 days of age for Ross 308 broilers that have received different levels of calcium and phosphorus throughout that period



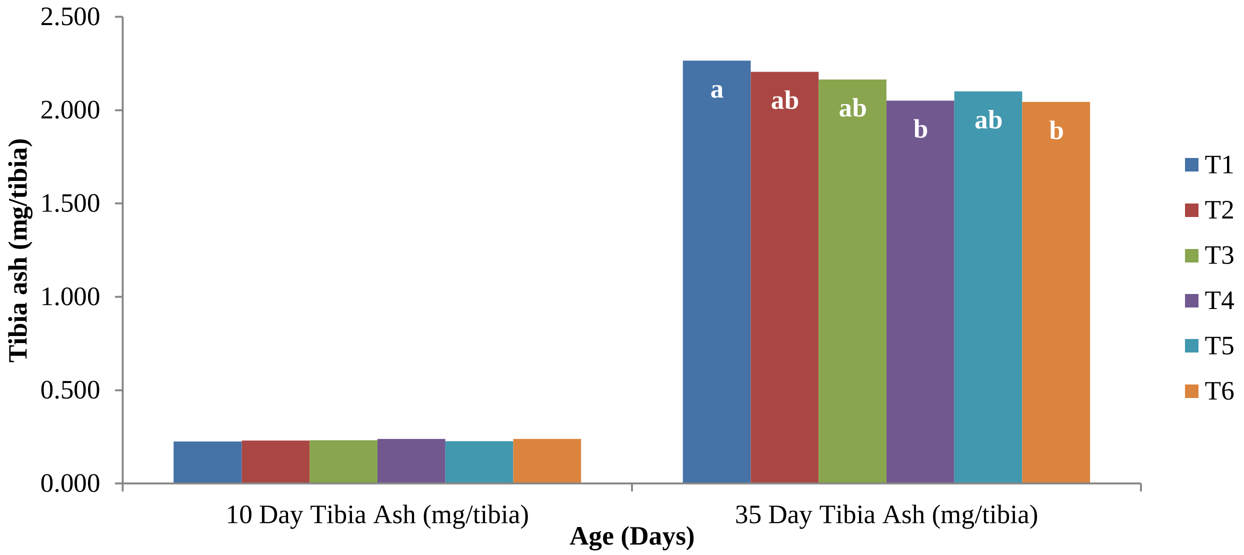
* **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.12 Correlation of Tibia Ash % at 10 days of age and at 35 days of age to treatment for Ross 308 broilers that have received different levels of calcium and phosphorus

The correlation between treatment and tibia ash can be seen in Figure 4.12. The correlations were strong and both relationships were found to be polynomial (3rd degree).

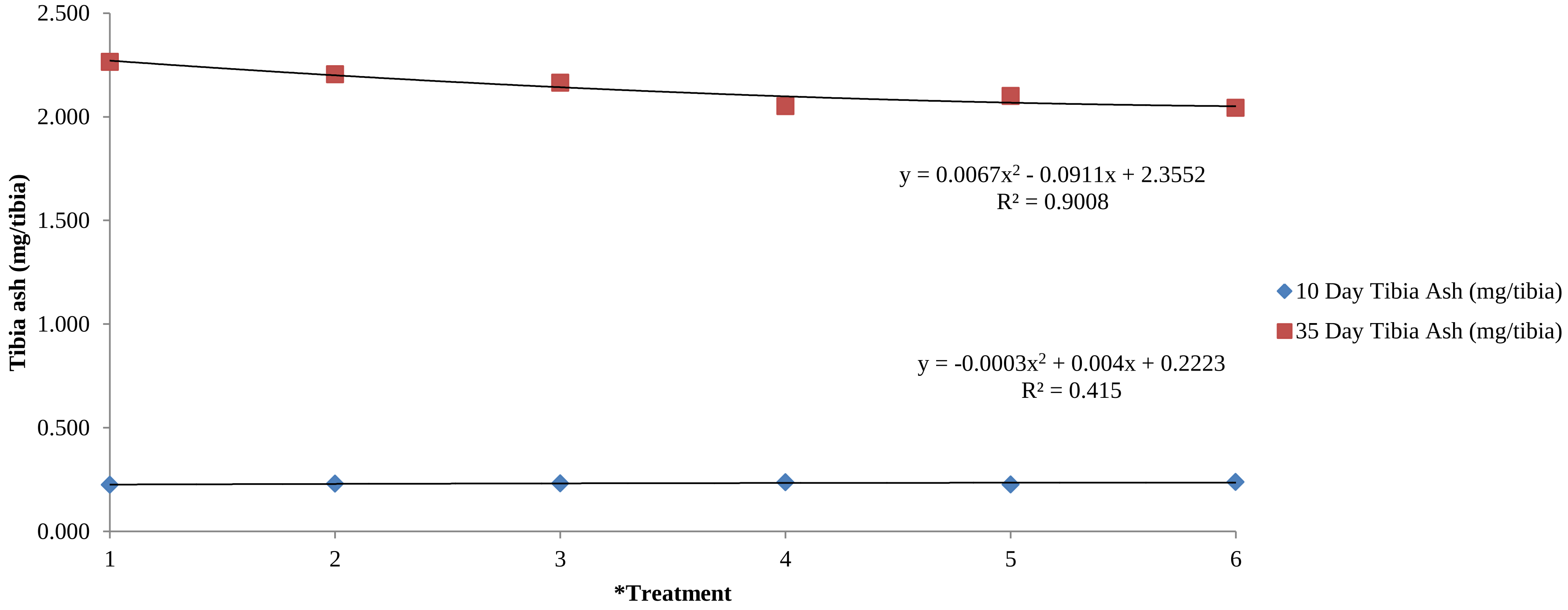
4.4.2 Tibia ash weight (mg/tibia)

