ABSTRACT

DALMAGRO, MARCELO RODRIGO. The Effects of Dietary Calcium and Phosphorus Levels on Performance, Mineral Retention, Bone Characteristics, Leg Abnormalities, and Walking Ability of Heritage Broilers. (Under the direction of Dr. Edgar O. Oviedo-Rondón).

This project aimed to develop a comprehensive understanding of the responses of Heritage broilers to dietary Ca and nPP concentrations. Three experiments were conducted to test combinations of dietary Ca and P levels during the most common feeding phases used in the USA (starter: 1-17 days; grower: 18-35 days; and finisher: 36-49 days). Performance, mineral retention, bone mineralization and strength, the incidence of bone and leg abnormalities, and walking ability were evaluated. Additionally, this seems to be the first time that the effects of mineral nutrition were evaluated on bone strength by assessing the incidence of femur breakages during mechanical deboning.

Heritage broilers showed optimum performance when provided 0.90-0.94% Ca and 0.42-0.44% nPP during the starter phase (Chapter II), while maximum bone mineralization and strength were obtained at the highest levels tested (1.04% Ca and 0.52% nPP). On the other hand, no significant effects of treatments were observed on the incidence of tibial dyschondroplasia (TD), leg abnormalities, and walking ability. Broiler capacity to retain P at 10 days was improved when using Ca levels higher than 0.85% combined with 0.37% nPP, and at 45 days retention was still influenced by the levels of Ca fed in the starter diet, retaining more P as dietary levels of Ca increased from 0.85 to 1.04%.

It was demonstrated that during the grower phase (Chapter III), dietary levels of Ca between 0.75 and 0.90% combined with 0.41 to 0.44% nPP resulted in the best growth performance. Similar to the results obtained in the first experiment, increments in Ca and nPP up to their

highest levels (0.94% Ca and 0.44% nPP) improved bone mineralization and strength. Nevertheless, these high levels were related to increased probability of incidence of bone and leg abnormalities. No significant differences between treatments were detected in walking ability. Moreover, broilers retained more Ca and P when fed diets containing 0.90-0.94% Ca and 0.33-0.35% nPP during the grower phase.

Ca and P levels fed during the finisher phase (Chapter IV) affected feed conversion ratio (FCR) during the same period, and optimum FCR was observed at Ca levels higher than 0.80% combined with nPP levels at about 0.27%. Increments in Ca and nPP dietary levels during the finisher phase still caused improvements in bone mineralization and strength. Levels of Ca lower than 0.45% and nPP lower than 0.25% reduced the probability of incidence of epiphyseal breakages during mechanical deboning and also the incidence of leg problems. Wide Ca:nPP ratios resulted in higher probability of incidence and severity of TD. The best retention of P was observed among broilers fed diets containing Ca levels higher than 0.70% and levels of nPP ranging from 0.26 to 0.37%.

In conclusion, data reported herein demonstrated that in order to obtain the optimum performance of Heritage broilers, the levels of Ca and nPP were as follows: 0.90-0.94% Ca combined with 0.42-0.44% nPP for the starter phase; 0.75-0.90% Ca combined with 0.41-0.44% nPP for the grower phase; and Ca higher than 0.80% combined with 0.27% nPP for the finisher phase. Bone mineralization and strength improved as dietary levels of Ca and nPP increased in all feeding phases. However, the incidence of bone and leg abnormalities increased as levels of Ca and nPP increased in the grower and finisher diets. Mineral

retention was affected by the levels of Ca and nPP in all dietary phases, and Ca and nPP levels fed in starter and grower diets influenced the retention of these minerals at market age.

The Effects of Dietary Calcium and Phosphorus Levels on Performance, Mineral Retention, Bone Characteristics, Leg Abnormalities, and Walking Ability of Heritage Broilers.

by
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DEDICATION

To my parents, João Carlos Dalmagro and Sirlei Scussel Dalmagro; and my brother, João Carlos Dalmagro Júnior, for all the love and encouragement that made me believe that I am the one who will build my future. Throughout my life you have shown me that my dreams could be as big as I wanted it to. I am blessed for having you as an example of integrity, kindness, and happiness.

To my wife, Isabela Leal Carmignan Dalmagro, for everything. I cannot describe in words all that you represent to me. Thanks for your love, dedication, friendship, support, and patience. You helped me to feel confident and to overcome obstacles. It is really good to know that as time passes, together we become better persons.

This is achievement is <u>ours</u>.

BIOGRAPHY

Marcelo was born and raised in São Miguel do Oeste, a small town in the southern state of Santa Catarina, Brazil. He obtained his degree in veterinary medicine with an honor for the highest performance student for the year at the Universidade Federal do Rio Grande do Sul, where he received a research scholarship from the Center of Diagnosis and Research on Avian Pathology. During his time at the veterinary school, Marcelo was founder of a research group in ostrich production and of a consulting company formed by students. As a veterinarian, Marcelo worked for 2 years at Sadia, one of the largest poultry companies in Brazil, supervising several broiler breeder farms. He also worked for an Argentinean pharmaceutical company, Vetanco, where he was in charge of sales and technical support for three states. In 2009, Marcelo and his wife decided to move to USA to pursue a Master degree at NC State University. Part of his research was focused on evaluating the responses of Heritage broilers to dietary concentrations of calcium and phosphorus. During his master program, Marcelo was awarded for his research presentations at the Annual Meeting of the Poultry Science Association in 2010 and 2011. After graduating, Marcelo is going to return to the industry, assuming a project leader position in a new business of Vetanco, in Buenos Aires, Argentina.

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This thesis is not only the outcome of two years of hard-work and study, but also a result of a wonderful life experience that you all participated in some way.

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CHAPTER I

Literature Review

INTRODUCTION

The United States of America (USA) is considered nowadays the world's largest poultry producer, and the second exporter of poultry meat, this activity being the major feed grain user in the country (USDA-NASS, 2011). The current status of USA in global poultry production is the result of a dramatic evolution during the last few decades, reaching 22.68 billion kg of broiler meat produced in 2010, which represents five times the amount that was being produced yearly in the 1970's (Table I-1 and Figure I-1).

Table I-1. Average live weight, heads slaughtered, and total live weight by type: total US poultry production in 2010 (USDA-NASS, 2011)

Commodity	Average live weight kg	Heads slaughtered x 1,000 Head	Total live Weight x 1,000 kg
Chickens	2.58	8,790,478	22,734,258
Turkeys	17.74	242,619	3,203,569
Ducks	3.10	23,627	73,304

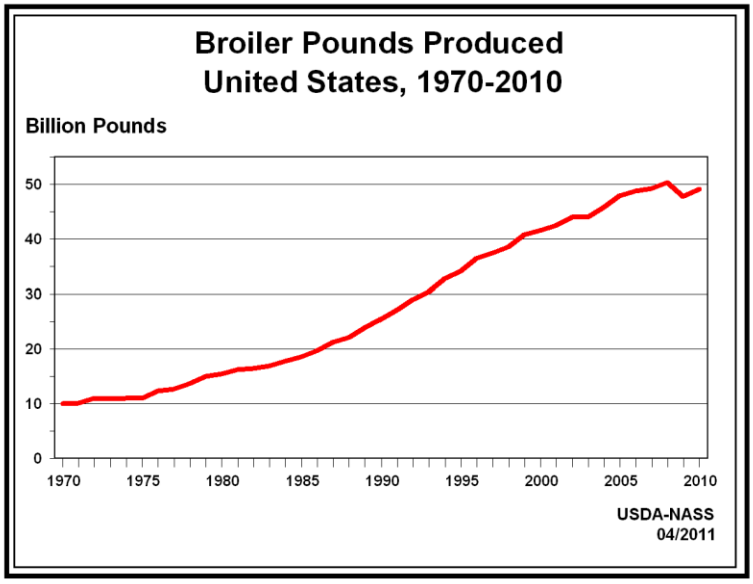

Figure I-1. Broiler meat produced in the USA, 1970 to 2010 (USDA-NASS, 2011)

The rapid and steady growth of broiler production is in good part due to the degree of specialization and technology that integrated companies have been using. Improvements in every portion of production, such as genetics, health, nutrition, management and environmental control, converged to make it possible to gradually obtain heavier broilers in less time, and converting feed-to-gain more efficiently (Havenstein, 2003).

Nutrition has a vital role and also a challenge in the current status of broiler production. According to some studies (Havenstein et al., 1994; Havenstein et al., 2003), nutrition has provided around 10-15% of the change that has occurred in broiler growth rate over the past 45 years, while genetic selection account with 85-90% of it. The genetic potential of broilers is being pushed to higher levels, which demands improvements on management, health, and nutrition. However, the business side is looking for reducing production costs. Since feed could represent 60-80% of the expenses in broiler production

(Goodwin, 2005; Murakami et al., 2007), this is surely the biggest target for cost-cutting. Among the nutrients of broiler diets, the three most expensive are energy, protein, and phosphorus (Summers, 1997; Biehl et al., 1998). Phosphorus (P) has been focused by both researchers and industry worldwide, not only for its effects on the cost of production, but also on broiler performance, processing plant efficiency, food safety, environmental concerns, leg health, and welfare. Calcium (Ca) can be considered an inexpensive ingredient; however, it is also crucial for the parameters mentioned above, as well as for its interrelationship with P. Since the metabolism of these minerals are closely related, it is necessary to discuss them together when determining their levels in broiler diets.

Because of the rapid growth of modern broilers, their requirement for oxygen, nutrients, enzymes, hormones, inductive agents, and growth factors has increased in comparison to earlier strains. Additionally, the supportive systems are challenged to maintain structure, function, and to satisfy demands of tissues during growth (Whitehead et al., 2003; Dibner et al., 2007). Thus, it is extremely important that their metabolism and bones support the increasingly heavier body weight (BW) and muscle mass that are obtained in younger ages as genetic selection for growth progresses. As discussed earlier, Ca and P levels in the diets affect performance results and the incidence of leg problems, directly compromising the welfare of the birds. Moreover, most of the poultry meat markets require animal welfare regulations, forcing companies to adapt their husbandry practices in a way to comply with those regulations. Ca and P metabolism involving bone formation also impacts processing plant downgrades due to bone breakages during processing and deboning, which results in physical food safety hazards when pieces of broken bones are left inside the meat. In addition

to these factors, environmental regulations on P excretion to the litter and further land application as organic fertilizer (EPA, 2003) have been important factors to consider when determining the Ca and P levels in broiler diets. It is also known that phosphate is one of the most expensive ingredients, so feed cost is largely affected by the levels of that mineral. Nutrient utilization has great variation among the different broiler genetic strains, as shown by their rates of growth and carcass characteristics. These variations should also be accounted for in the development of a feeding program.

The ideal scenario would be to use levels of Ca and P in broiler diets as close to their requirements as possible in order to minimize feed cost and P excretion to the litter. However, the challenge is to determine the minimum dietary levels of minerals, without adverse effects on broiler growth performance, health, welfare, processing plant efficiency, and food safety. In order to achieve the best economical outcomes of broiler production, this decision should be the result of an evaluation of how these parameters are affected by altering Ca and P levels in the diet, and the costs and benefits obtained by doing so.

The objective of this project was to evaluate the responses of Heritage broilers on performance, bone characteristics, mineral retention, leg health, and welfare when feeding different combinations of Ca and P. To accomplish this objective, three experiments were conducted in order to assess those parameters in each of the dietary phases commonly used by the USA industry. This Thesis contains a comprehensive literature review, and three additional chapters describing the experiment conducted for each dietary phase.

LITERATURE REVIEW

Calcium (Ca) and Phosphorus (P) together play important roles in most metabolic processes, especially those related to bone formation. Only a small percentage of the total body concentration of these minerals is found in the blood, but their presence in extracellular fluids is essential (Ansar et al., 2004). The skeleton of birds contains approximately 99% of the Ca and 80% of the P body reserves (Mendonça Júnior, 2000; Suttle, 2010). The responses of the fast-growing chicken to dietary Ca (Hurwitz et al., 1995) and P (Shafey and McDonald, 1990) can be altered by the modulation of growth by energy intake, genetic selection, sex, ambient temperature changes, or age. Some of these responses tend to be affected similarly by dietary Ca and P contents; others are affected differently by the respective minerals (Bar et al., 2003).

Ca is the most prevalent mineral in the body, and the dietary requirement of Ca is higher than those for any other mineral (De Matos, 2008). Even though Ca is the mineral with the highest concentration in common vegetable based broiler diets, the supplementation from inorganic sources is still required. Ca is an essential constituent of all living cells and extracellular fluids: it plays a role in the acid-base balance of body fluids; it is vital for the activity of several enzyme systems, including those necessary for the transmission of nerve impulses and the contractile properties of muscle; and it is required along with potassium and sodium for normal heart rhythm (Leeson and Summers, 2001). Since Ca is the major constituent of bones, the skeleton structure and health is directly affected by this mineral. Additionally, Ca affects blood coagulation, cellular motility, differentiation and proliferation, hormonal secretion, oxygen transport, and apoptosis (Brown, 2002).

Likewise, P has numerous roles in biological processes. It is necessary for cellular metabolism, cell signaling, as coenzymes, in nucleotide metabolism, in energy metabolism, in membrane function, bone mineralization, and for the formation of the bone organic matrix (Lyberg, 2006; Lamberg-Allardt et al., 2010). P is also essential for muscle coordination, metabolism of carbohydrate, amino acid and fat, nervous tissue function, normal blood chemistry, and transport of fatty acids and other lipids. P enters into the composition of important constituents of all living cells, and the salts formed from it are essential for keeping the acid-base balance (Leeson and Summers, 2001).

Calcium Control Mechanisms

According to De Matos (2008), from the 1% of the body Ca that is not in the bones, less than 10% of it is in the extracellular fluid, the rest being found intracelullarly. Despite its low concentration, the extracellular Ca, particularly in the ionized form which represents 20-60% of the extracellular Ca, is the physiologically active form with important roles in bone homeostasis and control of hormone secretions in response to changing demands. These important functions are some of the reasons for Klasing (1998) to describe Ca as the most metabolically active mineral, with a complex regulatory system. Ca is one of the most tightly regulated minerals, and the whole body Ca metabolism is controlled by a three tissue axis, as follows: absorption by the intestines, excretion and reabsorption by the kidneys, and bone resorption. The mechanisms for controlling hypocalcemia and hypercalcemia are summarized in Figure I-2.

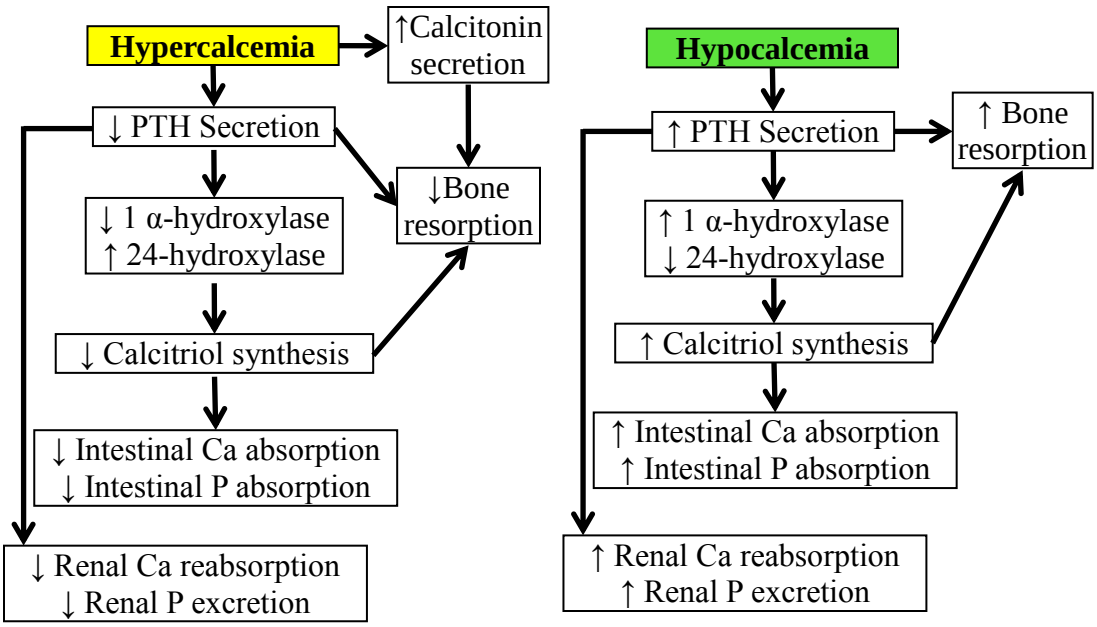

Figure I-2. Summary of the regulation of Ca homeostasis

To maintain Ca balance and bone health, the regulation of Ca absorption from the diet is an essential process, and depends on the presence of absorption pathways, digesta passage rate, and the solubility of Ca within the intestinal segment (Fleet and Schoch, 2010). Ca has to be partially solubilized in the acidic pH of crop, proventriculus and gizzard for the birds to absorb it. After solubilization, Ca transverses the intestine by both active (transcellular) and passive (paracellular) pathways. The transcellular pathway is saturable and occurs mainly in the proximal intestine. In contrast, the paracellular pathway is non-saturable, occurring throughout the length of the intestine as a linear function of luminal Ca concentration (Christakos et al., 2011; Ajibade et al., 2010). In monogastric species, one of the most important effects of calcitriol (the major metabolite of Vitamin D_3) is to stimulate active absorption of Ca from the intestinal tract via a vitamin D receptor (Shirley et al., 2003;

Breves and Schröder, 2010), acting differently in each portion of the intestine. Other hormones like parathyroid hormone (PTH) and growth hormone also influence Ca absorption directly, or indirectly through the regulation of renal calcitriol production (Fleet and Schoch, 2010). Both transcellular and paracellular Ca pathways are affected by 1,25-$(OH)_2$-D_3 (calcitriol). Vitamin D_3 is supplemented in broiler diets, but needs further activation by a two-stage hydroxylation process. The first occurs mainly in the liver forming 25-(OH)-D_3, which is not regulated by Ca and P levels in the plasma. The next step occurs when 25-(OH)-D_3 is transported to the kidney and converted either to calcitriol by the action of 1-α-hydroxylase, or to 24,25-(OH)-D_3, a less active metabolite of Vitamin D (De Matos, 2008). The hydroxylation to form calcitriol is a slow and well regulated process, with PTH, Ca, P, calcitriol itself, estrogens, prolactin, and growth hormone being involved. Depending on the plasma Ca concentration, 25-(OH)-D_3 is converted in the direction of a certain product (calcitriol or 24,25-(OH)-D_3). Hypocalcemia causes increment in PTH secretion, which results in up-regulation of hydroxylation at position 1, producing calcitriol. When the concentration of Ca is normal or high, hydroxylation at position 24 is up-regulated while 1-α-hydroxylase activity is reduced, increasing the production of 24,25-(OH)-D_3 (Taylor and Dacke, 1984).

The kidneys reabsorb almost 98% of the Ca filtered by the glomerulus, and are a major target tissue involved in the regulation of Ca homeostasis by calcitriol. The reabsorption of Ca can be passive or active, having the distal nephron as the key site for the dual regulation by calcitriol and PTH. A fall in plasma concentration of Ca stimulates the chief cells in the parathyroid to secret PTH (Dacke, 2000), which increases tubular

reabsorption of Ca and accelerates the formation of calcitriol by the kidney (Schröder and Breves, 2007; De Matos, 2008). In addition to PTH, calcitriol can regulate its own production by inhibiting the renal 1 α-hydroxylase enzyme (Ajibade et al., 2010). The effects of calcitriol in the kidney are dependent on the levels of circulating PTH. With hypocalcemia and in the presence of PTH, this metabolite decreases renal excretion of Ca by increasing tubular reabsorption. In the absence of PTH, Ca excretion is not affected. Calcitriol can down-regulate the production of PTH in the parathyroid gland directly and indirectly (De Matos, 2008).

The net flow of Ca into or out of the skeleton determines the regulation of circulating concentrations of ionic Ca, and consequently Ca absorption. The hormonal mechanisms that facilitate absorption also regulate Ca fluxes to and from the bone in a complex and multicenter way (Suttle, 2010). Nuclear receptors for calcitriol in the chondrocytes and osteoblasts facilitate Ca accretion. Osteoclasts respond to calcitriol via cytokines released by osteoblasts, increasing in sensitivity to PTH. The effects of calcitriol on bones are dependent on blood Ca and P levels. In individuals with normal plasma Ca levels, calcitriol stimulates bone formation by inducing synthesis of multiple bone proteins by the osteoblasts. In periods of hypocalcemia (increased levels of PTH) and hypophosphatemia (increased levels of growth hormones), the calcitriol has an opposite effect, promoting osteoclast differentiation and activation with release of Ca and P into circulation. Another hormone, calcitonin, has hypocalcemic and hypophosphatemic effects by limiting bone resorption when plasma levels of Ca are high. In birds, calcitonin function seems to lie specifically in the control of

hypercalcemia and in the protection of the bone against excessive Ca resorption (De Matos, 2008).

Phosphorus Control Mechanisms

Under normal physiological conditions, P homeostasis is determined by the balance between intestinal absorption of P from the diet, storage of P in the skeleton, and excretion of phosphate through the kidney (Bergwitz and Jüppner, 2010). Although specific sodium-dependent transporters for phosphate have been identified in the mucosa of the jejunum and kidneys of poultry, P deprivation only affects transporter expression in the kidneys (Huber et al., 2006). The regulation of Ca and P homeostasis are closely connected, but plasma P concentration is not as strictly regulated as Ca and may fluctuate throughout the day, especially after meals (Lyberg, 2006). Berndt and Kumar (2009) demonstrated that serum P concentrations can be altered significantly without changes in the absorption of P by the intestines, or in the excretion by the kidneys. According to these authors, P levels could be directly perceived, which is demonstrated by the presence of phosphate sensors in the intestine and in the parathyroid gland as well. The key role in P homeostasis is played by the kidney, although the movement of P from the extracellular fluid space into bone and into cells is also important in determining serum P concentrations (Lamberg-Allardt et al., 2010). On the hormonal side of the regulation, the more long-term adaptations to changes in P concentrations are likely to be mediated by the vitamin D endocrine system and PTH (Berndt and Kumar, 2009). Figure I-3 summarizes the P regulation mechanisms.

The digestive tract has several sections involved in P digestion and absorption in different ways. The crop, proventriculus, and gizzard region is where partial solubilization of the ingested compounds, and eventually the hydrolysis of phytates by exogenous phytase occur. The proximal small intestine is where a portion of the soluble compounds are absorbed, and in the distal small intestine certain compounds are insolubilized due to an increase in pH (Létourneau-Montminy et al., 2011). Similarly to Ca, P is solubilized in the gastric area, and absorbed by both active and passive mechanisms. The absorption rate is dependent on the solubilization and hydrolysis of the phytate P (PP) components of the feed (Ravindran et al., 1995). According to Breves and Scröder (1991), the large intestine is of minor importance for the total P absorption. Small amounts of P are secreted via gastric and pancreatic juice, bile and intestinal fluid, but their contribution to the gastrointestinal P turnover is low (Lyberg, 2006). P deficient animals absorb phosphate more efficiently because homeostatic regulation optimizes phosphate absorption when plasma concentrations are low. Although the major biologic function of vitamin D is to maintain Ca homeostasis, the calcitriol can also enhance the intestinal absorption of dietary P, especially from the proximal intestine (Leeson and Summers, 2001; Ajibade et al., 2010). P absorption is influenced by Ca serum concentration, where low Ca stimulates PTH secretion, inducing calcitriol production in the kidney (Lyberg, 2006). Calcitriol improves P absorption in the intestines (Lamberg-Allard et al., 2010) through an increase in the synthesis of a phosphate transport protein (Lyberg, 2006). After absorption, P is circulated throughout the body and readily withdrawn from the blood during bone development (Waldroup, 1995).

The reabsorption of P in the kidney is mediated by the type 2a sodium-phosphate and the type 2c sodium-phosphate co-transporters in the proximal tubular cells. An increase in serum P stimulates PTH secretion, which down-regulates the expression of type 2c co-transporter, decreasing renal P reabsorption. Additionally, the fibroblast growth factor 23 is synthesized in the osteocytes in response to hyperphosphatemia, and increases renal P excretion through down-regulation of both cotransporters (Lamberg-Allardt et al., 2010). In contrast, Berndt and Kumar (2009) demonstrated that animals fed a low-phosphate diet have decreased serum P concentration, which is associated with a reciprocal increase in circulating plasma Ca concentrations. This increase in plasma Ca inhibits PTH release, reducing the renal excretion of P. Additionally, low P diets and reductions in serum P are associated with increased calcitriol synthesis, which improves renal P reabsorption. While stimulation of calcitriol synthesis by the kidney in response to low levels of Ca is dependent on PTH, phosphate depletion may induce renal synthesis of calcitriol directly, independently of hormonal action (Leeson and Summers, 2001).

Bones play an important role in P homeostasis, since P can be driven in or out to adjust for body P needs. When calcitriol is released, it acts directly in the bones increasing the number of osteoclasts, thereby promoting the reabsorption of Ca and P. Ca levels in the blood also have a stimulatory effect on secretion of calcitonin, which inhibits the activity of osteoclasts and slows the rate of bone turnover (Lyberg, 2006). High serum P stimulates PTH secretion (probably by lowering Ca), which results in increased bone resorption combined with higher P excretion in the kidney. Finally, it causes an increase in serum Ca and a decrease in serum P concentrations (Bergwitz and Jüppner, 2010). Hayashibara (2007)

showed that restriction of dietary P also lowered serum concentration of PTH. Besides its effects on the intestines and kidneys, serum calcitriol promotes differentiation of osteoblasts and stimulates osteoblast expression of bone-specific alkaline phosphatase, osteocalcin, osteonectin, and a variety of other cytokines (Clarke, 2008).

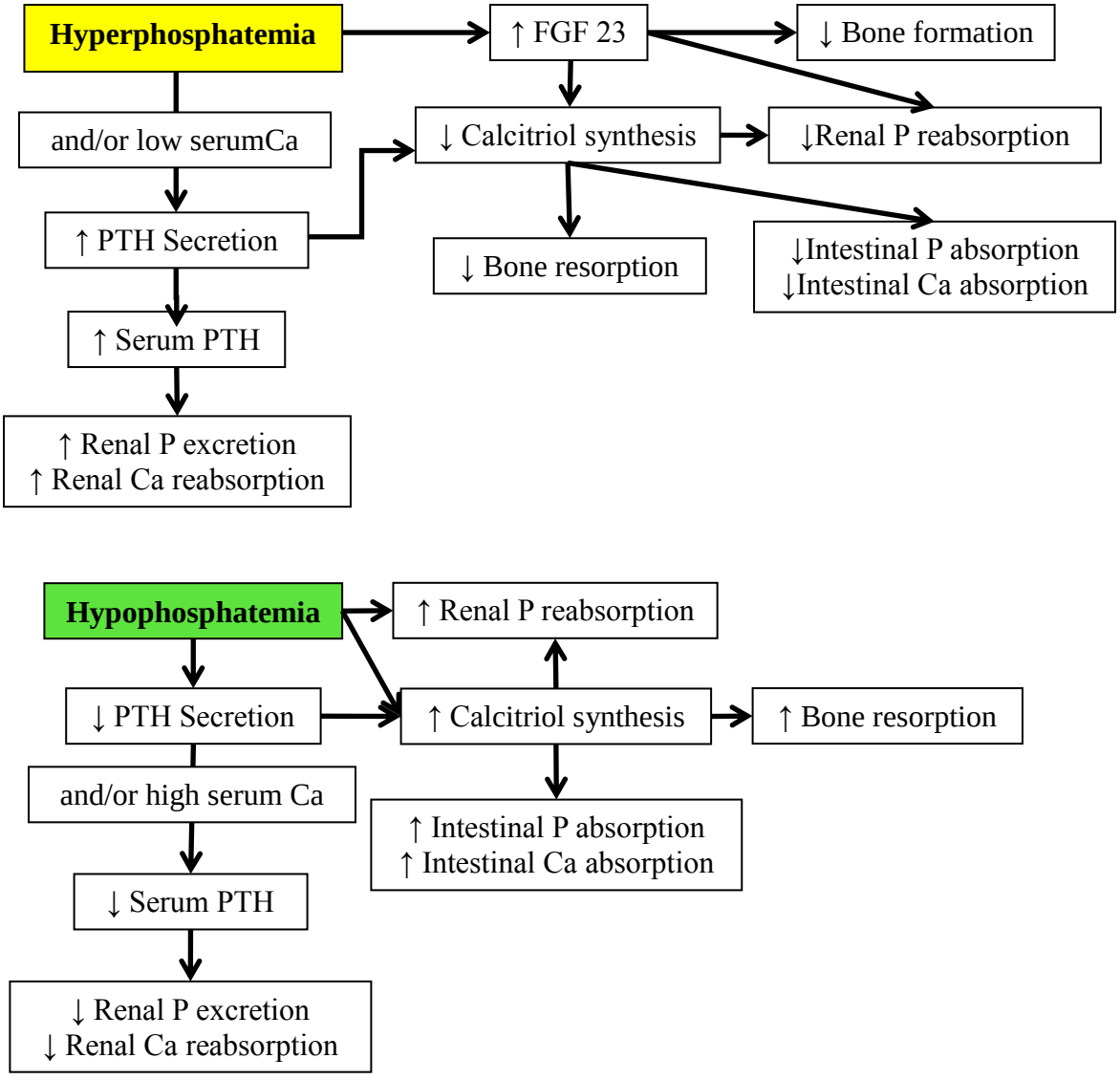

Figure I-3. Summary of the regulation of P homeostasis.

Metabolism Interactions

Ca and P regulation pathways cross in several ways, and their metabolism also has many other forms of interaction. Within seeds, the major form of P is myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (IP6), otherwise known as phytic acid, which contains 28.2% P (Ravindran et al., 1995). When the IP6 is in the anionic form (phytate), the phosphate groups are able to bind to positively charged cations, such as Ca, Mg, and K, forming the chelated molecule phytin, which accounts for about 50 to 80% of the total P in seed-based ingredients (Angel et al., 2002). The phytin molecule is often thought as an antinutrient, since the minerals that bind to it, as well as the attached phosphate groups are unavailable to the animal (Wodzinski and Ullah, 1996). Phytase breaks down the phytate making its components available for digestion, but it is scarce in birds, and generally supplemented in broiler diets.

Dietary Ca may decrease P retention by affecting phytase action. The optimal brush border phytase activity of the small intestine is reported to be between pH 5.5 and 6.5 (Maenz and Classen, 1998). However, the pH (7.39) reported by Shafey et al. (1991) obtained in a high Ca diet is greater than for optimal phytase activity. Ca also affects P digestion and absorption by other ways, such as being the dominant mineral chelating to phytate and forming insoluble complexes in the gut (Selle et. al., 2009), and inhibiting phytate P (PP) hydrolysis (Tamim and Angel, 2004). The effects of Ca concentration on the diet on P utilization are shown by Applegate et al. (2003), who demonstrated that a reduction in dietary Ca from 0.9% to 0.4% in broiler diets increased apparent ileal PP hydrolysis by 24% in one experiment (14-24 days), and by 12% in a second experiment (8-22 days).

Plumstead et al. (2008) also reported effects of Ca on P digestion, absorption, and consequent retention, observing a quadratic effect of Ca levels on P retention in broilers from 19 to 20 days. Ca levels ranging from 0.40 to 1.20% were tested in their experiment, and the breakpoint for P retention was found when using 0.74-0.99% Ca in the diet. These authors suggested that at low dietary Ca (before breakpoint), P appeared to be absorbed but was later excreted in the urine, presumably due to insufficient Ca for P retention to occur. Moreover, Schoulten et al. (2002) reported that P absorption is quadratically affected by dietary Ca concentration in broilers from 18 to 21 days, where the maximum P retention (78.9%) was observed at 0.88% Ca in the feed. Qian et al. (1997) demonstrated that P retention in broilers from hatch to 21 days increased quadratically from 58.4 to 61% (66 µg Vit D_3/kg), from 52.9 to 57.9% (660 µg Vit D_3/kg), and from 54.8 to 61.3% (6600 µg Vit D_3/kg) by reducing the dietary Ca:P ratios from 2.0:1 to 1.1:1, with no phytase supplementation. Reducing Ca in broilers starter diets (from 0.45% nPP and 0.90% Ca to 0.30% nPP and 0.60% Ca) improved P retention by 13% at 18 days, and an improvement in P retention was observed later at 32 days, suggesting that birds restricted in Ca and P levels during starter phase show an adaptation that is kept at later ages (Yan et al., 2005). It seems that keeping Ca and inorganic P levels in the minimum required improves phytate solubilization and phytase activity (mucosal and microbial), resulting in increased phytate degradation in the intestine (Mitchell and Edwards, 1996; Qian et al., 1997; Leske and Coon, 1999; Applegate et al., 2003).

The availability of Ca is also influenced by several dietary constituents, especially P and Ca itself (Scott et al., 1992). Schoulten et al. (2002) showed that Ca absorption was reduced linearly as the levels of Ca in the diet increased (0.46 to 1.30%) in broilers from 1 to

21 days of age. Similar results were found by Rostagno et al. (2000), who reported an increase in Ca absorption (54.12 to 66.10%) when dietary Ca was reduced from 1.00 to 0.68% and P from 0.70 to 0.56%. Earlier studies had already demonstrated that increased intestinal absorption of Ca (Fox et al., 1981) and P (Blahos et al., 1987) occurs in chicks given low P or Ca diets for a 10 to 15 day period. Ca utilization also is influenced by the lipid concentration of the diet, since binding occurs in the gut between these two dietary components, interfering with each other's absorption (Reid, 2004). There is evidence that in both humans and animals Ca intake increases the fecal excretion of fat, presumably via the formation of insoluble Ca fatty acid soaps in the gut or by binding of bile acids, which impairs the formation of micelles (Lorenzen et al., 2007). This reduction in fat absorption could negatively impact the energy value of the diet, thus affecting broilers performance. Earlier reports already suggested that a potential problem of adding fat to the diet of broilers is the formation of soaps during the process of digestion in the gastrointestinal tract, which would interfere with mineral metabolism, reducing Ca retention, and in some cases affecting bone ash and bone Ca contents (Whitehead et al., 1971). The reduction in the amount of Ca in broiler diets may improve performance and increase profitability; however, this should not be at the expense of increased leg problems (Driver et al., 2005).

Besides the importance of Ca and P individual levels on nutrients absorption and utilization, the metabolism of these minerals is largely affected by the Ca:P ratio. Broilers adapt to a wide range of diet P concentration, as long as a balance with Ca is maintained. Furtado (1991) suggests that an adequate P absorption is only obtained if Ca dietary concentration is ideal. Ca deficiency limits the utilization of the P absorbed, and the excess

could react with P forming insoluble complexes in the intestinal lumen. In order to prevent the negative effect of Ca on P availability, Letourneau-Montminy et al. (2007) suggested that in young chicks the recommended dietary nPP levels can be lowered with a concomitant reduction in dietary Ca concentration (from 0.69% Ca and 0.39% nPP to 0.60% Ca and 0.28% nPP) with no effects on performance in broilers from 5 to 15 days of age. However, this reduction in Ca and P levels decreased tibia ash concentration by 7% and tibia breaking strength by 18%.

Even small deviation from optimal supply of Ca and P in the diet may produce changes in their serum levels. Ansar et al. (2004) observed higher serum Ca concentration in birds fed high Ca:P ratios. When normal Ca:P ratios (2:1) were fed, higher serum P concentration was found, compared to higher ratios. It indicates that a diet with increased Ca:P ratio induces hypophosphatemia. It may be a problem especially in cases where birds are fed low P combined with high Ca diets, which would reduce even more P serum levels. In the case of P deficiency, the metabolism may react with anti-homeostatic responses, such as an increased Ca absorption, finally aggravating the hypercalcemia and damaging the kidneys (Page et al., 1979). According to Hurwitz et al. (1995), higher Ca intake leads to an initial increase in plasmatic Ca levels, which suppresses PTH secretion, and consequently reduces the production of calcitriol, reducing Ca and P absorption in the intestines in a situation where it should be increased to keep an adequate Ca:P ratio.

Calcium and Phosphorus Dietary Supplementation

Broiler diets are seed based, formulated basically using corn, soybeans, and in some cases wheat. Cereals are usually poor in Ca and rich in P; however, most of the P is stored in the seed coat in the form of phytate complexes with Ca, magnesium and potassium (Selle et al., 2003). Since the levels of Ca and the ability of the chicken to utilize PP in these macro-ingredients are insufficient to meet their requirements, poultry diets are supplemented with inorganic sources of these minerals, which have higher bioavailability.