The tibia ash (mg/tibia) is shown in Figure 4.13 for 10 days and 35 days of age. At 10 days of age, as with tibia ash %, no significant differences (P > 0.05) were noted. At 35 days of age, the trend was very similar to tibia ash % at 35 days of age. Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) was found to be significantly higher (P ≤ 0.05) than Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). No other significant differences (P > 0.05) were found between the treatment means for tibia ash (mg/tibia).



abc Column means with the same superscripts are not significantly (P<0.05) different from each other. **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.13 Tibia ash (mg/tibia) at 10 days and at 35 days of age for Ross 308 broilers that have received different levels of calcium and phosphorus



* **T1**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%; **T2**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T3**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%;**T4**: PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%; **T5**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%; **T6**: PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%

Figure 4.14 Correlation of tibia ash (mg/tibia) at 10 days of age and at 35 days of age to treatment for Ross 308 broilers that have received different levels of calcium and phosphorus

As presented in Figure 4.14, tibia ash (mg/tibia) had a strong relationship to treatment for 35-day tibia ash, however, not a strong relationship with 10-day tibia ash (mg/tibia). Both relationships in this figure are polynomial (2nd degree).

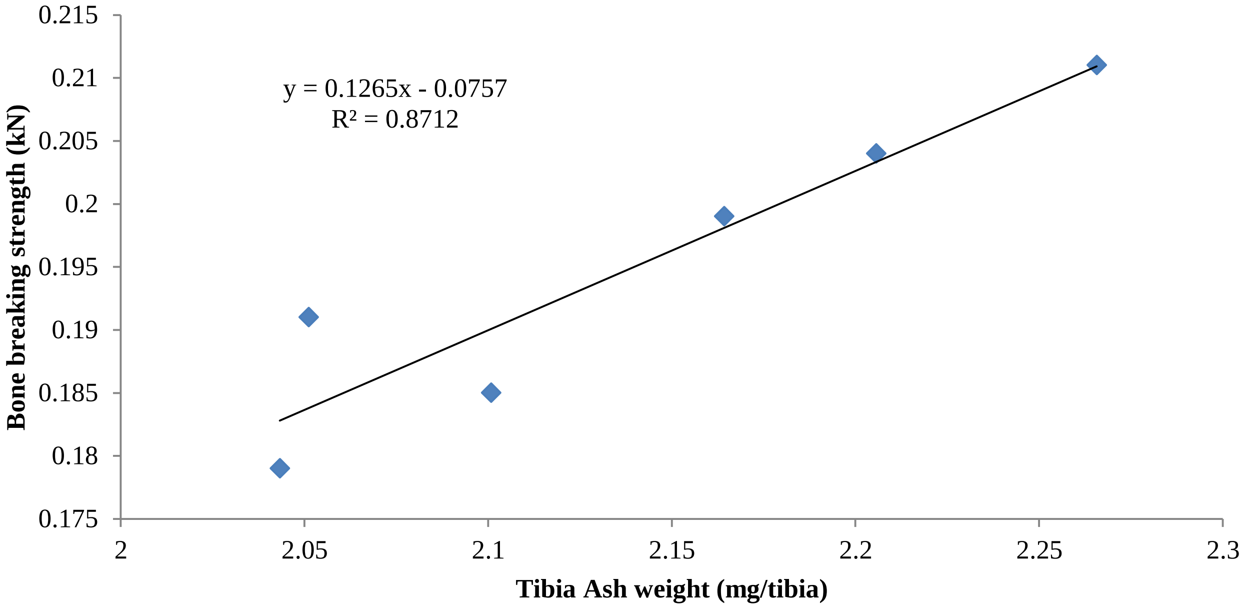


Figure 4.16 Correlation between tibia ash weight (mg/tibia) and bone breaking strength (kN)

The correlation between tibia bone ash (mg/tibia) and bone breaking strength (kN) is depicted in Figure 4.16 and shows a strong linear relationship with a correlation coefficient of 0.87.

Chapter 5

Discussion

5.1 Performance data

Countless studies in broiler nutrition have been done on Ca and P and the effects of the concentrations thereof on bone ash % and bone structure. However, few studies concentrated on the effect of Ca and P on the performance of broilers. In this study, the treatment means for body weight only showed significant differences ($P \leq 0.05$) at 28 days of age. This may be due to the levels of Ca and P not being greatly different during the pre-starter and starter phases, and that body weight is not as sensitive of an indicator for mineral deficiencies as bone ash (Nelson & Walker, 1964). Gillis *et al.* (1948) showed that body weight at 28 days of age could be used to determine the relative bioavailability of P and was a sensitive indicator as such, which agrees with the data in this trial as the lowest body weight gains are evident in the broilers that received the lowest Ca and P during the trial period. The body weight results showed that the Ca and P could safely be lowered during the finisher phase to 0.55% Ca and 0.25% RP. During the post finisher phase, Ca could be lowered to 0.52% and RP to 0.25% without any negative effect on the body weight of broilers. Such a reduction in Ca and P levels in broiler feed will result in substantial economic gains per ton of feed sold.

At 28 and 35 days of age, the treatment means for the broilers of Treatment 5 were not significantly lower ($P \leq 0.05$) than those that received feed according to the Ross 308 guidelines, which again indicates that the Ca and P levels can be significantly lowered from the starter phase without affecting the body weight significantly ($P \leq 0.05$). Both Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had significantly ($P \leq 0.05$) lower body weight at 35 days of age, which may be due to the extremely low Ca and P levels in the finisher and post finisher diets. Even though the growth rate of the broilers that received Treatment 4 and 6 was significantly poorer ($P \leq 0.05$) at these low levels compared to the broilers that received feed according to the Ross 308 guidelines, the mortality was not affected and the bone breaking strength for the broilers that received Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) was not significantly ($P \leq 0.05$) lower than those that received the Ross 308 guidelines.

The average daily gain was only significantly affected ($P \leq 0.05$) at 21 days of age. This may also be due to the levels of Ca and P not being greatly different at those phases. At 21 days of age, the broilers that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and

P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) were significantly lower ($P \leq 0.05$) than the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%). This may be due to the lowered Ca and P levels in the grower phase. Ruangpanit *et al.* (2015) showed that feed intake and body weight gain were negatively impacted when low levels of available P was present in the feed, which agrees with the data seen in the current trial. Conversely, Narcy *et al.* (2009) showed that Ca levels of 0.6% and AvlP levels of 0.39% in the starter feed resulted in the best daily gain for broilers. Suttle (2010) concluded that Ca levels above 7 g/kg were unnecessary in broiler feed.