Calcium Sources

The basic components of broiler diets, corn and soybean meal, contain low levels of Ca. Therefore, supplemental Ca is needed, and its sources differ in origin (animal, plant, or mineral), particle size, and structure (Guinotte et al., 1991; Ajakaiye et al., 1996), resulting in different physic-chemical properties (Reid and Weber, 1976). Generally, Ca is considered an inexpensive ingredient in poultry rations. The most common source of supplemental Ca in poultry diets is in some form of limestone. A limestone that contains 38-40% Ca is referred to as Ca carbonate, while those that contains 33-37% Ca is called ground limestone. Limestone varies not only in Ca content, but also in particle size and magnesium concentration. It is produced by mining the natural geological deposits, crushing, drying and screening (Axe and Liu, 2002). Dicalcium phosphate, generally used as P supplemental source in broiler diets, also contributes to Ca levels to some degree. However, other sources such as marine shells (oysters, bivalves, etc.), calcium citrate, Ca citrate-malate (CC-M), and meat and bone meals could be substitutes, according to their availability and price. Among

sources, there are variations in Ca bioavailability. Even not considering the bioavailability of certain Ca source, several other factors influence the absorption and retention of Ca, such as solubility (Roth-Bassell and Clydesdale, 1992), particle size and retention time (Leeson and Summers, 2001), Ca and P concentration in the diet (Scott et al., 1992; Applegate, 2003), and the presence of other nutrients, such as fats and their fatty acid composition (Reid, 2004). In a study comparing Ca carbonate, oyster shell, and bivalve shell Ca supplements reported no significant effects on feed intake (FI), dietary ME, or broiler body weight (BW); however, bone ash response varied when different Ca sources were used in broiler diets, and less excreta fat present as soap was found when Ca carbonate was used (Ajakaiye, 2003). Henry and Pesti (2002) did not observe more bioavailability of CC-M for tibia ash and tibia Ca content when compared to broilers fed Ca from Ca carbonate, despite the increase in BW gain and feed conversion ratio (FCR). These authors concluded that CC-M is a good source of Ca for young growing chicks, and that the only limiting factor on its use is the price, significantly higher than limestone. The particle size is also very important and influences the availability of Ca. Guinotte et al. (1991), testing three Ca sources (P-treated shell, oyster shell, and marble), three particle sizes (ground – less than 150 µm, medium – 600 to 1180 µm, and coarse – greater than 1180 µm), and three levels of Ca (0.5, 0.7, and 0.9%) with ground limestone as reference, reported that ground particles of Ca carbonate (<150 µm) significantly improved Ca retention, 28 days BW and tibial ossification characteristics in growing chickens. Bone breaking strength and tibia ash were not affected by Ca source. Conversely, the origin of Ca sources slightly affected Ca utilization and chick performance, with limestone having the lowest Ca retention compared to the shell sources and the lowest

feed conversion as compared to the shells and marble sources. In another experiment, Guinotte et al. (1995) demonstrated that coarse sources (> 1180µm) of Ca increased the amount of insoluble Ca in the gizzard content of chicks, causing a reduction in intestinal Ca retention and in bone mineralization. Manangi and Coon (2007) tested Ca carbonate particle sizes from 28 to 1306 µm in the diet of broiler chickens raised up to 28 days of age. The authors reported that particle size ranging from 137 to 388 µm improved performance and tibia ash content. Chickens fed the biggest particle size (1306 µm) gained less weight, had the poorest FCR, and the highest mortality. Retention of Ca was not affected by the particle size. Similarly, P retention (%) was not affected; however, total P intake and total retention were higher and could be a cause of higher performance in birds that consumed 137-388 µm Ca carbonate particles. Furthermore, the smallest Ca particles size (28 µm) had the highest solubility and limited PP hydrolysis reducing P available for growth and bone mineralization.

Phosphorus Sources

In broiler production, dietary P is originated primarily from plant and animal feedstuffs, and since it generally does not meet requirements for chickens, inorganic P is supplemented (Van der Klis and Versteegh, 1996; Waldroup, 1999). According to Waldroup (1999), P in plant feedstuffs may be partitioned into two separate groups: organically-bound P present as salts of phytic acid (PP) and P present in other forms (nPP). Within seeds the major form of P is the phytic acid or IP6 (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate), which contains approximately 28.2% P (Ravindran et al., 1995). The availability of the P in the diet varies according to the source used (Coffey et al., 1994; Van der Klis and

Versteegh, 1996; Hemme et al., 2005), and to the concentration of other minerals, especially Ca (Edwards and Veltmann, 1983). Broilers are probably capable of using a portion of the phytate-bound P, whereas the availability of inorganic P is less than 100% (Van der Klis and Versteegh, 1996). In animal nutrition, most supplemental P comes from several forms of phosphates, mainly dicalcium phosphate (22% Ca and 18.5% P), monocalcium phosphate (16% Ca and 21% P) and defluorinated phosphate or tricalcium phosphate (33% Ca and 18% P). In poultry feed, the most common inorganic P supplements are dicalcium and monocalcium phosphate (Newman and Leeson, 1997). Since fluorine binds to Ca-phosphates making it poorly available (Lima et al., 2000) and also impairs bone metabolism by binding to Ca in bone (Garzillo et al., 1997), P sources used in animal feeds should not contain more than one part of fluorine to 100 parts of P (AAFCO, 2010). In some countries, meat and bone meals are widely used and represent a good source of P, containing from 2 to 14% P (Axe and Liu, 2002)

The bioavailability of P in defluorinated rock phosphate has been reported to range from 83 to 100% (Dilworth and Day, 1964; Sullivan, 1966; Coffey et al., 1994; Hemme et al., 2005), which is similar to the bioavailability of dicalcium phosphate (Nelson and Walker, 1964; Sullivan, 1966; Leeson and Summers, 2001). Tricalcium phosphate is usually considered to be a highly available form of P. Despite the differences, it has been reported that there is no effect of inorganic P source on broiler performance (Dilworth and Day, 1964; Coffey et al., 1994; Hemme et al., 2005), however, the efficiency of feed manufacturing can vary according to the P source used. Finally, the cost is still the main factor to determine which source of inorganic P is going to be used when formulating diets for broilers.

Phosphates are found in the form of phosphate rock, which is processed by reacting primarily with sulfuric acid to produce phosphoric acid (H_3PO_4), the feedstock for other products. Phosphoric acid is then reacted with phosphate rock and soda ash to produce tricalcium phosphate, or with Ca carbonate ($CaCO_3$) to produce dicalcium/monocalcium phosphate (Axe and Liu, 2002). Because of its large livestock and poultry industry, North America is the second-largest consumer of all three Ca-phosphates (U.S. Geological Survey, 2011). World production of phosphate rock in 2009 was concentrated in three countries (which account for 66% of the world total): China (60.2 million ton), USA (26.4 million ton), and Morocco (23 million ton). In the USA, phosphate rock is mined by 6 firms at 12 mines in 4 states (Florida, Idaho, North Carolina, and Utah). Florida is the most important mining state, accounting for 65% of total annual domestic production. Phosphate rock in 2009 was used primarily for production of wet process phosphoric acid for fertilizer applications, which represented more than 95% of USA consumption. The remainder was used in the manufacturing of animal feed supplements, for direct application to soil, and for elemental P production (U.S. Geological Survey, 2011).

Ca and P Requirements

Since P is considered to be one of the most important minerals economically and environmentally, researchers and industry have focused efforts on studying diverse methods to reduce its use in animal diets, as well as its excretion to the environment. Angel (2011) described that according to its form, P can be referred as total P (tP), encompassing any and all forms of P; available P (aP), referring to the P that is absorbed from the diet into the

animal; inorganic P (iP), as any P not bound to an organic molecule; phytate P (PP), being an organic P that is part of a six carbon ring structure called phytic acid; and non-phytate P (nPP), which refers to the tP minus PP, or the P in the feed or ingredient that is not bound to the phytic acid molecule.

As discussed before, Ca is also essential for several metabolic processes, and it is especially important because of its interrelationship with P absorption and utilization. In an evaluation of Ca and P requirement their ratio is extremely relevant, and to establish the optimum levels it would be ideal to test combinations of these minerals, instead of their individual levels.

It is known that the Ca and P requirements decrease as broilers chickens grow (Angel, 2011). Consequently, when more dietary phases are used, broiler requirements can be met with more precision. In some countries it is a common practice for poultry companies to make use of 4- or 5-feeding-phases, however there are few data establishing requirements of Ca and P for each specific phase. Not only the levels within each period are important, but also the carry over effect from the previous nutrition should be considered. Moran and Todd (1994), tested low aP diets in the starter (0.40% aP), grower (0.35% aP), and finisher phases (0.30% aP), while keeping Ca similar to the control diets. These researchers found that continuously feeding low concentrations of dietary aP led to processing losses. However, other reports demonstrated that when broilers were fed diets containing adequate amount of Ca and P during the starter, grower and finisher phases, the removal of any added nPP and most of the Ca in the withdrawal phase had no deleterious effects on performance (Skinner et al., 1992). This is in agreement with Fritts and Waldroup (2006), who suggest that the nPP

levels can be markedly reduced in the latter stages of broiler production provided that adequate levels are fed during the starter and early grower phases. Bone is a dynamic tissue and is constantly being remodeled according to the needs of the body. Thus, birds that were fed sufficient P during earlier phases would have P mobilized from the bones, in case a restriction in later phase occurs (Angel et al., 2000a). It could be one of the reasons that Skinner et al. (1992) did not find any difference in performance yet saw effects in bone breaking strength when inorganic P was removed from the diet, compared to birds fed NRC (1994) recommended nPP concentrations from 42 to 56 days of age.

Earlier periods of deficiency could make the birds more efficient on using nutrients that were previously restricted. As shown by Yan et al. (2005), broilers exposed at an early stage of growth to a moderate Ca and P deficiency compared to NRC (1994) diets are able to adapt to it, showing compensatory growth and improvement in bone parameters (tibia ash, and tibia and shank densitometry parameters) in a later growth phase (18 to 32 days of age). Those authors demonstrated that restricted birds improved ileal absorption of P and Ca, and increased ileal PP disappearance after exposure to the deficient diet. The degree and length of mineral deficiency are important as both of these determine the structural integrity of the bone. Animals respond to nutrient restriction by increasing absorption rates and utilization efficiency, and as a consequence the excretion of those nutrients is reduced. These effects may last not only during the restriction phase, but also for ulterior periods, even if birds are fed adequate diets thereafter (Fox et al., 1981; Blahos et al., 1987; Bar et al., 2003; Yan et al., 2005). This adaptation process might involve increased production of 1,25-dihydroxy-vitamin D_3 (Blahos et al., 1987), calbindin (Morrissey and Wasserman, 1971; Bar et al.,

2003), and intestinal NaP cotransporter (Yan et al., 2007); however, the factor that initiates all these processes is still unknown.

When determining Ca and P requirements, variations should be considered in the optimum levels for each of the parameters evaluated, such as bone mineralization and breakage, leg abnormalities, performance, and mineral excretion. Research results show that BW gain changes are not very sensitive measurements of Ca and P requirements (Waldroup et al., 2000; Yan et al., 2001; Dhandu and Angel, 2003), thus, most research on Ca and P requirements is done based on bone ash. However, the levels of Ca and P required to maximize bone mineralization may be much higher than for performance. It was exemplified by Yan et al. (2001), who reported that the nPP requirement for 21-to-42-days-old male broilers in diets containing 0.90% Ca was 0.186% nPP based on BW, whereas for tibia ash percentage it was 0.332% nPP. For poultry companies, though, the requirements for maximum bone ash do not match the business objectives, being more adequate to evaluate broiler performance and carcass yield, feed cost, processing losses and food safety issues due to bone breakage, and in some cases, the P excretion. Thus, the applicability of bone ash is limited to where its reduction starts to impact those parameters (Angel, 2011). The ideal would be to evaluate Ca and P not as individual levels, but as their combinations, reporting the requirements for each specific objective.

The last NRC (1994) official publication was released more than 15 years ago, and contains recommendations for a three-phase feeding program. Nevertheless, modern broilers are more efficient in absorbing and retaining dietary nutrients, have a faster growth rate, and reach a heavier BW in less time, differing considerably from older strains in both

performance and carcass conformation (Havenstein et al., 1994ab, Williams et al., 2000). Recent estimations of the P requirements of broilers (Yan et al., 2000; Fritts and Waldroup et al., 2006; Dhandu and Angel, 2003) illustrate that the NRC (1994) recommendations exceed the requirements of modern broiler chickens.

It is interesting to note that not only the NRC recommendations seem to be overestimated for the modern broilers needs, but also the recommendations of the genetic companies and the levels commonly used in the USA industry seem to be higher than those normally found in scientific reports (Table I-2).

Table I-2. Comparison of Ca and P levels recommended by the NRC and genetic companies, the levels normally used by the US poultry industry, and requirements determined in scientific reports.

Dietary phase	Criteria	NRC[1]	Genetic companies[2]	Average US companies[3]	Top 5 US companies[3]	Research summary[5]
Starter 0-21 d	Performance	1.00% Ca 0.45% aP	0.90-1.05% Ca 0.45-0.50% aP	1.03% Ca 0.51% aP	1.14% Ca 0.53% aP	0.89% Ca 0.35% nPP
	Mineralization					0.89% Ca 0.39% nPP
Grower 22-35 d	Performance	0.90% Ca 0.40% aP	0.85-1.05% Ca 0.42-0.48% aP	0.91% Ca 0.44% aP	1.03% Ca 0.42% aP	0.83% Ca 0.30% nPP
	Mineralization					0.82% Ca 0.31% nPP
Finisher 35-42 d	Performance	0.90% Ca 0.40% aP	0.85-0.95% Ca 0.40-0.45% aP	0.84% Ca 0.39% aP	0.95% Ca 0.39% aP	0.83% Ca 0.30% nPP
	Mineralization					0.82% Ca 0.31% nPP
Withdrawal > 42 d	Performance	0.80% Ca 0.35% aP	0.80-0.85% Ca 0.40-0.42% aP	0.79% Ca 0.37% aP	0.90% Ca 0.41% aP	0.60% Ca 0.11% nPP
	Mineralization					0.72% Ca 0.16% nPP

[1]Adapted from NRC (1994)
[2]Adapted from Hubbard (2007), Cobb 500 (2008), and Ross 708 (2009) nutritional guidelines
[3]Adapted from Agristats (2011)
[4]Adapted from Angel (2011), based on male broiler chickens only

Ca and P effects on Live Performance

Due to their roles in the animal metabolism, Ca and P affect broiler performance in several ways. In general, nutritionists have no concerns about excess Ca in broilers diets due to its low cost, and also because it does not show clear toxicity effects. However, Bar et al. (2003) and Schoulten et al. (2003) reported that BW gain was negatively affected as levels of Ca in the feed increased from 0.40 to 2.00% and from 0.55 to 0.85%, respectively. On the other hand, P is an issue among researchers and industry, because of its effects on feed cost, broiler performance, mineral metabolism, and environmental issues. Research reports on Ca and P effects on live performance are very diverse and sometimes even contrasting. As discussed earlier, broiler response to the levels of these minerals depends on genetics, age, overall nutrition, and environment conditions. Therefore, all these factors should be taken into consideration when comparing and establishing the levels of Ca and P in broiler diets. Some studies comparing levels of Ca and P and their effects on live performance are summarized in the Table I-3.

Table I-3. Summary of studies that evaluated the effects of Ca and P levels on performance during the starter, grower, and finisher phases.

Reference	Strain/Sex	Age, d	Levels tested	Summary of the results
Starter phase				
Waldroup et al., 2000	Cobb Male	0-21	0.10 to 0.50% nPP with 1.00% Ca with or without phytase	Requirement for BWG was 0.32-0.34% nPP Requirement for FCR variated from 0.17-0.27% according to nPP concentration in corn
Schoulten et al., 2003	Hubbard Mixed	1-21	0.46, 0.67, 0.88, 1.09, and 1.30% Ca combined with 0.42% aP	Increasing Ca from 0.46% to 1.30% led to a linear reduction in BWG, however FCR and FI were not affected
Onyango et al., 2003	Ross Male	7-22	0.51% Ca with 0.13% nPP; 0.67% Ca with 0.24% nPP; and 1.00% Ca with 0.50% nPP	FI, BWG, and FCR increased linearly with increase in levels of Ca and nPP
Yan et al., 2005	Hubbard Male	0-19	0.90% Ca with 0.45% nPP and 0.60% Ca with 0.30% nPP	No effects on BW at 8 days. At 14 and 19 days birds fed higher levels were heavier FI from 8 to 19 days was lower in the birds fed lower levels
Driver et al., 2005	Cobb Mixed	0-18	0.90% Ca / 0.35%nPP (0.68% tP) 0.60% Ca / 0.24% nPP (0.47% tP)	Broilers fed higher Ca and nPP showed improved BWG, FI, and FCR
Coto et al., 2008	Cobb 500 Male	0-18	0.35, 0.40, 0.45 and 0.50% nPP each nPP level combined with Ca in the ratio of 2:1 and other 3 Ca levels (0.20% less Ca than 2:1 ratio, and 0.20% and 0.40% more Ca than the 2:1 ratio)	No effects of nPP levels on BWG and FCR BWG was higher when using 0.20% more Ca than the 2:1 ratio (0.9-1.2%) FCR was higher when using 2:1 ratio or 0.40% more Ca than the 2:1 ratio.
Powell et al., 2011	Ross	0-14	0.40, 0.45, 0.50, 0.55, and 0.60% nPP with Ca:nPP of 2.2:1	No differences in ADG and FCR, however FI increased linearly with increments in nPP levels
Powell et al., 2011	Ross	0-21	0.50 or 0.60% nPP with Ca:nPP of 1.9:1	Feeding 0.60% decreased ADG during the starter phase. In the overall performance (0-49d) FCR was better for broilers fed 0.50% in the starter phase compared to the 0.60%.
Grower phase				
Ziaei et al., 2010	Ross	14-39	0.83% Ca with 0.66% tP 0.73% Ca with 0.55% tP 0.63% Ca and 0.55% tP 0.53% Ca and 0.55% tP	BWG and FCR were not affected FI was reduced when levels of Ca were below 0.73%
Bar et al., 2003	Cobb Mixed	29-43	0.69% tP with 0.40-2.00% Ca	BWG reduced progressively as Ca increased
Cardoso Jr. et al., 2010	Cobb	8-35	0.37, 0.32, and 0.27% nPP combined with 0.85, 0.75, 0.65, and 0.55% Ca	No effects on performance
Finisher phase				
Dhandu and Angel, 2003	Ross Male	32-42	0.33, 0.27, 0.20, and 0.15% nPP combined with 0.69% Ca	No effects on BWG, FI, and FCR
Chen and Moran, 1994	Ross x AA x Hubbard	42-49	0.79% Ca and 0.54% tP 0.59% Ca and 0.38% tP	No efffects on live performance
Gomes et al., 2004	Hubbard Mixed	43-53	0.15, 0.22, 0.29, 0.36, 0.43, and 0.50% nPP with 0.89% Ca	No effects on live performance

Ca and P Effects on Mineral Retention

In many regions of intensive animal production, the amount of excreta generated is higher than the demand for it as organic fertilizer (Takemasa and Takagi, 2001). Therefore, regulations have been adopted limiting the use of animal waste for land application, focusing mainly on P and nitrogen. The quantity of P released into the environment is a global concern, and arises from the fact that P contributes to the eutrophication of water and contamination of watersheds. It has been an increasing issue in USA, mainly in areas where poultry production is concentrated (Sharpley, 1999). The USA Environmental Protection Agency (EPA) has passed federal regulations that limit the amount of poultry litter that can be applied to soils, based mainly on litter P content (EPA, 2003). As an example, there are areas of northwestern Arkansas and eastern Oklahoma (lying in the Tulsa, OK watershed) where farmers are prohibited from applying poultry litter on land until a P index has been established (Cody, 2003). These environmental concerns and regulations have led poultry companies and universities to conduct vigorous research on finding nutritional strategies to reduce P excretion that include reducing levels of P in the feed, the use of dietary enzymes, supplementation of diets with higher levels of vitamin D_3, utilization of ingredients with lower concentrations of PP, and feeding low levels early in life for birds to adapt and improve absorption in later stages (Li et al., 2000; Waldroup et al., 2000; Yan et al., 2001; Dhandu and Angel, 2003; Yan et al., 2005; Ashwell and Angel, 2010).