At 28 days of age, the trend continued, however, the body weight gains of the broilers that received Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) were found to be significantly lower ($P \leq 0.05$) than those that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%). This may be due to the significant drop in Ca and P for Treatment 6 in comparison to the levels found in Treatment 1(PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%). However, at 35 days of age, there were no significant differences ($P > 0.05$) between the treatment means for body weight gains. Walk *et al.* (2016) discovered similar results when lowering Ca and P in the late phases of feeding broilers as the broilers adapt to the low levels of Ca and P by increasing the uptake and retention of the supplied Ca and P, therefore, making them more efficient. Ruangpanit *et al.* (2015) also found that a decrease of 0.15% P had a negative effect on broiler performance, bone mineralisation and P retention; however, with the addition of phytase, these downfalls were avoided given the same P levels.

The cumulative feed intake showed a similar trend to the average daily growth rate. Significance ($P \leq 0.05$) between treatment means were only seen at 21 and 28 days of age and were not seen at 35 days of age, assuming that the broilers adapted their feed intake to the levels of Ca and P and have adjusted the uptakes and retention of the minerals accordingly as described by Walk *et al.* (2016). The cumulative feed intake of the broilers that received Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) was significantly higher ($P \leq 0.05$) at 21 days of age. This may be due to the lowered Ca and P levels supplied at the grower phase, consequently increasing their feed intake to supply the required Ca levels needed by the broiler. Despite that, Treatment 3, Treatment 4 and Treatment 5 also had these lowered levels during the grower phase but were not significantly ($P > 0.05$) different to the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%).

At 28 days of age, the cumulative feed intake of the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) was significantly lower ($P \leq 0.05$) than the broilers that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (LOW1 Ca and P from PS to G and LOW2 Ca and P for F and PF.). The higher intakes in this week did not result in higher daily growth rates, which then had a negative effect on the FCR and thus the efficiency of growth. These higher intakes can be explained by the low Ca and P levels in the grower phase for these treatments as seen in the research done by Wilkinson *et al.* (2012). He stated that the modern broiler has a particular appetite for Ca and will consume feed until that requirement is met. Akter *et al.* (2015) concluded that high Ca levels negatively affected FI and BWG and showed an interaction between Ca and phytase which had a significant effect on the feed intake of broilers.

The feed conversion ratio (FCR) is a calculation which describes the amount of feed in kg needed to produce a kg of live weight. The treatment means for the FCR results of the current trial only showed significance at 21 days of age. At 21 days of age, the broilers that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%), Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had significantly higher ($P \leq 0.05$) FCRs than those that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%). This may be due to the lower Ca and P levels of the aforementioned treatments at the grower phase as the FI was found to be higher for the broilers that received these treatments and the body weight gain showed no parallel increase. Conversely, Akter *et al.* (2015) stated that the FCR was not affected by different levels of Ca, P and phytase.

PEF is beneficial in taking various performance factors into consideration and will reflect the highest performing broilers. However, it does not take economics into consideration as broilers are produced on a least cost basis and not a maximum performance basis. Therefore, the objective would be to get the best return per unit time as the resources are often limited. Where resources are unlimited, the objective would be to maximise the return per unit of production (per broiler). PEF is however still used as a valuable performance factor as it gives an overall assessment of a flock.

As PEF incorporates all the performance parameters already discussed, as well as mortality, it can be deduced that the trend for PEF will be similar to the rest of the parameters. As with the other performance results, there were no significant differences ($P > 0.05$) found before 21 days of age as the pre-starter and starter diets did not deviate drastically from each other concerning Ca and P concentrations. At 21 and 28 days of age, the broilers that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had a significantly lower ($P \leq 0.05$) PEF than the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%). The PEF of the broilers that received Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) was not significantly lower ($P>0.05$) than those that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) at 21 days of age, and may be due to the higher P level and the lower Ca level in the pre-starter diet which have been shown to benefit the broiler chick early in life (Sacranie, *et al.*, 2013; Hopcroft, *et al.*, 2016). At 35 days of age, the broilers that received Treatment 1 and Treatment 2 had a significantly higher ($P < 0.05$) PEF than those that received the rest of the treatments.

The results for the cumulative mortality showed no significant differences ($P > 0.05$) between the treatment means throughout the entire trial period. The mean mortality over all the treatments was remarkably low at 2.68% for an open-sided commercial broiler house stocked at 20 birds/m^2 in winter. The cumulative mortalities of the broilers that received Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had consistently low mortality rates, despite the low Ca and P levels that those broilers received. These results show that the Ca and P levels used in this trial did not significantly ($P > 0.05$) affect the mortality rate which agrees with previous research on the matter (Angel, *et al.*, 2002b).

5.2 Phosphorus excretion

Inorganic phosphorus is the third most expensive feed ingredient in broiler diets, and due to the uncertain absorption efficiencies and the fear of undersupplying this important mineral, nutritionists have thus been oversupplying P in the attempt to feed the modern broiler (Waldroup, 1999). In South Africa, chicken manure is a common ingredient used as fertiliser in our phosphorus-deficient soils. However, there are large amounts of phosphorus found in chicken manure due to the overfeeding of this mineral. This results in leaching of P into local groundwater which may result in very dangerous, yet easily avoidable, pollution (Sharpley, 1995).

In this trial, the P levels that were found in litter from broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%) were significantly higher ($P \leq 0.05$) than those of Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%). Plumstead *et al.* (2007) found that by reducing the NPP by 0.1%, the P excreted reduced by 18% in broiler breeder pullets raised on litter. In this trial, there was a 0.12% decrease in RP which in turn resulted in a 16% decrease in Total P in the litter. Research by Applegate *et al.* (2003) also showed that a reduction in nPP with the addition of phytase reduced the amount of total P and water-soluble P and which better met the requirements of P for broilers.

Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and treatment 4 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had relatively high levels of Ca and P, but were then drastically decreased in the finisher and post-finisher phases. This sudden change could have been countered with an up-regulation of P uptake in the small intestine to make up for the sudden reduction in P resulting in a decline in P excretion (Rama Rao *et al.*, 2003).

The reduction of total P in the litter was observed to be greatest when the RP was only reduced from the finisher phase of feeding (Treatment 2). There were no additional benefits in significantly ($P \leq 0.05$) lower total P in the litter when reducing P from 0.25% to 0.20% in the finisher and post-finisher. Similar results were noted by Plumstead *et al.* (2007).

5.3 Bone breaking strength

During the determination of bone breaking strength, a few parameters were measured which can be useful to understand the properties of the bone. Firstly, the maximum load was measured in kN to determine the amount of force needed to result in a fracture of the tibia. Many authors have used bone breaking strength as a criterion for Ca and P deposition in the bone. Rowland *et al.* (1967) was the first to use this criterion and found that the correlation between tibia ash and bone breaking strength was 0.98. In the current experiment, a linear relationship of tibia ash (mg/tibia) to bone breaking strength (kN) with a correlation coefficient of 0.87 was found.

The time (sec) in which it took for the bone to break was also recorded. The time criterion may be a good indicator of the flexibility of the bone, but may not be an indicator of Ca and P depositions in the bone. Velleman (2000) found that the organic component of bone is important for tensile strength and flexibility. It is the combination of compressive strength and flexibility of the bone that contributes to its bone breaking strength (Rath *et al.*, 1999).

The amount of load the tibia could withstand before breaking differed significantly ($P \leq 0.05$) between some treatments and showed an interesting trend. The bone breaking strength of the broilers that received Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) could withstand a significantly ($P \leq 0.05$) lower load than those that received Ca and P levels according to the Ross 308 guidelines. Similar results were found in research done by Leske and Coon (2002), they postulated that the decrease in bone strength was due to bone resorption to mobilise Ca back into the plasma due to the low levels of Ca provided in the trial feed. It was found that 0.39% RP was sufficient in the 0-3 week phase of a broilers life for optimum bone breaking strength. In this trial, a 5 phase feeding programme was used but showed that 0.32% at the grower phase (18-27 days) was not sufficient for bone breaking strength. Skinner *et al.* (1992) found a decrease in bone breaking strength when limestone was removed from the diet in late phase feeding. In the current experiment, a drop in Ca was apparent from starter to post-finisher for Treatment 6 which may explain the lowered bone breaking strength in comparison to Treatment 2.