Based on research publications, Powell et al. (2008) suggested that it is possible to reduce the P concentration of the diet without affecting broiler growth performance, and potentially mitigate the P excretion to the litter. The economic issues behind P use in poultry

nutrition, in addition to the fact that feeding lower levels of P reduce the concentration of total P in the excreta make it important to feed broilers P levels as closer to their requirements as possible. It could be done by using precision formulation tools and by minimizing the safety margins (Dhandu and Angel, 2003). The reduction in P excretion by using lower levels of P in the diet was shown by Waldroup et al. (2000), who reported that as nPP concentration increased from deficient (0.10%) to excessive (0.50%) with 1.00% Ca in the diet of male Cobb chickens, the excreta P concentrations increased gradually until the point at which tibia ash percentage was at a maximum, and then increased steeply. This is in agreement with Yan et al. (2005), who reported improved Ca and P retention at 18 days when birds were fed starter diets containing low levels of these minerals (0.60% Ca and 0.30% nPP vs. 0.90% Ca and 0.45% nPP). In the same study, broiler chickens were then exposed to two different grower diets (0.60% Ca and 0.30% nPP vs. 0.80% Ca and 0.40% nPP), and birds fed low levels of Ca and P in both the starter and grower phase showed higher ability to retain Ca and P at 32 days, as compared to birds fed the higher levels during the starter and grower periods. Different results were described by Ziaei et al. (2008), who tested diets with 0.9% Ca and 0.72% tP as a control, and three diets with lower tP concentration (0.61%) with different Ca concentration (0.8, 0.7 and 0.6%) in broilers from 11 to 21 days. These researchers reported that lowering the level of P in the feed did not affect P excretion. Moreover, when Ca levels were reduced below 0.73%, FI and skeletal strength were impaired. As discussed before, Ca and P metabolisms are interrelated, and Ca also can modulate P excretion, as shown by Plumstead et al. (2008). These researchers reported that although low levels of dietary Ca consistently increased phytate hydrolysis and the amount of

P absorbed from the intestines, overall P retention at 17 and 20 days responded positively to increasing dietary Ca concentrations (from 0.47 to 1.16%) fed to broiler chickens from 16 to 21 days of age. Similar interactions were reported by Qian et al. (1997) and Schoulten et al. (2003).

Ca and P Effects on Bone Characteristics

Ca and P are well known for their roles in bone development. The concentrations of these minerals in broiler diets, as well as their ratio, have effects on the bone inorganic matrix formation and on the reserves that can be used in case birds are not receiving their requirements through the feed. Bone formation is especially important during early growth and is highly dependent on nutrition (Rath et al., 2000), especially on dietary Ca and P levels. During the grow-out, most of Ca consumed is used for bone formation, and P deposition in the bone follows Ca to form hydroxyapatite. Barreiro et al. (2009) suggested that the bone tissue presents a plasticity, which makes it able to respond to stimuli, adapting itself to the pathological or physiological conditions that are submitted. This is especially important for modern broilers, since their market age is relatively short (in some cases, as short as 28 days), and sometimes are sent to the processing plant even before the skeletal system is fully developed. Bone development poses several challenges to broiler production and management due to the large body mass that the immature skeleton has to support. The role of nutrition in this case is to provide the best nutrient balance, in order for the bones to develop as fast and correctly as possible, with Ca and P levels being essential for that.

Logically, bone development has effects on broiler performance, mainly limiting the growth when it is not adequate. Fragility of bones due to abnormal development is correlated to the incidence of bone fragments in deboned products and to the incidence of bloody meat during processing of the carcass (Gregory and Wilkins, 1992; Driver et al., 2006). Chen and Moran (1995) reported that these facts may be caused by reductions in Ca and P levels in the diet. Eventually, bone disorders may affect the efficiency of the processing plant, increasing downgradings, costs for parts removal, and the risk for food safety. Broiler processing plants in the USA are highly automated, and most of the companies have machinery to perform mechanical deboning of several parts, most commonly thighs and drumsticks. When mechanical deboning is used, the strength of bones to resist to the forces applied during processing is vital for this process to occur correctly.

Bone status is commonly used as an indicator of mineral adequacy in poultry diets (Onyango et al., 2003), and consequently it is used to assess the adequacy of dietary Ca and P levels. There are several invasive and noninvasive methods of evaluating bone mineralization in poultry. Bone ash, bone breaking strength, bone weight, and bone volume are examples of invasive methods. Photon absorptiometry (bone densitometry) can be used as both invasive and noninvasive (Rao et al., 1993).

Considering that tibia ash is a very sensitive measurement, it has been the most common method used to evaluate Ca and P requirements based on the degree of mineralization. Driver et al. (2006) demonstrated that tibia ash is not only related to bone strength parameters, but also has strong correlation to the incidence of bloody breast muscles. Although tibia ash is widely used to evaluate Ca and P adequacy, it is time- and labor-

intensive. In order to reduce cost and time to obtain results, bone breaking strength via shear force, has been used as an alternative method to assess bone mineralization (Shaw et al., 2010a). In this evaluation, several parameters could be estimated. Some of them were described by Crenshaw et al. (1981) as follows: breaking strength is the force required to break the bone (expressed in N); and stress is the force per unit area (expressed in Pa). Wilson and Mason (1992) showed that shear values are well correlated with bone ash, but they are liable to variation based on bone storage, handling, and site of shearing. Shaw et al. (2010b) found no differences in breaking forces between the right and left tibia of broilers, with weekly analyses from hatch to 28 days of age, suggesting that both tibiae grow and mineralize at the same rate. It was suggested by Driver et al. (2006) that short bones, such as clavicles, are more sensitive to short-term fluctuations in the Ca and P status of the bird, whereas the integrity of the tibia and femur depend more on the Ca and P status of the chick during early life, when bone development is more active.

Other methods of assessing bone mineralization are the determination of Bone Mineral Content (BMC) and Bone Mineral Density (BMD) using Dual X-ray Absorptiometry (DEXA). BMD measures the projection density (g/cm^2) of bones and does not necessarily reflect bone size, whereas BMC is a function of density and size. Onyango et al. (2003) reported that the correlation between bone densitometry and tibia ash percentage in broilers is 86%. These measurements were described by Yan et al. (2005) as good indicators of Ca and P nutrition, with BMC being more sensitive than BMD. These researchers also suggested that analysis of tibiae seemed to be more sensitive than those of the shanks. Higher BMC only indicates more total bone mineral content and does not necessarily means stronger

bones, because it can be a result of relatively larger bones. There are also normal alterations in bone density and contents according to the BW of the birds, as showed by Schreiweis et al. (2004), who stated that larger birds had higher BMD and BMC. Moreover, bone mineralization occurs differently between sexes. Some reports showed that females had higher tibia ash percentage than males (Alves et al., 2002; Schoulten et al., 2003). It could be a result of the precocious sexual maturity that occurs in females, caused by the action of the estrogen hormones, which help improve calcification in young birds. Different results were shown by (Teixeira, 1994) who found no differences between sexes in tibia mineralization.

Skeletal Abnormalities

Skeletal abnormalities represent a great concern within the poultry industry not only for their effects on production (Venalainen et al., 2006), but for being one of the most prevalent welfare problems in broilers (Julian, 2004; Mench, 2004). As production problems, these abnormalities potentially reduce feed efficiency and growth, slow down automatic processing lines, increase the requirement of manual trimming during deboning (Oviedo-Rondón, 2007), and eventually maximize the risk of food safety problems due to pieces of bone in the meat caused by the breakage of fragile bones.

According to Newman and Leeson (1997), within the skeleton there are three types of bone tissue of birds: the cortical bone, which is found in the diaphysis of the long bones; the cancellous bone in the vertebrae; and the epiphysis of the long bones. At the end of the long bones there is the metaphysis, the growth plate, and the epiphysis (which is covered in articular cartilage). The chondrocytes populate the growth plate in a parallel arrangement to

the long axis of the bone. It can be divided in several zones from the proximal to the distal border, as follows: the resting/reserve zone, containing stem cells; the proliferative zone, with stacks of flattened cells; the hypertrophic zone, containing hypertrophic chondrocytes; and the degenerative zone with a partially calcified matrix and invading capillaries (Pines et al., 2005). The bone-forming cells proliferate in the growth plate, and then enlarge, followed by the mineralization of the cartilage, which becomes replaced by the so called bone. Any factor interfering with the correct development of bone structures may cause bone or leg abnormalities. Although it has been proposed that modern broilers have a high capacity to adapt to P or Ca deficiency (Hurwitz et al., 1995; Bar et al., 2003; Yan et al., 2005), it was also reported that numerous skeletal disorders are associated with dietary imbalances and deficiencies (Cook, 2000; Long et al., 1984a; Long et al., 1984b; Whitehead et al., 2003; Williams et al., 1999; Shafey, 1993; Coto et al., 2008).

In growing chicks, tibial dyschondroplasia (TD) is a major skeletal problem, and it is associated with genetic (Sheridan et al., 1978) and nutritional factors, especially Ca and P levels and their ratio (Sanders and Edwards, 1990). TD lesions can affect the welfare of broilers, possibly causing pain and lameness. Broilers affected generally have bowed legs, sit on their hocks and are reluctant to move. In most cases, only a very small percentage of the affected birds show clinical signs (Henry and Pesti, 2002); however, both clinical and subclinical cases can result in economic loss due to trimming and downgrading of carcasses (Burton et al., 1981). In summary, TD is a skeletal abnormality that occurs in the proximal ends of the tibia-tarsus and tarsus-metatarsus of growing birds, where prehypertrophic cartilage cells derived from the growth plate fails to undergo normal maturation and

vascularization, remaining as cartilage instead of converting to bone tissue (Riddell, 1975; Poulos et al., 1978). The TD lesion is characterized by a mass of white, opaque, unmineralized, unvascularized cartilage (Henry and Pesti, 2002), and a system to score its incidence and severity was developed by Edwards and Veltmann (1983). By their methodology, a score 0 means tibiae with no visible TD lesions, while scores 1, 2, and 3 mean increasing degrees of TD lesions. Earlier studies conducted by Riddell (1975) demonstrated that male chicks are more susceptible to the development of TD. Nutrition has an important role in the development of TD, and Edwards and Veltmann (1983) proposed that high levels of Ca in the feed might prevent TD incidence. These authors reported that the incidence of TD was 13% in chickens fed a diet containing 1.1% Ca and 0.55% aP, while 39% of broilers fed diets low in Ca and high P (0.8% Ca and 0.75%) developed TD. Not only the total levels of Ca and P affect this disorder, but also the ratio of Ca and aP seem to be of prime importance in the expression of TD lesions (Hulan et al., 1985). It was proven by Riddell and Pass (1987), who evaluated the incidence of TD in broiler chickens fed different starter diets (0.8% Ca and 0.75% aP; 1.1% Ca and 0.55% aP; and 1.4% Ca and 0.55% aP) up to 4 weeks of age. After this period, all birds were fed a common grower feed (1.1% Ca and 0.55% aP). In this study, chickens fed the narrowest Ca:aP ratio had the highest incidence and severity of TD, while the widest ratio resulted in the lowest incidence severity of TD.

Oviedo-Rondón et al. (2006) categorized leg abnormalities as infectious and non-infectious. Among the non-infectious, valgus and varus are the most frequent causes of lameness and skeletal problems in broiler and turkey flocks. Valgus is the most common deformity, and it is defined as a lateral, and varus as a medial angulation of the distal

tibiotarsus resulting in deviation of the lower part of the leg (Julian, 1984; Leterrier and Nys, 1992). When the deviation becomes so severe that the bird is unable to rise, particularly in the case of valgus deformity, this is called "twisted legs" (Thorp, 1992). Another deformity common in poultry flocks is named crooked toes, with a not well understood pathogenesis, that could be explained by the shortening of digital flexor tendons (Crespo and Shivaprasad, 2003), which results in laterally or medially crooked toes (Herenda and Franco, 1996). Broilers that exhibited crooked toes showed greater heterophil:lymphocyte ratio, indicating that crooked toes prevalence could be associated with increased stress and welfare issues in broilers (Campo and Prieto, 2009).

Locomotion Disorders

The whole metabolism of modern broilers is challenged by their rapid growth, and one of the most affected is the skeletal system, which consists of bones, cartilage, ligaments, and tendons. Abnormalities in the development of the skeletal system will affect directly the walking ability of the birds. Broilers with locomotion problems have poor growth performance (Kestin et al., 1999; Kestin et al., 2001), and they are likely to be downgraded at slaughter, reducing the profitability of the companies. The walking ability also reflects the welfare status, since leg abnormalities could cause pain and discomfort (Weeks et al., 2000), hindering the access to feeders and drinkers, and making birds unable to express their normal behavior. These locomotion disorders, and consequently changes in feeding and drinking pattern, lead to loss in weight gain (Savory and Kostal, 2006). Therefore, gait-scoring systems are currently used as a welfare evaluation. Additionally, lame birds spend more time

lying in the litter (Oviedo-Rondón et al., 2009), making them more susceptible to breast blisters, scratches, inflammatory processes, muscle atrophy (Julian 1998; Vaillancourt and Martinez, 2002), and other diseases. It leads to an increment in the contaminants carried into the processing plant, which threatens food safety (Oviedo-Rondón, 2007).

Modern broilers have different performance, carcass conformation, and body composition as compared to former strains. While the performance results have been improved, the incidences of leg problems have also increased. Venalainen et al. (2006) states that genetic selection of modern broilers imposes stress on the skeletal system, resulting in poorer walking ability and increased incidence of lameness. Although economic losses from leg disorders are difficult to estimate, industry data (not shown) suggest that 0.3-1% of all broilers in commercial flocks are affected.

Several researchers developed ways to measure the lameness, evaluating and scoring the walking ability or the willingness of birds to stand (Mench, 2004). The most known method is the one described by Kestin et al. (1992), which proposed a gait-scoring-system with 6 levels, having the score 0 for a normal walking bird, and score 5 for birds unable to walk. In a way to facilitate the assessment of the gait-scoring-system in the field, Webster et al. (2008) validated a correspondence between the Kestin and a simpler method, containing only three levels. It is known that walking ability deteriorates as age progresses (Venalainen et al., 2006; Brickett et al., 2007), thus, comparisons should be done only between flocks of the same age. Additionally, females are reported to have lower gait scores than males (Venalainen et al., 2006; Brickett et al., 2007). There is a strong positive correlation between the gait score and BW (Kestin et al., 1992; Venalainen et al., 2006; Talaty et al., 2010);

however, its correlation with specific leg problems (TD, varus, valgus, and crooked toes) is low, indicating that other factors are involved in the walking ability of broilers (Lynch et al., 1992; Kestin et al., 1999; Sanotra et al., 2001). Talaty et al. (2010) reported that when using BW as a covariate, all bone traits (BMC and bone measures), except BMD, did not differ between birds with poorer walking ability (gait score of 3) and those with good walking ability (gait score 0 or 1). Similarly, other studies showed that gait scores are not related to bone strength, ash, radiographic density, and bone traits (Yalcin et al., 1998; Venalainen et al., 2006).

In the present research, all the previous factors were evaluated in a comprehensive scope, aiming to obtain data to support the decision-making process in regards to the levels of Ca and P to be used in Heritage broiler diets.