Remarkably, the bone breaking strength of the broilers that received Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%), Treatment 2 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%), Treatment 3 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 4 (PS: Ca of

1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) were not significantly different (P > 0.05) from each other. The only difference between these 4 treatments and Treatment 5 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.55% and P of 0.25%, PF: Ca of 0.52% and P of 0.25%) and Treatment 6 (LOW1 Ca and P from PS to G and a level below LOW1 Ca and P for F and PF) are the pre-starter and starter Ca and P levels. This suggests that the correct Ca and P levels and ratio early in a chick's life can affect the bone integrity later in life. It can also be seen that drastically lowering the Ca and P in the late phases does not significantly decrease (P > 0.05) the bone breaking strength once the broilers have had sufficient levels thereof at a young age. Leske & Coon (2002) found that the RP must at least be 0.39% in the starter diet for adequate bone strength later in the bird's life. It was also postulated by Leske & Coon (2002) that the lowered bone strength seen in the treatments with low Ca and RP was due to bone resorption to attempt to maintain the Ca levels in plasma.

A strong correlation coefficient of 0.99 was found between treatment (Ca and P levels) and Maximum load (kN) that the tibia could endure before breaking as observed in research by Leske & Coon (2002). As discussed before, the Ca and P levels early in a chick's life appeared to affect the bone breaking strength at 35 days of age. Thus, the treatment in its entirety, from pre-starter to post-finisher including the Ca and P interactions that were found (Faridi *et al.*, 2015), must be considered when observing correlations. Biological systems are complex and no one factor affects only a parameter and must be considered in unity with other contributors.

5.4 Tibia ash

The tibia is the fastest growing bone in the body and as such rapidly shows a response to a phosphorus deficiency (McLean and Urist, 1961). The tibia ash was expressed in 2 ways, tibia ash % which is the weight of ash (dry matter), divided by the weight of the dry defatted bone, and mg ash per tibia.

At 10 days of age, there were no significant differences (P > 0.05) found between treatment means for both ash parameters, ash % and mg ash per tibia. Shastak *et al.* (2012) found that at 11 days of age, tibia ash % did not show significant differences (P > 0.05) even though drastically different levels of nPP were supplied. The results found in this trial, therefore, agree with those of Shastak *et al.* (2012). However, when mg ash per tibia was analysed, Shastak *et al.* (2012) did find significant differences (P < 0.05) at 11 days of age. The levels used in pre-starter and starter phases in this trial, however, were not drastically different and may be the reason why no significant differences were found between the treatments for tibia ash weight or tibia ash % at 10 days of age.

At 35 days of age, the results showed that drastically lowering Ca and P levels from the finisher phase of feeding, as seen for Treatment 4 and 6, resulted in a significantly lowered (P ≤ 0.05) tibia ash % compared to the broilers that were fed Treatment 1 (PS: Ca of 1.05% and P of 0.50%, S: Ca of 0.96% and P of 0.48%, G: Ca of 0.87% and P of 0.44%, F: Ca of 0.80% and P of 0.40%, PF: Ca of 0.76% and P of 0.38%). The results of this trial further showed that reduction of Ca and P levels throughout the broiler's production cycle should not affect the bone structure and hence the health of the broiler. The broilers fed Treatment 6 (PS: Ca of 1.00% and P of 0.55%, S: Ca of 0.82% and P of 0.37%, G: Ca of 0.64% and P of 0.32%, F: Ca of 0.49% and P of 0.20%, PF: Ca of 0.47% and P of 0.20%) had a significantly lower ($P \leq 0.05$) ash % than those that received Treatment 5. Conversely, the results obtained from the ash weight (mg/tibia) did not show a significant difference ($P < 0.05$) between Treatment 5 and Treatment 6 as it was corrected for the weight of the tibia. Applegate & Lilburn (2002) concluded that the tibia weight increased with the body weight of a broiler. A heavier tibia will result in a heavier tibia ash weight (mg/tibia). The ash % may be lower, however, the tibia itself was larger, thus containing more minerals within it and thus had a higher tibia ash weight.

The tibia ash weight (mg/tibia) had a very similar trend to the tibia ash %. However, the differences between the treatment means were smaller for tibia ash weight than it was for ash %. Tibia ash weight has been considered a more accurate parameter when determining the effect of mineral supplementation in the diet as it considers the variation in bone size and weight, whereas ash % may result in bones with large differences in bone weight and size with very similar ash % values (Skinner & Waldroup, 1995; Applegate & Lilburn, 2002; Shim *et al.*, 2012). No other significant differences ($P > 0.05$) were found between the broilers that received different Ca and P levels for 35-day tibia ash weight, as tibia ash weight considers the variation in bone weight. Ruangpanit *et al.* (2015) found that tibia ash increased by 4.5% when AvlP was lowered in feeds in the presence of phytase. It was also found that decreasing P supplementation by 0.15% resulted in lowered bone mineralisation and P retention in the absence of phytase, however, this was again corrected with the use of Phytase (Ruangpanit *et al.*, 2015). The results reported by Ruangpanit *et al.* (2015), concurs with the results of this trial as no significant differences were found for tibia ash weight when lowering AvlP in the broiler feed, most likely due to the addition of phytase in all of the treatments.