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CHAPTER II

Effects of Calcium and Phosphorus Levels in Starter Diets on Heritage Broiler Performance, Mineral Retention, Bone Characteristics, Leg Abnormalities, and Walking Ability

ABSTRACT

One experiment was conducted to evaluate the effects of Ca and nPP levels in starter diets (0-17 days) on performance, mineral retention, bone and leg characteristics, and walking ability of Heritage broilers. 1,920 male and female chicks were distributed in 64 pens with 15 chicks of each sex per pen. Starter diets were corn-soybean meal based, containing 16 combinations of 4 Ca(0.62, 0.76, 0.90, and 1.04%) and 4 nPP levels (0.31, 0.38, 0.45, and 0.52%). Common grower and finisher diets were fed from 18 to 35 days and 36 to 49 days, respectively. BWG, FI, and FCR were assessed for each dietary phase. Ca and P retention were evaluated at 10 and 45 days. At 42 days walking ability, leg abnormalities, and individual body weights were recorded. Tibia strength was determined at 17 days using the 3-point bending test, thighs collected at 49 days were used to evaluate bone breakage during mechanical deboning, and bone densitometry was assessed using shanks collected at 49 days. TD evaluation was done at 17 and 49 days and tibia ash percentage determined at 17 days. Data were analyzed within a randomized complete block design by response surface methodology. BWG, FI, and FCR during the starter phase and female BWG from 0-49 days were affected quadratically ($P \leq 0.05$) by Ca levels. P retention at 10 days had a quadratic ($P \leq 0.05$) effect of nPP and at 45 days it was affected quadratically ($P \leq 0.05$) by Ca and linearly ($P \leq 0.05$) by nPP levels in starter feed. Tibia ash percentage increased linearly ($P \leq 0.05$) with increments in dietary Ca. Male breaking strength was affected linearly ($P \leq 0.01$) by Ca. BMC showed a quadratic effect ($P \leq 0.05$) of nPP for both sexes. Female BMD increased linearly ($P \leq 0.05$) as nPP levels increased, while male BMD was affected linearly

($P \leq 0.05$) by Ca and quadratically ($P \leq 0.05$) by nPP. Bone breakage during mechanical deboning was affected quadratically by Ca ($P \leq 0.05$) and nPP ($P \leq 0.01$) levels fed during starter phase. In summary, levels around 0.90-0.94% Ca and 0.42-0.44% nPP improved performance during the starter phase. The retention of P at 10 days has higher when broiler chicks were fed lower levels of Ca and P (around 0.65% Ca and 0.37% nPP), and maximum tibia ash occurred at the highest levels of Ca and nPP. No significant treatment effects were detected on flock uniformity, leg abnormalities, TD, or walking ability.

Key words: Calcium, phosphorus, starter, broilers, bones, mineral retention

INTRODUCTION

Dietary concentrations of calcium (Ca) and phosphorus (P) have effects on most metabolic processes of broilers, especially during earlier stages of life when bone formation is more active. From a practical standpoint, besides its effects on performance and bone parameters (Brown, 2002; Coto et al., 2008; Suttle, 2010), P plays a critical role on feed cost and on environmental impact when poultry litter is used as fertilizer (Waldroup et al., 2000; Knowlton et al., 2004; Ziaei et al., 2008). Even though Ca is considered an inexpensive ingredient, the deficiency or excess of this mineral may negatively affect broiler performance, bone development, and P utilization (Edwards and Veltmann, 1983; Shafey, 1993; Qian et al., 1997; Sebastian et al., 1997; Cabral, 1999; Alves et al., 2002). The dietary levels of Ca and P may also have consequences in broiler flock uniformity, an important parameter for the efficiency in poultry production and processing. Since Ca and P interact in many biological functions, their dietary requirements are interdependent (Qian et al., 1997;

Rama Rao et al., 2003; Applegate et al., 2003; Tamim and Angel, 2003; Yan et al., 2005), which highlights the importance of establishing recommendations of Ca and P concomitantly.

Several researchers showed that Ca and P recommended levels for starter diets by the NRC (1994) (0.45% nPP and 1.00% Ca) seem to be higher than the needs of the birds for optimum performance and bone characteristics (Yan et al., 2003; Waldroup et al., 2000; Dhandu and Angel, 2003). Nonetheless, according to Agristats (2011), the USA industry seems to be using higher levels of these minerals in broiler diets. In most cases, the importance of P has led nutritionists to add a significant margin of safety, in order to reduce the likelihood of problems caused by inadequate P intake (Knowlton et al., 2004), while Ca levels can be oversupplied because it does not cause major changes in the cost of the feed (Schoulten et al., 2003).

The dietary levels of Ca and P fed early in life have effects not only within this particular stage, but also in the subsequent phases of the grow-out. Powell et al. (2011) demonstrated that higher nPP levels (0.55 and 0.60% vs. 0.40, 0.45, and 0.50%) fed during starter diets (0-14 days) have effects in the subsequent dietary phase (14-28 days), by improving bone characteristics at 28 days of age in birds that were also fed higher levels of nPP during the grower phase (0.35% vs. 0.30%). However, those higher levels of nPP fed in the starter phase reduced the ability of broilers to adapt later to a lower level of nPP (0.30% vs. 0.35%) in the grower feed. It also has been demonstrated by these authors that Ca and P levels could be reduced in the grower diet without harming broiler performance and bone characteristics as long as the birds were fed the lower levels of nPP previously during the

starter phase. This could be explained by the mineral resorption from bones that occurs to supply Ca and P for other metabolic requirements during phases when these minerals are not supplied adequately (Angel et al., 2000). In this perspective, Skinner et al. (1992) reported that broilers fed diets containing Ca and P levels recommended by the NRC (1994) from hatch to 42 days and then transferred to finisher diets containing no inorganic supplementation of Ca and P from 42 to 49 days had live performance results not different than broilers fed finisher diets that met the levels recommended by the NRC (1994). However, in the same study the authors found significant differences in tibia measurements and strength. Similarly, Fritts and Waldroup (2006) demonstrated that levels of 0.40% nPP and 0.90% Ca in starter diets supported later reductions of Ca and P levels up to 0.30% nPP and 0.80% Ca for the grower, 0.20% nPP and 0.60% Ca for the finisher, and 0.10% nPP and 0.50% Ca for the withdrawal phases. In contrast, Yan et al. (2005) showed that feeding Ca and P deficient diets (0.60% Ca and 0.30% nPP) during the starter phase (1-19 days) negatively affected performance and bone parameters during this particular period; however, it made birds to become more efficient on using nutrients during the subsequent grower phase (19-32 days), even compensating for growth and bone parameters, while reducing P excretion. Additionally, Ashwell and Angel (2010) reported that even when fed moderately P deficient diets (0.59% Ca and 0.25% aP) only for a short period of time (90 hours after hatch) broilers were better able to handle a deficiency in P in the grower/finisher phase (0.40% Ca and 0.12% aP). The earlier restricted birds not only were heavier at 38 days of age, but also were more efficient in converting feed to gain, had higher tibia ash, and higher P retention.

These findings emphasize the importance of Ca and P nutrition during the starter

phase and demonstrate that the levels to be used must be decided considering the whole feeding program. Even though there are several publications on Ca and P requirements for the starter phase, most of them evaluate these minerals individually, or does not consider the effects of the early Ca and P nutrition on parameters at market age. Additionally, there are few publications that assess performance, bone mineralization, bone strength, leg health, and walking ability in a comprehensive way. The objective of this project was to test the effects of different combinations of Ca and P levels used in starter diets on the parameters mentioned above for Heritage broilers.

MATERIAL AND METHODS

Birds and Management

The animal work was conducted at the North Carolina State University Poultry Research Unit (Raleigh, NC) between January 12th and March 3rd 2010. All practices regarding bird care were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Heritage broiler chicks were hatched from fertile eggs provided by a local hatchery (Perdue Farms Inc., Candor, NC). The experimental house was curtain-sided, with exhaust fans and forced air heating system combined with upward blowing ventilation fans. There were a total of 64 pens, divided into 4 blocks within the house. Chicks were feather-sexed at hatching, and a total of 1920 birds (960 males and 960 females) were individually identified with neck tags. They were then weighed in groups of 15 males and 15 females; and 30 birds (one group of each sex) were randomly allocated to each of the 64 pens. Pens were 1.22 x 3.96 m (approximately 6 birds/m^2, or a final density of

21 kg/m^2), and covered with a 15 cm layer of new pine wood shavings to avoid Ca and P intake from used litter, equipped with 2 tube feeders and 2 bell-shaped drinkers. Brooding temperatures at placement were set at 35-36 °C and kept like this for the first night. Temperatures were reduced gradually to 32.2-33.5 °C from 1 to 7 days, 29.4 °C from 8 to 14 days, 26.7 °C from 15 to 21 days, and to ambient temperature thereafter. Observed house temperatures were recorded twice a day in 8 different points within the house, and are reported in Appendix A.

The lighting program consisted of 23 hours of light per day during the first week, 4 hours of darkness per day during the second week, and 16 hours of light per day from 22 to 49 days. During this last light period, supplemental light was provided from 10:00 pm to 2:30 am, in order to obtain the 16 hours of light. Feeders were shaken once a day until 21 days of age and twice a day thereafter.

Dietary Treatments

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit. The composition of the diets utilized throughout the trial is shown in Table II-1. The starter basal diet was corn-soybean meal based, into which limestone, dicalcium phosphate, and washed sand was added in order to obtain the 16 treatments. The starter dietary treatments were formulated to contain combinations of 4 levels of Ca (0.62, 0.76, 0.90, and 1.04%) and 4 levels of nPP (0.31, 0.38, 0.45, and 0.52%). Celite was added as an indigestible marker to the starter and finisher diets at 1%, for further calculation of mineral retention. Since more than 90% of the broiler diets in the USA contain phytase (Agristats, 2011), this

enzyme was added to the common grower and finisher diets; however, starter diets did not contain phytase to avoid mixing or pelleting variability and effects on enzyme activity among the different treatments. Each treatment had one pen randomly assigned within each block, with a total of 4 replicate pens per treatment. Feed and water were provided for *ad libitum* consumption during the whole grow-out period; however, the amount of feed per feeding phase was limited to mimic the management normally used in the industry. Approximately 900 g of starter feed were offered as crumbles from placement up to approximately 17 days of age. A common grower diet was fed as pellets to broiler chickens of all treatment groups from 18 to 35 days (about 2,700 g/bird), containing 0.72% Ca and 0.36% nPP. The finisher diet was provided *ad libitum* as pellets from 36 to 49 days of age for all chickens, with 0.62% Ca and 0.31% nPP. In the days before each new period (16 and 34 days of age), feed was adjusted for the estimated feed intake (FI) of the mortality, so all birds within pens had access to approximately the same amount of feed calculated for each period.

Data Collection

Feed samples from each diet were collected after pelleting/crumbling-cooling process and analyzed for Ca, tP, crude protein and acid insoluble ash. Group body weights (BW) per sex were obtained at hatch, 17, 35, and 49 days of age, and individual BW at 42 days to assess flock uniformity as the coefficient of variation (CV%). FI was documented at the end of each dietary phase (17, 35, and 49 days), and mortality BW was recorded daily for the adjustment of feed conversion ratio (FCR). FI, BW gain (BWG) and FCR were calculated for each period (0 to17, 18 to 35, and 36 to 49 days of age) and for the whole grow-out (0 to 49

days). At 42 days of age, all birds were evaluated for the prevalence of leg abnormalities (crooked toes, valgus, varus, and twisted legs), and walking ability was assessed using the gait score system of Kestin et al. (1992). The 6 walking ability scores assessed by the Kestin method were then grouped in 3 gait score categories (Webster et al., 2008) for statistical analysis, with score 1 representing birds not affected, 2 representing birds with some degree of lameness, and 3 representing birds with severe lameness. At 17 and 49 days, one chicken of each sex per pen were randomly selected, euthanized, and legs were collected. Approximately 100 g of fresh fecal samples per pen were collected at 10 and 45 days of age, frozen overnight, and then freeze-dried (Virtis Freezemobile - Model 12XL, Warminster, PA) for further acid insoluble ash (AIA) determination and Ca and P analysis.

Analytical Methods

The drumsticks collected at 17 days were manually deboned, remaining tibiae measured (length and diameters), wrapped in cheese-cloth, and then kept frozen in bags at -20°C to maintain bones moist for further analyses. Tibiae were thawed by leaving them inside the plastic bags at room temperature for 6 hours, and the 3-point bending test was then performed to evaluate bone strength. Bones were sheared midshaft using a crosshead speed of 5.0 mm/min to minimize splintering (Crenshaw et al., 1981). The resistance of bones to automatic deboning forces was tested with the 49 days thighs by using a mechanical deboning machine (Meyn-D40, Oostzaan, The Netherlands). Afterwards, tibiae collected at 17 and 49 days of age were evaluated for the incidence and severity of tibial dyschondroplasia (TD) according to the TD scoring system developed by Edwards and

Veltmann (1983). Bone ash percentage was determined with 17 days tibiae (Hall et al., 2003). Bone mineral content (BMC) and bone mineral density (BMD) were determined in the shanks collected at 49 days by Dual Energy X-ray Absorptiometry (DEXA) at the USDA-ARS in Beltsville, MD.

The AIA analysis was performed by weighing 5 g of feed or 3 g of freeze-dried feces in pre-weighed porcelain crucibles, and digesting the samples in 50 mL of acid (4 *N* HCl) on a hot plate at approximately 95°C for 45 minutes. The solution was filtered using a 125 mm hardened ashless filter paper (Whatman, International Ltd, Maidstone, England) dried at 70°C for 4 hours, and ashed at 600°C overnight in a muffle furnace. The ash weight was then used to calculate the percent AIA of the samples (procedure adapted from Scott and Boldaji, 1997). For Ca and P analysis, 2.5 g of feed or fecal samples were weighed into pre-weighed porcelain crucibles and ashed at 600°C overnight. Ash content were digested in 4 mL of acid (6 N HCl) on a hot plate at approximately 100°C until fully evaporated. Samples were then resuspended in 8mL 6 N HCl, rinsed into 100mL volumetric flasks, and filled to volume with deionized water. Flasks were covered with parafilm, vigorously agitated, and 13 mL of the suspension was filtered into 15mL conical centrifuge tubes using 125 mm hardened ashless filter paper (Whatman, International Ltd, Maidstone, England) to remove particulates (procedure adapted from Leske and Coon, 2002). Inductively-Coupled Plasma Optical Emission Spectroscopy (Perkin Elmer 2000 DV ICP-OES, Waltham, MA) was used to determine Ca and P concentrations from each sample, and corrected to a corn-based standard and a negative control. Ca and P retention were calculated using the formula exemplified by Brenes et al. (2003), as follows: 1 - [(AIA concentration in feed / AIA

concentration in excreta) × (Mineral concentration in excreta / Mineral concentration in feed)].

Data Analysis

Data were analyzed using the response surface methodology within a randomized complete block design, containing 16 treatments (4 levels of Ca x 4 levels of nPP) and 4 replicates per treatment. Blocks represented the pen distribution within the house and it was considered as a random effect. In order to satisfy the normality assumption, all percentage data were converted by an arcsine-square root transformation prior to the analyses. Pens were the experimental unit for performance and mineral retention data analysis, and JMP 9 (SAS Inst. Inc., Cary, NC) was used for the statistical analyses. The categorical data (mechanical deboning, leg abnormalities, gait scores, and TD evaluations) was statistically analyzed using each broiler chicken as an experimental unit. For these parameters, data were treated as binomial, for each condition the response take the value 0 (absence) or 1 (presence). The GLIMMIX Procedure of SAS (SAS Inst. Inc., Cary, NC) was used, having the linear and quadratic effects of levels of Ca and P in the diet and their interaction effects as fixed, and considering the pens as random effects nested within treatment combinations. The results, as log odds of a certain condition, were modeled within the effects to obtain the probability of observing each condition individually.

Statistical model used for the response surface methodology:

$$Y_{ijkl} = \mu + Ca_i + nPP_j + Ca*nPP_{ij} + Ca^2_i + nPP^2_j + P_l\,(T_{ij}) + B_k + \varepsilon_{ijkl}$$

Where:

Y_{ijkl}: Variable response

μ: Overall mean

Ca_i: Linear effect of the i^{th} Ca level (i = 1-4)

nPP_j: Linear effect of the j^{th} nPP level (j = 1-4)

$Ca*nPP_{ij}$: Effect of the first order interaction between Ca level i and nPP level j

Ca^2_i: Quadratic effect of the i^{th} Ca level

nPP^2_j: Quadratic effect of the j^{th} nPP level

$P_l\,(T_{ij})$: Random effect of dietary treatment combinations nested within pens (l = effect of pen, l = 1-4)

B_k: Random effect of the block (k = 1-4)

ε_{ijkl}: The experimental error associated to each observation.

Table II-1. Composition of broiler diets (%) and formulated nutrient contents

Ingredients	Starter basal 0-17 days	Grower 18-35 days	Finisher 36-49 days
	----------%----------		
Corn	49.39	60.05	60.15
Soybean meal, 48%	30.41	23.06	19.91
Distillers dried grains with solubles	10.00	10.00	12.00
Poultry fat	4.36	3.43	4.04
Salt (NaCl)	0.42	0.37	0.36
Limestone	0.40	1.27	1.24
Dicalcium phosphate, 18.5%	0.34	0.66	0.31
DL-methionine, 99%	0.31	0.27	0.21
L-lysine-HCl, 78,8%	0.22	0.28	0.25
Choline chloride, 60%	0.20	0.20	0.20
Sodium bicarbonate	0.18	0.12	0.09
L-threonine, 98%	0.09	0.14	0.13
Coccidiostat[1]	0.06	0.05	0.05
Mineral premix[2]	0.05	0.05	0.03
Vitamin premix[3]	0.05	0.04	0.03
Phytase[4]	-	0.02	0.02
Filler[5]	3.52	-	1.00
Total	100.00	100.00	100.00
Nutrient composition			
Metabolizable energy, kcal/kg	3,065	3,140	3,175
Crude protein, %	22.24	19.3	18.25
Calcium, %	0.31	0.72	0.62
Total phosphorus, %	0.45	0.49	0.42
Non phytate phosphorus, %	0.20	0.36	0.31
Digestible lysine, %	1.20	1.05	0.96
Digestible total sulfur amino acids, %	0.93	0.81	0.74
Digestible threonine, %	0.75	0.68	0.63
Sodium, %	0.25	0.23	0.22
Potassium, %	0.94	0.78	0.74
Chloride, %	0.32	0.31	0.30
Dietary electrolyte balance, mEq/100 g	268	225	210

[1]Monteban® 45 (Narasin), Elanco Animal Health, Greenfield, IN, at 60 g/ton in the starter and 54 g/ton in the grower diet.
[2]Trace minerals provided per kilogram of premix: manganese (MnO_2), 220 g; zinc (ZnO and $ZnSO_4$), 250 g; iron ($FeCO_3$), 75 g; copper (CuSO4 and $CuCl_2$), 10 g; iodine ($Ca(IO_3)_2$), 5 g; selenium (Na_2SeO_3), 1 g.
[3]Vitamins provided per kilogram of premix: vitamin A, 18,739,292 IU; vitamin D3, 6,613,868 IU; vitamin E, 66,139 IU; vitamin B12, 33 mg; riboflavin, 22,046 mg; niacin, 88,185 mg; d-pantothenic acid, 30,865mg; menadione, 3,968 mg; folic acid, 2,646 mg; vitamin B6, 7,716 mg; thiamine, 5,512 mg; biotin, 176 mg.
[4]Ronozyme® P CT at 185 g/ton to provide 930 FYT (DSM Nutritional Products, Parsippany, NJ).
[5]Celite, Celite Corp., Lompoc, CA, at 1 g/kg of feed in the finisher diet. Filler also contained the amounts of dicalcium phosphate, limestone, and washed sand used to obtain the 16 treatments.