Correlations were also examined in the current trial between the treatment (Ca and P levels) and tibia ash %. A strong correlation was found at both 10 and 35 days of age. At 10 days of age, the correlation coefficient was 0.84 and at 35 days of age, the correlation coefficient was 0.80. Both of these relationships were polynomial (3^{rd} order) as they are biological systems and are not necessarily linear in nature. Correlations were also made between the treatment and tibia ash weight. It was found that at 10 days of age the tibia ash was better explained by tibia ash % rather than tibia ash weight as the correlation coefficient of tibia ash weight was low at 0.42. However, at 35 days of age, tibia ash weight was a better criterion to explain mineral deposition in the bone as the correlation coefficient to treatment was very strong at 0.90. The relationship between 35-day tibia ash weight and treatment was polynomial (2^{nd} degree).

Chapter 6

Conclusion

Calcium and phosphorus are vitally important macro minerals and are the most abundant minerals in the broiler. They are needed by broilers for many functions, most importantly for bone mineralisation. Phosphorus also plays an important role in broiler growth as it is prevalent within nucleic acids, nucleotides, phospholipids and phosphorylated proteins. It is therefore essential to have the correct levels of Ca and P in the broiler diet, especially with the fast growth rate and the rapid production cycles that the modern broiler is exposed to. However, there is a large likelihood that nutritionists have been over supplementing these minerals due to the lack of understanding of absorption rates and bioavailability of these minerals and how they interact with each other. Excessive levels of Ca and P have been shown to negatively affect growth efficiency and increase bone strength. New research has suggested that the Ca and P levels in a broiler diet can be safely reduced as nutritionists have been cautiously over supplementing these minerals. With the addition of phytase in most broiler diets and its positive effect on P availability, a less conservative approach can be used when supplementing Ca and P. To determine the precise Ca and P levels for broiler nutrition is extremely challenging and calls for further exploration, but if achieved could yield benefits beyond mineral nutrition.

The alternate hypothesis of this trial was to show that lowering Ca and P levels throughout the broiler rearing period based on recommendations made by Dr R. Angel in consultation with AFGRI Animal Feeds (14/10/2015) compared to Ca and P levels currently recommended by Ross, will not reduce broiler performance and bone integrity. Performance parameters such as body weight, average daily gain, feed intake, feed conversion ratio and PEF were affected by the lowering of Ca and P. Body weight at 35 days of age was significantly lower ($P \leq 0.05$) when the Ca and P levels were reduced to a level below Dr Angel's recommendations for the finisher and post-finisher phases. No other significant differences ($P > 0.05$) were found for body weight between treatment means, thus concluding that Ca and P levels can be lowered throughout the rearing period without significantly lowering ($P \leq 0.05$) the body weight at 35 days of age. Lowering the Ca and P levels from the grower phase resulted in a significantly lower ($P \leq 0.05$) average daily gain at 28 days of age. Nonetheless, at 35 days of age, the treatment means did not differ significantly ($P > 0.05$). Feed intake was affected by the lowered Ca and P levels in the diet. The trend showed that the broilers that received lower levels of Ca and P had higher feed intakes, possibly to make up for the lack of minerals. The broilers that received treatments with lowered Ca and P levels from the grower phase had significantly higher ($P \leq 0.05$) feed intakes than those that received levels described by the Ross 308 guidelines. However, the feed intake was not affected when the Ca and P levels were lowered from the finisher phase. At 35 days of age, there were no significant differences ($P > 0.05$) between the treatment means for feed intake. At 35 days of age, the feed conversion ratio of the broilers was affected by the different levels of Ca and P. The broilers that received the treatments that had lowered Ca and P levels from grower to post-finisher phase had significantly higher ($P \leq 0.05$) FCRs than those that received levels according to the Ross 308 guidelines. However, the broilers that received the treatment that had lowered Ca and P levels from the finisher phase was not significantly different ($P \leq 0.05$) from those that received Ca and P levels according to the Ross 308 guidelines. The same trend was again seen for the PEF results obtained. However, the PEF results still showed that the Ca and P can be lowered from the finisher phase without a significant difference ($P > 0.05$) to the broilers that received the Ross 308 guidelines. There were no significant differences ($P > 0.05$) observed between the treatment means for cumulative mortality, therefore, a reduction in Ca and P throughout the rearing period did not significantly ($P > 0.05$) affect the mortality rate. There was no particular trend regarding the mortality throughout the rearing period.