RESULTS AND DISCUSSION

Ca and P analyses of feed samples indicated that formulated values were in agreement with obtained lab results. The correlation coefficients between formulated and analyzed values for Ca and P were 0.83 and 0.97, respectively.

Performance during the Starter Phase (0-17 days)

The BWG during the starter phase for both male and female Heritage chickens was quadratically affected ($P \leq 0.01$) by Ca levels in the diet (TablesII-2 and II-3). For females, the maximum BWG (502 g) from 0 to 17 days was estimated at 0.90% Ca and 0.43% nPP (Ca:nPP 2.09:1), and keeping Ca levels at 0.90%, the nPP could vary between 0.40 and 0.45% without changing significantly the BWG response (Figure II-1 A). For males (Figure II-1 B), the maximum BWG (559 g) was obtained at 0.94% Ca and 0.44% nPP (Ca:nPP 2.14:1), and the range of nPP to get the same BWG response was wider than for females (0.38-0.48% nPP at 0.94% Ca). Similar requirements were reported by Runho et al. (2001) when performance was the criteria. These researchers reported that for Hubbard males and females, the requirements from hatch to 21 days were 1.00 % Ca and 0.45% nPP to obtain a BWG of 689 and 613 g for males and females, respectively. In contrast, Brenes et al. (2003) demonstrated that for growth performance in straight-run Cobb chickens, the requirements from hatch to 21 days could be lower (0.35% nPP and 0.82% Ca for reaching 587 g BWG) than those found by Runho et al. (2001). Moreover, in a literature review with 158 treatments from 14 references Létourneau-Montminy et al. (2010) estimated that starter diets containing 0.60% Ca and 0.31% nPP allowed similar live performance and bone mineralization to those

obtained using the NRC (1994) recommendations of 1.00% Ca and 0.45% nPP. Using higher levels of nPP, Powel et al. (2011) did not observe differences in the performance of straight-run Ross 708 chickens during the starter period when birds were fed levels varying from 0.40 to 0.60% nPP with a Ca:nPP of 2.2:1. Coto et al. (2008) also found no effects of dietary P (0.35 to 0.50%) in Cobb broiler chickens from 0 to 18 days on BWG, independently of the Ca levels used.

Ca levels also affected quadratically ($P \leq 0.05$) FCR during the starter phase (Figure II-2 B), and the optimum FCR (1.37) was estimated at 0.93% Ca and 0.42% nPP (Tables II-2 and II-3). Kornegay et al. (1996) reported that for broilers fed diets containing phytase, the best feed efficiency from hatch to 21 days was obtained at lower Ca (0.88%) and nPP (0.20%) levels than those estimated herein. Panda et al. (2007) demonstrated that low nPP levels (0.30% nPP) in broiler chicken diets with constant Ca levels (1.00%) from 1 to 21 days depressed BWG, FI, and FCR. In this case, even though the performance in the chicks fed 0.35% nPP improved significantly as compared with the 0.30% nPP diet, it was significantly lower than those of 0.40% nPP. However, no further improvement was observed in the 0.45% nPP as compared to the 0.40% nPP diet.

The FI during the starter phase had a quadratic effect ($P \leq 0.05$) of both Ca and nPP dietary levels, the maximum FI (779 g) being estimated at 0.88% Ca and 0.44% nPP (Tables II-2 and II-3). Levels of Ca higher than 0.99% seem to decrease FI and this reduction is pronounced with wider Ca:nPP (Figure II-2 A). This is in agreement with Shaw et al. (2010), who reported a lower FI in birds fed 0.25% nPP as compared to those fed 0.35 or 0.45% nPP up to 28 days. In an experiment testing levels of Ca from 0.65 to 1.25% combined with

0.45% nPP during the starter phase for Starbro broilers, Alves et al. (2002) observed a quadratic effect of Ca levels on FI, and the maximum was observed at 0.67% Ca; however, BWG decreased as levels of Ca were increased. Qian et al. (1997) reported that for Peterson x Arbor Acres male broilers, the higher the Ca levels in starter diets (from 0.56 to 1.02%), the lower was the FI obtained, which was reflected in a reduction of BWG. Similarly, Yan et al. (2005) showed that male Hubbard-Isalic chicks receiving 0.95% Ca and 0.43% nPP from hatch to 14 days had depressed FI and BWG than those receiving 0.63% Ca and 0.23% nPP. No significant effects of treatments were observed on mortality during the starter phase.

Overall Performance (0-49 days)

As demonstrated in the Figure II-3, female BWG for the whole grow out was affected quadratically ($P = 0.059$) by Ca levels fed in starter diets (Tables II-2 and II-3), the maximum BWG (3,011 g) being estimated at 0.77% Ca and 0.49% nPP. In the present experiment, broiler chickens were fed a common grower diet containing 0.72% Ca and 0.36% nPP. Yan et al. (2005) reported that broilers fed higher levels of Ca and nPP during the starter phase (0.90% Ca and 0.45% nPP vs. 0.60% Ca and 0.30% nPP) had better performance results at 19 days; however, by the end of the grower phase (32 days) no differences were detected. During the grower phase, those researchers found no negative effects of feeding lower levels of Ca and nPP (0.60% Ca and 0.30% nPP vs. 0.80% Ca and 0.40% nPP) when birds were fed adequate levels of Ca and P in the previous phase (0.90% Ca and 0.45% nPP). According to Angel et al. (2000), when birds are fed sufficient amounts of minerals during earlier phases and less than the required in the later phases, then minerals from bone will be used to meet

the other needs of the body. Thus, it seems that if in the experiment reported herein broilers were fed diets lower in Ca and nPP during the grower and finisher phases, they would require higher levels of these minerals during the starter period to reach the optimum overall performance. For males, the BWG from 0 to 49 days only showed a tendency to reduce linearly (P = 0.076) as nPP levels fed in the starter diet increased. It could be due to increased needs of nPP during the subsequent phases in broilers fed higher nPP levels in the starter diet, as suggested by Powell et al. (2011). These authors reported that broilers fed higher levels of nPP in the starter phase (0.60% vs. 0.40%) required higher levels (0.35% vs. 0.30%) of nPP in the grower phase to obtain similar performance from those that received lower nPP in the starter phase. The overall FCR, as well as the flock uniformity (CV%) at 42 days were not affected by Ca and nPP levels used in the starter diets.

The effects of treatments on mortality were observed during the whole grow-out, where male mortality was affected quadratically ($P \leq 0.05$) by Ca levels fed in the starter phase (Table II-4 and Table II.5). The highest mortality was observed at the lowest nPP level (0.31%) combined with Ca levels between 0.75 and 0.90%. The mortality was reduced when the lowest levels of Ca (0.62%) were combined with the highest levels of nPP (0.52%), and when the highest levels of Ca (1.04%) were combined with 0.38 to 0.45% of nPP.

Table II-2. Response surface regression coefficients and predicted optimal values for the effects of dietary Ca and nPP levels during the starter phase on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of male and female Heritage broilers during the starter period (0-17 days) and the whole grow-out (0-49 days).

	BWG 0-17 d		FI	FCR	BWG 0-49 d	
	Females	Males	0-17 d	0-17 d	Females	Males
Intercept	222.07**	182.62**	409.72**	1.71**	2958.43**	3723.89**
Ca	458.71**	569.05**	429.26	-0.87**	-34.04	94.20
nPP	328.94	509.00	809.04	0.27	150.76	-265.00
Ca x Ca	-235.59**	-325.55**	-246.41*	0.42*	-852.79*	6.38
Ca x nPP	-82.53	92.12	9.99	0.20	-1042.96	356.12
nPP x nPP	-298.24	-681.20	-936.19*	-0.54	-1408.59	1403.06
Predicted value	502 max	559 max	779 max	1.37 min	3011 max	-
Ca at predicted	0.90	0.94	0.88	0.93	0.77	-
nPP at predicted	0.43	0.44	0.44	0.42	0.49	-

*$P \leq 0.05$, ** $P \leq 0.01$

max predicted value was a maximum, min predicted value was a minimum

Table II-3. Effects of dietary Ca and nPP levels in starter diets on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of male and female Heritage broilers during the starter period (0-17 days) and the whole grow-out (0-49 days).

Ca	nPP	BWG 0-17 d*		FI 0-17 d	FCR 0-17 d	BWG 0-49 d*	
		Females	Males			Female	Male
-----%-----		----------g----------			---g/g---	----------g----------	
0.62		478[b]	524[b]	753[b]	1.404[a]	2943	3670
0.76		492[a]	545[a]	764[ab]	1.371[b]	2988	3727
0.90		497[a]	556[a]	771[a]	1.368[b]	2971	3674
1.04		492[a]	553[a]	762[ab]	1.366[b]	2943	3732
Pooled SEM		5	4	5	0.007	20	19
	0.31	487	537	754	1.379	2937	3737
	0.38	491	552	765	1.371	2976	3700
	0.45	492	544	769	1.389	2964	3688
	0.52	489	545	763	1.369	2969	3679
Pooled SEM		5	4	5	0.007	20	19
0.62	0.31	476	530	741	1.391	2925	3708
0.62	0.38	473	517	753	1.417	2937	3667
0.62	0.45	482	520	761	1.426	2980	3704
0.62	0.52	481	529	756	1.384	2929	3602
0.76	0.31	491	533	758	1.380	2941	3785
0.76	0.38	493	562	773	1.363	2963	3695
0.76	0.45	491	551	775	1.388	3019	3737
0.76	0.52	495	536	752	1.353	3030	3692
0.90	0.31	491	547	758	1.376	2941	3657
0.90	0.38	504	560	777	1.360	3000	3715
0.90	0.45	498	553	774	1.367	2973	3630
0.90	0.52	493	566	775	1.370	2972	3695
1.04	0.31	490	540	758	1.370	2941	3796
1.04	0.38	493	569	758	1.345	3005	3725
1.04	0.45	498	551	766	1.378	2883	3679
1.04	0.52	488	550	768	1.373	2944	3727
Pooled SEM		8	8	9	0.014	36	43
Source of Variation		----------*P*-values----------					
Ca		0.005	<0.001	0.036	<0.001	0.174	0.102
nPP		0.801	0.069	0.068	0.131	0.376	0.291
Ca*nPP		0.967	0.124	0.724	0.357	0.264	0.516

[a,b] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

*The average BW at hatch was 43 g for females and 44 g for males.

Table II-4. Response surface regression coefficients for the effects of dietary Ca and nPP levels during the starter phase (0-17 days) mortality of female and male Heritage broilers during the whole grow-out (0-49 days).

	Mortality 0-49 days	
	Female	Male
Intercept	71.80 *	-26.59 *
Ca	-84.92	163.57
nPP	-166.27	-136.37
Ca x Ca	37.21	-106.28 *
Ca x nPP	49.33	23.79
nPP x nPP	148.85	127.61

*$P \leq 0.05$, ** $P \leq 0.01$

Table II-5. Effects of dietary Ca and nPP levels in starter diets on mortality of female and male Heritage broilers during the whole grow-out (0-49 days).

Ca	nPP	Mortality 0-49 days	
		Female	Male
-----%-----		----------%----------	
0.62		3.33	6.66
0.76		2.91	8.75
0.90		0.41	10.83
1.04		2.91	4.58
Pooled SEM		1.16	2.24
	0.31	3.75	10.00
	0.38	0.41	5.83
	0.45	2.91	8.33
	0.52	2.50	6.67
Pooled SEM		1.16	2.24
0.62	0.31	8.34	8.33
0.62	0.38	0.00	5.00
0.62	0.45	0.00	10.00
0.62	0.52	5.00	3.33
0.76	0.31	3.33	10.00
0.76	0.38	1.67	5.00
0.76	0.45	5.00	13.33
0.76	0.52	1.67	6.67
0.90	0.31	0.00	16.66
0.90	0.38	0.00	10.00
0.90	0.45	1.67	6.66
0.90	0.52	0.00	10.00
1.04	0.31	3.34	5.00
1.04	0.38	0.00	3.33
1.04	0.45	5.00	3.33
1.04	0.52	3.34	6.66
Pooled SEM		2.16	3.99
Source of Variation		----------*P*-values----------	
Ca		0.2038	0.1305
nPP		0.1603	0.4322
Ca*nPP		0.3101	0.7307

Mineral Retention

Mineral Retention during the Starter Phase (10 Days of Age)

The mineral retention data is presented in Tables II-6 and II-7. Ca retention at 10 days of age was affected quadratically ($P \leq 0.05$) by nPP and linearly ($P \leq 0.05$) by Ca dietary levels. Broilers retained more Ca when fed diets containing low levels of Ca (≤ 0.65%) combined with nPP levels around 0.45%. Using levels of nPP higher than this affected Ca retention negatively (Figure II-4 A). Rostagno et al. (2000) reported an increase in Ca absorption (54.12 to 66.10%) when dietary Ca was reduced from 1.00 to 0.68% and tP from 0.70 to 0.56%. As expected, increased levels of Ca reduced its retention, and above 0.85% Ca the combination with high levels of nPP (> 0.45%) impairs the retention of Ca even more (Figure II-4). This is also in agreement with Schoulten et al. (2003), who demonstrated that Ca absorption was reduced linearly as the levels of Ca in the diet increased (0.46 to 1.30%) in broilers from 1 to 21 days of age. The excretion of Ca (mg) during the starter phase (0 to 17 days) increased as the levels of Ca increased in the diet (Table II-7), in agreement with the reduction on Ca retention (%) as dietary Ca increased.

The retention of P at 10 days (Figure III-2) had a quadratic effect of nPP levels in the feed ($P \leq 0.05$). P retention improved as levels of nPP increased from 0.32% up to 0.37%, where a maximum retention (around 59%) was observed. Further increments in nPP levels decreased P retention (Figure II-5 A). It was also observed that when dietary nPP levels increased to more than 0.38%, P excretion increased up to 32.48 mg (at 0.52% nPP). Catalá-Gregori et al. (2007) reported that birds fed phytase supplemented starter diets containing high P (0.29% nPP and 0.52% Ca) retained more P than those fed low P (0.20% nPP and

0.61% Ca) at 21 days. However, most of reports on P retention demonstrate that it is improved as nPP levels are reduced in the feed, as an adaptation to better utilize the scarce nutrient. Similarly, Yan et al. (2005), who demonstrated that birds fed lower levels of nPP (0.30 vs. 0.45%) during the starter phase exhibited a higher ability to absorb P (49.5 vs. 56.0%) at 18 days of age. The retention of P may also be affected by Ca levels in the diet. It could be explained physiologically by effects of minerals in the intestinal mucosa. The optimal brush border phytase activity of the small intestine is reported to be between pH 5.5 and 6.5 (Maenz and Classen, 1998), and high Ca diets increase the pH, decreasing efficiency of phytase activity (Shafey et al., 1991). This could be one of the reasons that the lowest P retention occurred when using Ca levels higher than 0.95% and nPP levels higher than 0.50%. According to Hurwitz et al. (1995), high dietary Ca also leads to an increase in plasmatic Ca levels, which suppresses PTH secretion, and consequently reduces the production of calcitriol, reducing Ca and P absorption and causing a reduction in P retention. In the present study the highest P retention was found when using Ca levels higher than 0.90% and nPP levels around 0.37%. Schoulten et al. (2003) reported similar influence of Ca on P retention, where the maximum P retention was observed when using 0.88% Ca, and both increasing and decreasing Ca negatively affected P retention.

Mineral Retention during the Finisher Phase (45 Days of Age)

The Ca retention during the finisher phase evaluated at 45 days of age, as demonstrated in Tables II-6 and II-7, was quadratically affected ($P \leq 0.05$) by the levels of Ca used in starter diets. Broilers retained less Ca as the levels of this mineral increased from

0.62 to 0.79%, and the lowest Ca retention at 45 days (0.33%) was estimated when feeding birds with 0.79% Ca and 0.43% nPP during the starter phase (Figure II-4 B). It was expected that a linear reduction in Ca retention would occur as levels of Ca during the starter phase increased. However, using levels of Ca higher than 0.87% during the starter phase improved the retention of this mineral at market age.

P retention at 45 days (Tables II-6 and II-7) had a quadratic effect ($P \leq 0.05$) of Ca and a linear effect ($P \leq 0.05$) of nPP levels used during the starter phase. At low levels of Ca (≤0.80%), increments in nPP improved P retention. However, when Ca was higher than 0.80%, the chickens retained more P and its retention was less influenced by nPP levels (Figure II-5 B). Similar results were reported by Plumstead et al. (2008), who demonstrated that overall P retention at 16-17 and 19-20 days responded positively to increasing dietary Ca concentrations (from 0.47 to 1.16%) fed to broiler chickens from 16 to 21 days of age. However, different results were reported by Yan et al. (2005), who stated that broiler chickens exposed to a moderate deficiency of Ca and P during the starter phase are more able to retain these minerals in the subsequent phases. These authors reported that feeding low Ca and P levels in starter diets (0.30% nPP and 0.60% Ca vs. 0.45% nPP and 0.90% Ca) from 0 to 18 days improved Ca and P retention during this period, and this difference was maintained up to 32 days of age.

Table II-6. Response surface coefficients and predicted optimal values for the effects of dietary Ca and nPP levels in starter diets (0-17 days) on Ca and P retention (%) at 10 and 45 days, and on Ca and P excretion (g) from 0 to 17 days for Heritage broilers.

	Ca retention		P retention		Excretion 0-17 d	
	10 d	45 d	10 d	45 d	Ca	P
Intercept	-0.09**	1.64**	-0.13**	0.70**	24.398*	53.172
Ca	0.02**	-1.98	0.32	-0.92**	19.613**	9.100
nPP	3.92	-2.11	2.95*	0.60*	-88.335	-249.838**
Ca x Ca	0.15	1.33**	-0.03	0.72**	-17.627	-4.087
Ca x nPP	-1.11	-0.28	-0.60	-0.42	25.394	88.915
nPP x nPP	-3.60*	2.70	-3.22*	-0.12	145.680	214.917
Predicted minimum		33.00				
Ca at predicted		0.79				
nPP at predicted		0.43				

*$P \leq 0.05$, ** $P \leq 0.01$

Table II-7. Effects of dietary Ca and nPP levels in starter diets (0-17 days) on Ca and P retention (%) at 10 and 45 days, and on Ca and P excretion (g) from 0 to 17 days for Heritage broilers.

Ca	nPP	Ca retention		P retention		Excretion 0-17 d	
		10 d	45 d	10 d	45 d	Ca	P
-----%-----		----------%----------				--------mg--------	
0.62		67.58^{a}	45.08	54.42^{ab}	52.84^{ab}	15.18^{c}	25.95
0.76		66.93^{a}	42.30	57.71^{a}	50.34^{b}	19.18^{b}	25.51
0.90		61.04^{b}	42.04	53.22^{b}	53.36^{ab}	26.97^{a}	27.67
1.04		61.19^{b}	49.57	57.14^{ab}	56.40^{a}	30.92^{a}	25.64
Pooled SEM		1.35	3.44	1.38	1.87	0.97	0.79
	0.31	61.87	46.99	56.93^{a}	51.60	24.14	21.18 ^{c}
	0.38	67.19	44.72	56.72^{a}	52.49	21.01	23.63 ^{c}
	0.45	64.78	41.93	57.28^{a}	54.27	22.96	27.48 ^{b}
	0.52	62.89	45.35	51.56^{b}	54.58	24.13	32.48 ^{a}
Pooled SEM		1.35	3.44	1.38	1.87	0.97	0.79
0.62	0.31	67.11^{ab}	48.84	55.13^{abc}	50.03	15.30^{f}	20.90 ^{def}
0.62	0.38	66.41^{ab}	45.68	54.71^{abc}	52.37	15.70^{ef}	23.76 ^{cdef}
0.62	0.45	67.16^{ab}	40.43	55.33^{abc}	53.51	15.49^{ef}	27.07 ^{abcde}
0.62	0.52	69.65^{a}	45.35	52.53^{abc}	55.44	14.25^{f}	32.08 ^{ab}
0.76	0.31	63.96^{ab}	42.78	63.76^{a}	48.27	20.75^{cdef}	18.78 ^{f}
0.76	0.38	69.34^{ab}	36.54	58.15^{ab}	46.81	18.00^{def}	24.19 ^{cdef}
0.76	0.45	70.35^{a}	45.48	56.80^{abc}	53.55	17.44^{def}	27.74 ^{abcd}
0.76	0.52	64.06^{ab}	44.43	52.12^{abc}	52.72	20.53^{cdef}	31.35 ^{abc}
0.90	0.31	49.62^{c}	44.55	46.15^{c}	54.04	34.35^{a}	25.90 ^{bcdef}
0.90	0.38	69.79^{ab}	42.98	51.71^{abc}	55.21	21.28^{bcdef}	25.95 ^{bcdef}
0.90	0.45	61.97^{abc}	38.16	60.24^{a}	51.26	26.47^{abcd}	26.85 ^{bcde}
0.90	0.52	62.78^{abc}	42.49	54.80^{abc}	52.95	25.78^{abcde}	32.00 ^{ab}
1.04	0.31	66.80^{ab}	51.80	62.67^{a}	54.05	26.19^{abcd}	19.17 ^{ef}
1.04	0.38	63.24^{abc}	53.68	62.32^{a}	55.56	29.06^{abc}	20.62 ^{def}
1.04	0.45	59.66^{abc}	43.66	56.77^{abc}	58.79	32.46^{ab}	28.25 ^{abcd}
1.04	0.52	55.06^{bc}	49.13	46.80^{bc}	57.20	35.96^{a}	34.51 ^{a}
Pooled SEM		2.81	5.01	2.46	2.67	2.05	1.53
Source of variation		----------*P*-values----------					
Ca		0.001	0.059	0.037	0.006	<0.001	0.181
nPP		0.056	0.427	0.003	0.176	0.130	<0.001
Ca*nPP		0.002	0.682	<0.001	0.505	0.004	0.054

[a,b,c,d,e,f] Means within a column without a common superscript differ significantly (P<0.05) when tested with Tukey's honestly significant-difference test.

Bone Mineralization

The bone mineralization data is presented in Tables II-8 and II-9. Male and female tibia ash percentage at 17 days increased linearly ($P \leq 0.05$) as levels of Ca in the diet were increased (Figures II-6 A and B). The highest values of tibia ash percentage (47.54% for females and 45.50% for males) were observed when the highest level of Ca (1.04%) and nPP (0.52%) were used. Similar results were reported by Brugalli et al. (1999) and Li et al. (2000), who estimated the Ca and P requirements from hatch to 21 days based on tibia ash as 1.00% and 0.45%, respectively. A study testing higher levels of nPP was conducted by Powell et al. (2011), and these authors reported that tibia ash at 14 days increased linearly (49.72 to 52.68%) as nPP levels increased from 0.40 to 0.60% (Ca:nPP 2.2:1). Brenes et al. (2003), compared levels of 0.25% aP (0.66% Ca) and 0.35% aP (0.82% Ca) in broilers from 1 to 21 days of age and observed an increment in tibia ash percentage from 44% to 45.5% by increasing the aP level from 0.25 to 0.35% during the starter phase.

The BMC of the shanks at 49 days showed a quadratic effect ($P \leq 0.05$) of nPP levels used in the starter diet for both sexes. The female BMC increased quadratically as nPP level increased up to 0.39%, reaching the maximum (4.92 g) when combined with 0.77% Ca (Figure II-7 A). Males responded differently, decreasing quadratically as levels of nPP were increased up to 0.41%, where the minimum BMC (6.48 g) was estimated in combination with 0.69% Ca (Figure II-7 B). The maximum male BMC seems to occur at the highest level of Ca (1.04%) combined with the lowest level of nPP (0.31%). Female shank BMD at 49 days increased linearly ($P \leq 0.05$) as nPP levels were increased in the starter phase (Figure II-8 A). In contrast, male shank BMD was affected linearly ($P \leq 0.05$) by Ca and quadratically

($P \leq 0.05$) by nPP levels in the starter diet. The male BMD responded similarly to BMC. The response surface (Figure II-8 B) indicated that the maximum BMD for the males was found at the highest level of Ca (1.04%) combined with the lowest level of nPP (0.31%). Onyango et al. (2003) reported that BMC and BMD at 22 days increased as Ca and nPP levels increased from 0.51% Ca and 0.13% nPP to 1.00% and 0.50% nPP.

Table II-8. Response surface coefficients and predicted optimal values for the effects of dietary Ca and nPP levels in starter diets (0-17 days) on tibia ash at 17 days, and bone densitometry (BMC and BMD) of shanks at 49 days for male and female Heritage broilers.

	Tibia ash		BMC		BMD	
	Females	Males	Females	Males	Females	Males
Intercept	36.97**	47.48**	-0.42**	7.75	0.208**	0.281**
Ca	-11.20**	4.29**	0.26	-1.96	0.069	0.050**
nPP	39.50	-36.24	11.47	-29.42	0.101*	-0.763
Ca x Ca	11.21	-9.38	-1.56	2.09	-0.043	0.059
Ca x nPP	1.89	40.43*	5.47	-2.31	0.030	-0.268
nPP x nPP	-42.41	2.50	-19.99*	37.65**	-0.084	1.189**
BW	-	-	0.001**	0.002**	0.001	0.001**
Predicted value			4.92 max	6.47 min		
Ca at predicted			0.77	0.69		
nPP at predicted			0.39	0.41		

*$P \leq 0.05$, ** $P \leq 0.01$

max predicted value was a maximum, min predicted value was a minimum

Table II-9. Effects of dietary Ca and nPP levels in starter diets (0-17 days) on tibia ash at 17 days, and bone densitometry (BMC and BMD) of shanks at 49 days for male and female Heritage broilers.

Ca	nPP	Tibia ash		BMC		BMD	
		Females	Males	Females	Males	Females	Males
-----%-----		--------%--------		--------g--------		--------g/cm^2--------	
0.62		44.07[b]	42.62[c]	4.72	7.25	0.268	0.291
0.76		43.08[b]	42.88[bc]	4.85	7.24	0.271	0.293
0.90		46.77[a]	44.84[a]	4.72	7.36	0.272	0.300
1.04		46.66[a]	44.46[ab]	4.74	7.49	0.272	0.306
Pooled SEM		0.55	0.48	0.08	0.13	0.003	0.004
	0.31	44.21	43.74	4.73	7.50	0.265	0.303
	0.38	45.50	43.74	4.85	7.23	0.268	0.293
	0.45	45.21	43.68	4.87	7.08	0.275	0.290
	0.52	45.67	43.64	4.58	7.53	0.276	0.304
Pooled SEM		0.55	0.48	0.08	0.13	0.03	0.004
0.62	0.31	43.33	43.51	4.62	7.47	0.261	0.295
0.62	0.38	45.17	43.33	5.21	7.21	0.274	0.282
0.62	0.45	44.46	42.73	4.60	7.18	0.267	0.286
0.62	0.52	43.33	40.91	4.46	7.16	0.270	0.303
0.76	0.31	41.75	42.91	5.02	7.14	0.269	0.290
0.76	0.38	42.98	42.58	4.75	7.06	0.259	0.292
0.76	0.45	43.08	42.62	4.95	6.81	0.278	0.288
0.76	0.52	44.52	43.42	4.69	7.95	0.278	0.304
0.90	0.31	45.31	44.59	4.66	7.78	0.255	0.307
0.90	0.38	47.13	45.01	4.73	7.07	0.277	0.298
0.90	0.45	45.93	44.86	5.04	7.20	0.277	0.292
0.90	0.52	48.73	44.89	4.43	7.38	0.279	0.301
1.04	0.31	46.44	43.95	4.62	7.63	0.273	0.318
1.04	0.38	46.74	44.04	4.71	7.58	0.262	0.302
1.04	0.45	47.36	44.51	4.88	7.12	0.278	0.295
1.04	0.52	46.13	45.36	4.74	7.63	0.277	0.309
Pooled SEM		1.19	0.94	0.17	0.29	0.007	0.008
Source of variation			----------*P*-values----------				
Ca		<0.001	0.002	0.650	0.613	0.830	0.062
nPP		0.336	0.999	0.108	0.106	0.110	0.054
Ca*nPP		0.748	0.599	0.164	0.553	0.450	0.899
BW		-	-	0.002	0.003	0.905	0.005

[a,b,c] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

Bone Breaking Strength

The force needed to break the male tibiae at 17 days using the three-point bending test increased linearly ($P \leq 0.01$) with increments in the Ca levels used during the starter phase (Figure II-9), while in the females no significant effects were detected (Tables II-10 and II-11). Runho et al. (2001) found similar results, estimating the requirements of male Hubbard broilers of 1.00% Ca and 0.45% nPP from 0 to 21 days when breaking force was the criterion. Powell et al. (2011) showed that breaking force at 14 days for Ross 708 chickens increased linearly as the levels of Ca and nPP increased from 0.40 to 0.60% with a Ca:nPP of 2.2:1 during the starter phase (0 to 14 days). A lower requirement was reported by Rama Rao et al. (2003), who estimated the optimum strength for Cobb males at 0.70% Ca and 0.40% nPP from 0 to 21 days of age.

Mechanical Deboning

To our knowledge, this is the first time that the effects of Ca and nPP have been tested on automatic deboning machines. The partial breakage (breakages at the level of the epiphysis) during mechanical deboning of the thighs collected at 49 days was affected quadratically by the levels of Ca ($P \leq 0.05$) and P ($P \leq 0.01$) fed during the starter phase (Tables II-10 and II-11). The highest probability of incidence of partial breakage (around 50%) seems to be with the intermediate level of nPP (0.40-0.47%) combined with higher levels of Ca (> 1.00%). However, reducing the Ca levels to less than 0.65%, while keeping the nPP level around 0.43%, also increases the chances of having partial breakages (around 40%). The total breakage (breakages at the diaphysis) during mechanical deboning of the

thighs collected at 49 days was affected quadratically ($P \leq 0.05$) by Ca levels fed during the starter phase (Tables II-10 and II-11). The highest probability of incidence of total breakage seems to be when using levels of Ca around 0.80% combined with levels of nPP lower than 0.36%, and the lowest probability occurred at the maximum level of Ca 1.04% combined 0.45% nPP. Driver et al. (2005) also reported an increase when evaluating the incidence of broken tibias and clavicles at processing (35 days) when broilers were fed lower levels of Ca and nPP (0.60% Ca and 0.24% nPP vs. 0.90% Ca and 0.35% nPP) from hatch to 18 days.

Table II-10. Response surface regression coefficients for the effects of dietary Ca and nPP levels in starter diets (0-17 days) on the probability of incidence of bone breakage during mechanical deboning of thighs at 49 days and on tibia breaking strength at 17 days.

	Mechanical deboning	Breaking strength - Force	
	Diaphyseal breakage	Females	Males
Intercept	-10.02	-82.79	-12.42
Ca	40.43*	187.59	312.41**
nPP	-7.42	509.36	-128.15
Ca x Ca	-23.77*	-62.91	-139.67
Ca x nPP	-3.13	-166.30	-67.48
nPP x nPP	30.27	-459.53	279.27
Sex	0.58	-	-

*$P \leq 0.05$, ** $P \leq 0.01$

Table II-11. Effects of dietary Ca and nPP levels in starter diets (0-17 days) on the probability of incidence of bone breakage during mechanical deboning of thighs at 49 days and on tibia breaking strength at 17 days.

Ca	nPP	Mechanical deboning	Breaking strength - Force	
		Diaphyseal breakage	Female	Male
-----%-----		Probability	----------N----------	
0.62		0.36	95	108[b]
0.76		0.53	103	115[ab]
0.90		0.62	100	131[a]
1.04		0.30	103	127[ab]
Pooled SEM		0.10	5	6
	0.31	0.62	97	114
	0.38	0.50	109	126
	0.45	0.32	96	112
	0.52	0.38	99	130
Pooled SEM		0.10	5	6
0.62	0.31	0.37	91	108
0.62	0.38	0.50	109	112
0.62	0.45	0.37	90	95
0.62	0.52	0.24	91	118
0.76	0.31	0.63	89	102
0.76	0.38	0.63	101	122
0.76	0.45	0.37	106	106
0.76	0.52	0.50	114	132
0.90	0.31	0.88	106	120
0.90	0.38	0.63	115	138
0.90	0.45	0.50	82	131
0.90	0.52	0.37	97	136
1.04	0.31	0.50	101	127
1.04	0.38	0.24	110	134
1.04	0.45	0.12	106	116
1.04	0.52	0.45	93	133
Pooled SEM		0.20	10	11
Source of variation		----------*P*-values----------		
Ca		0.169	0.658	0.013
nPP		0.251	0.217	0.053
Ca*nPP		0.879	0.365	0.960
Sex		0.106	-	-

[a,b] Means within a column without a common superscript differ significantly ($P<0.05$) when tested with Tukey's honestly significant-difference test.

Tibial Dyschondroplasia, Leg Abnormalities, and Walking Ability

No significant effects of Ca and nPP levels fed during the starter phase were observed on TD incidence and severity at 17 and 49 days, or leg abnormalities occurrence and walking ability at 42 days of age for Heritage broilers under the conditions of this experiment. Coto et al. (2008) reported that TD incidence and severity in male Cobb chickens at 18 days were affected by dietary Ca, but not nPP levels, suggesting that adequate dietary Ca levels are required for minimizing the incidence and severity of TD. These researchers tested diets containing levels of nPP ranging from 0.35 to 0.50% combined with levels of Ca that represented a Ca:nPP ratio of 2:1, - 0.20%, + 0.20%, and + 0.40% of the 2:1 Ca:nPP ratio. In that experiment, broiler chickens fed the lowest Ca level (0.2% less Ca than the 2:1 Ca: NPP ratio) had a significantly higher incidence of TD as compared to those fed the other Ca levels. The incidence of TD tended to be lower as the Ca level increased, which is in agreement with Ledwaba and Roberson (2003), who observed a higher incidence of TD in Ross cockerels fed a suboptimal calcium concentration (0.65%) as compared to those fed a marginal Ca level (0.85%). Coto et al. (2008) demonstrated that TD severity responded similarly to TD incidence, where birds fed 0.2 % less Ca than the 2:1 Ca: nPP had a significantly higher severity of TD than birds fed the higher Ca levels. Although there was no significant difference in TD severity among birds fed the other Ca levels, there was a trend to reduced TD severity as the calcium level increased. Earlier studies conducted by Riddell and Pass (1987) evaluated combinations of Ca and aP during the starter phase (hatch to 4 weeks). These authors tested levels ranging from 0.8 to 1.4% Ca and 0.55 to 0.75% nPP. They reported that broiler chickens fed diets containing 1.4% Ca and 0.55% aP had the lowest

incidence and less severe TD lesions at 4 weeks, while chickens fed 0.8% Ca and 0.75% (Ca:aP 1.07) had not only depressed growth, but also the highest incidence and most severe lesions of TD. Panda et al. (2007) reported an increase in leg abnormalities incidence in chicks fed diets containing 0.30 and 0.35% nPP when compared to diets with 0.40 and 0.45% nPP from 1 to 21 days at a constant Ca level of 1.00%. Nelson et al. (1990) did not find effects of levels of Ca and aP fed from 1 to 21 days (0.80% Ca and 0.40% aP vs. 1.00% Ca and 0.50% aP) on the incidence of crooked legs at 21 days of age. That researcher also did not find significant effects when the levels varied from 0.77 or 0.97% Ca and 0.33 to 0.48% aP in broiler diets fed from 1 to 21 days when evaluating crooked legs incidence at 42 days of age. Genetic lines differ significantly from each other in growth characteristics, body composition, and the incidence of bone and leg problems. Consequently, this can be one of the reasons of the differences between the experiment herein and other research reports.

Conclusions

In summary, the present data indicate that for optimum performance during the starter phase, the levels of Ca and nPP ranged between 0.90-0.94% and 0.42-0.44%, respectively. Bone mineralization represented by tibia ash percentage was maximized with the highest levels of Ca and nPP tested (1.04% Ca and 0.52% nPP). In general, bone densitometry and tibia strength improved when using the highest level of Ca. Broiler chickens retained more P during the starter phase when fed approximately 0.37% nPP combined with Ca levels higher than 0.85%. However, at market age it was less influenced by nPP levels, and increasing Ca levels from 0.85 to 1.04% in the starter diet improved P retention at 45 days of age. No

significant effects of treatments were observed on flock uniformity, leg abnormalities, and walking ability at 42 days, as well as on TD incidence and severity at 17 or 49 days of age.