A downward trend was observed for P excretion with decreasing Ca and P supplementation in the diets. When including Ca and P according to the Ross 308 guidelines the highest P excretion was noted, which was significantly higher ($P > 0.05$) than the P excretion of the broilers that received the treatments which had lower Ca and P from finisher to post-finisher phase. Thus, decreasing the Ca and P during the late phases reduced the amount of P excreted, therefore reducing the wastage of minerals which also resulted in a reduction in pollution. Bone breaking strength is a sensitive criterion for mineral deposition and thus showed a very strong correlation to the Ca and P levels that the broilers received. The bone breaking strength had a stepwise decline in maximum load as the Ca and P levels were decreased in the diets. The tibias of the birds that were fed according to the Ross 308 guidelines had a significantly higher ($P > 0.05$) load bearing strength (kN) or bone breaking strength than those of the treatments that started with lower Ca and P from pre-starter to post-finisher. This showed that the mineral intake during the early phases had an impact on the bone strength at slaughter age. However, broilers that received the treatments that started off with Ca and P levels according to the Ross 308 guidelines and finished with lowered Ca and P in the late phases were not significantly different ($P > 0.05$) to those that received Ca and P levels according to the Ross 308 guidelines throughout the trial. The results for tibia ash % and ash weight for the broilers that received different levels of Ca and P only showed significant differences ($P \leq 0.05$) at 35 days of age. The bone ash % of the broilers that received treatments that had LOW2 Ca and P levels were significantly lower ($P \leq 0.05$) than those that received the Ross 308 guidelines. This may be due to the very low Ca and P levels supplied in the finisher and post finisher phases. However, the rest of the treatments were not significantly different ($P > 0.05$), even though they tended to decrease in tibia ash % with decreasing Ca and P levels supplied. The tibia ash weight (mg/tibia) gave a steadier trend, again with the tibia ash weight decreasing with lowered Ca and P levels supplemented. No other significant differences ($P > 0.05$) were seen between treatment means for tibia ash weight.

In conclusion, this trial showed that it would still be safe practice to use the Ross 308 guidelines from the pre-starter to grower phases for optimum performance and bone integrity. However, Ca can be decreased by 0.25% and 0.24% and P by 0.15% and 0.13% in the finisher diet and post-finisher diets respectively compared to the Ross 308 guidelines without causing a detrimental effect on performance parameters. The decreased levels drastically reduced the P excretion, which resulted in less wastage and curbing the pollution problem. These levels also did not affect the bone breaking strength, bone ash % or tibia ash weight and therefore did not hinder bone mineralisation or bone integrity.

Chapter 7

Critical Review and Recommendations

1. Future research should determine the digestible Ca and P indexes on a standardised platform in order that research can be compared internationally and across different laboratories. Large discrepancies and variations have been observed between laboratories that analyse Ca and P due to unstandardised methods. Available research is based on nPP value which is used due to its convenience but does not give valuable results as nutritionists do not formulate to nPP value but to a retainable or digestible value.

2. Extended research can be done across different breeds, for example, Cobb as it is a common breed used in South Africa with very little research concerning the Cobb breed. A similar trial can be done for layer production as calcium and phosphorus play an important role in the production of eggs.

3. This trial was done on recommendations from experts in the field of mineral absorption which have done countless trials on finding the best Ca to P ratio and optimum levels in their conditions. However, methods and procedure that work well in the United States of America may not necessarily work in South Africa or under typical South African conditions (such as management, rearing period, weather, water quality and feed ingredient variation). Therefore, it may be worth doing independent research in South Africa to find the optimum Ca and P levels and ratio that is best suited for our environment.

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