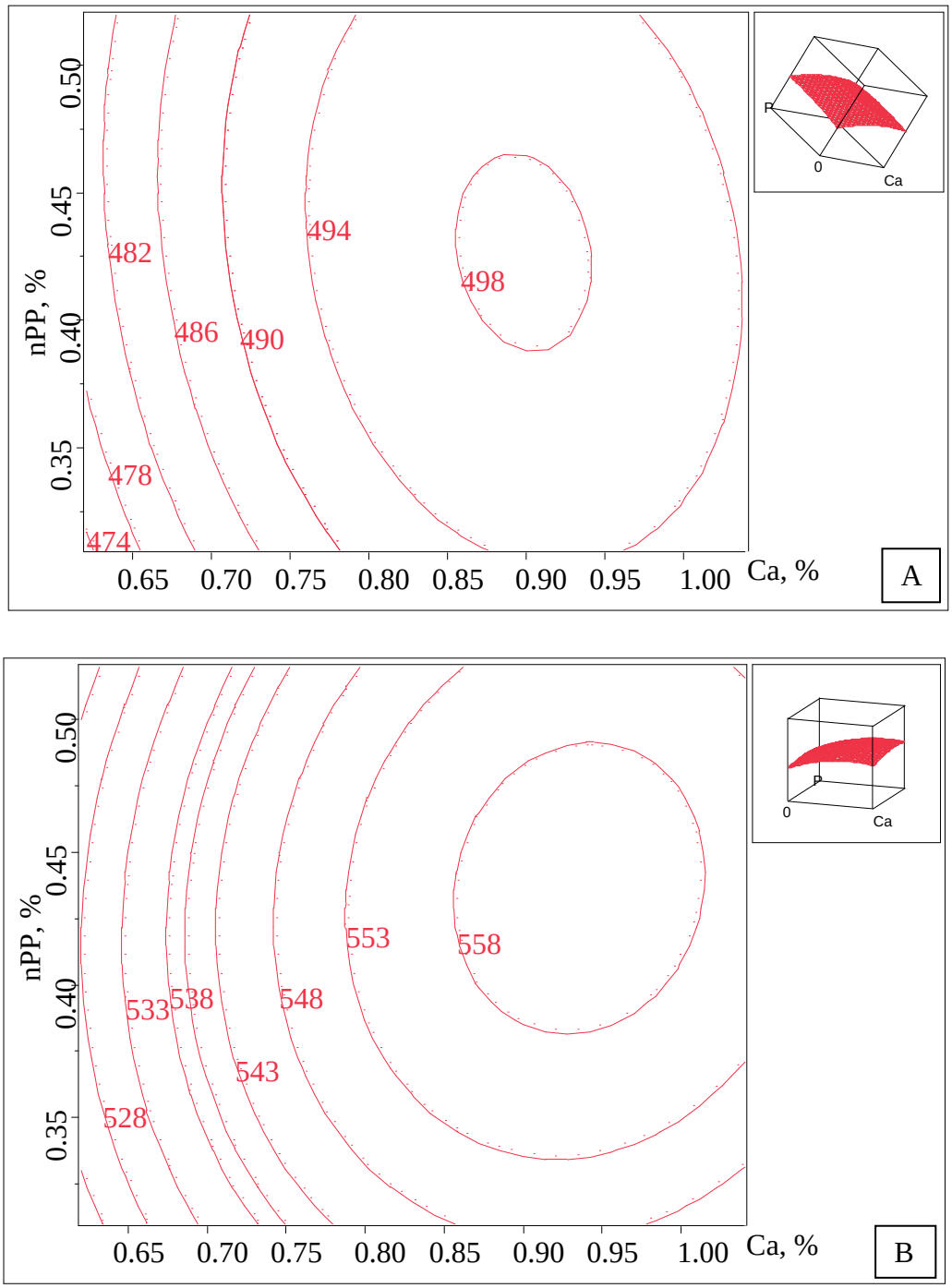

Figure II-1. Response contours of female (A) and male (B) body weight gain (g) from hatch to 17 days with varying dietary levels of Ca and nPP during the starter phase (0-17 days).

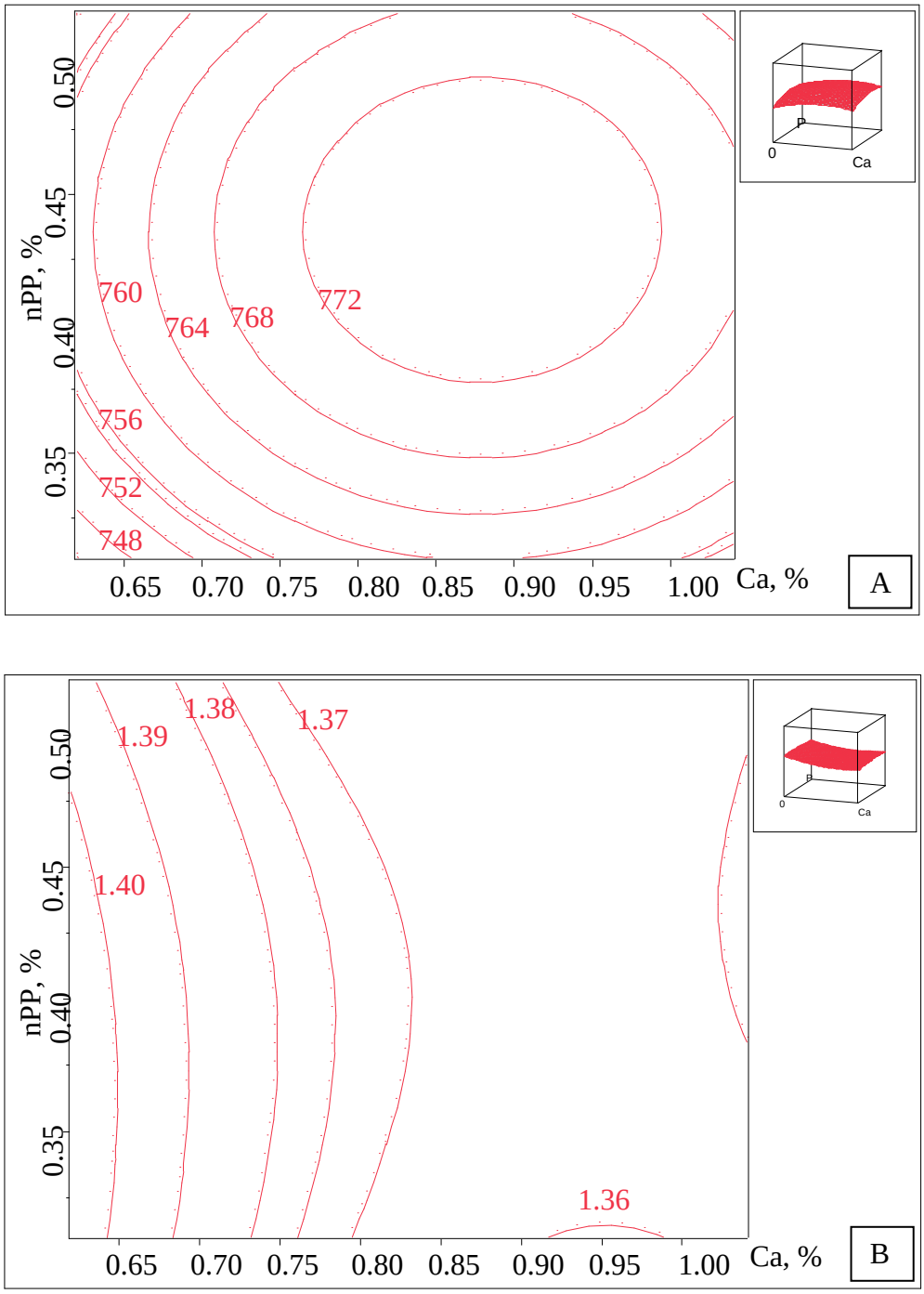

Figure II-2. Response contours of (A) feed intake (g) and (B) feed conversion ratio (g:g) from hatch to 17 days with varying dietary levels of Ca and nPP during the starter phase (0-17 days).

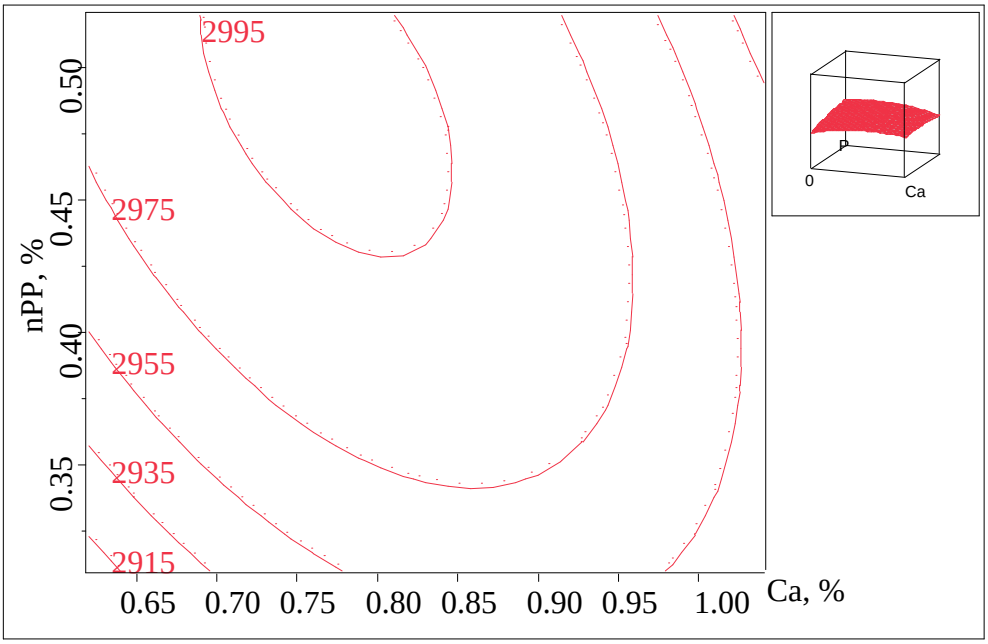

Figure II-3. Response contours of female body weight gain (g) from hatch to 49 days with varying dietary levels of Ca and nPP during the starter phase (0-17 days).

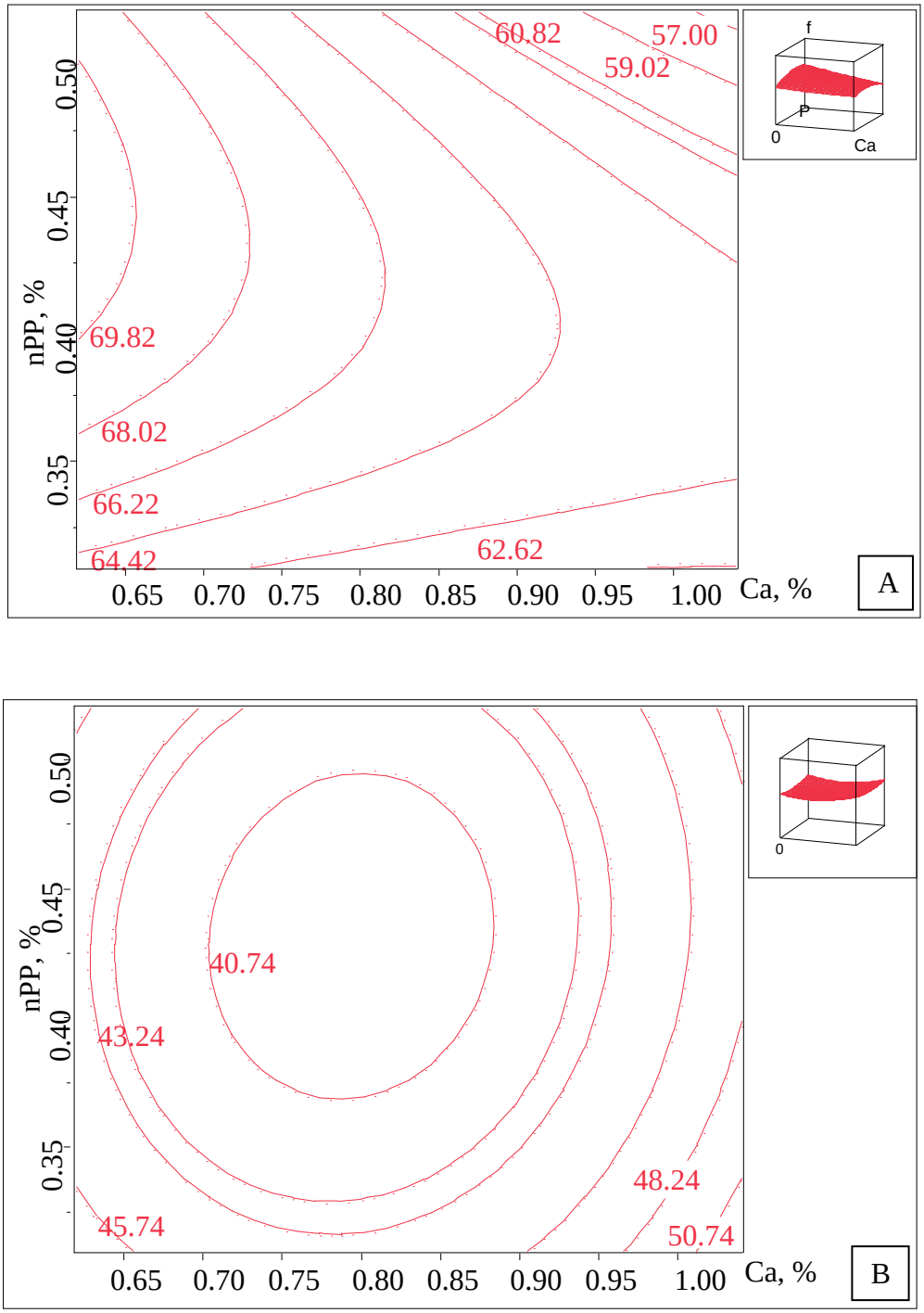

Figure II-4. Response contours of Ca retention (%) at 10 days (A) and 45 days (B) with varying dietary levels of Ca and nPP during the starter phase (0-17 days).

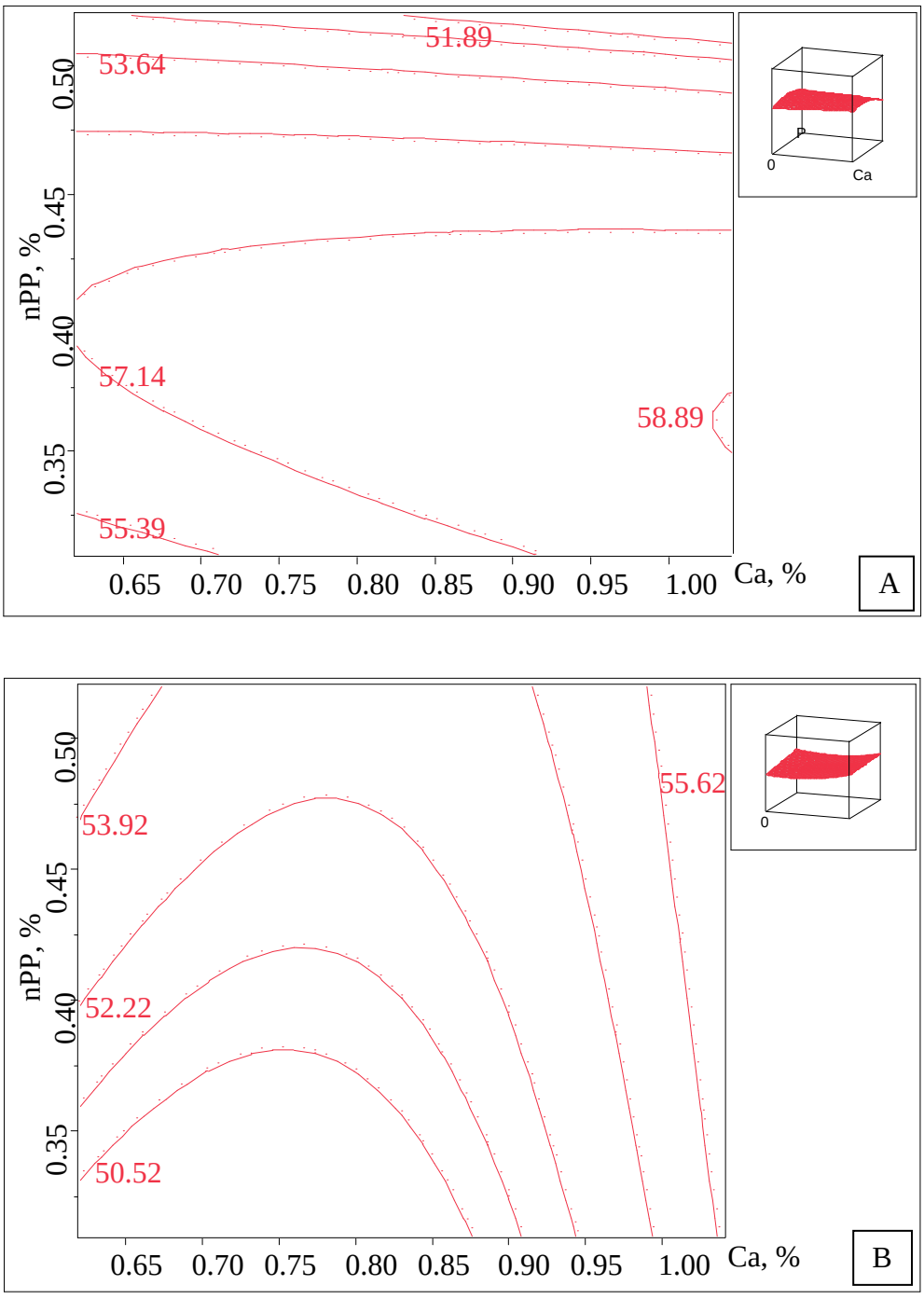

Figure II-5. Response contours of P retention (%) at 10 days (A) and 45 days (B) with varying dietary levels of Ca and nPP during the starter phase (0-17 days).

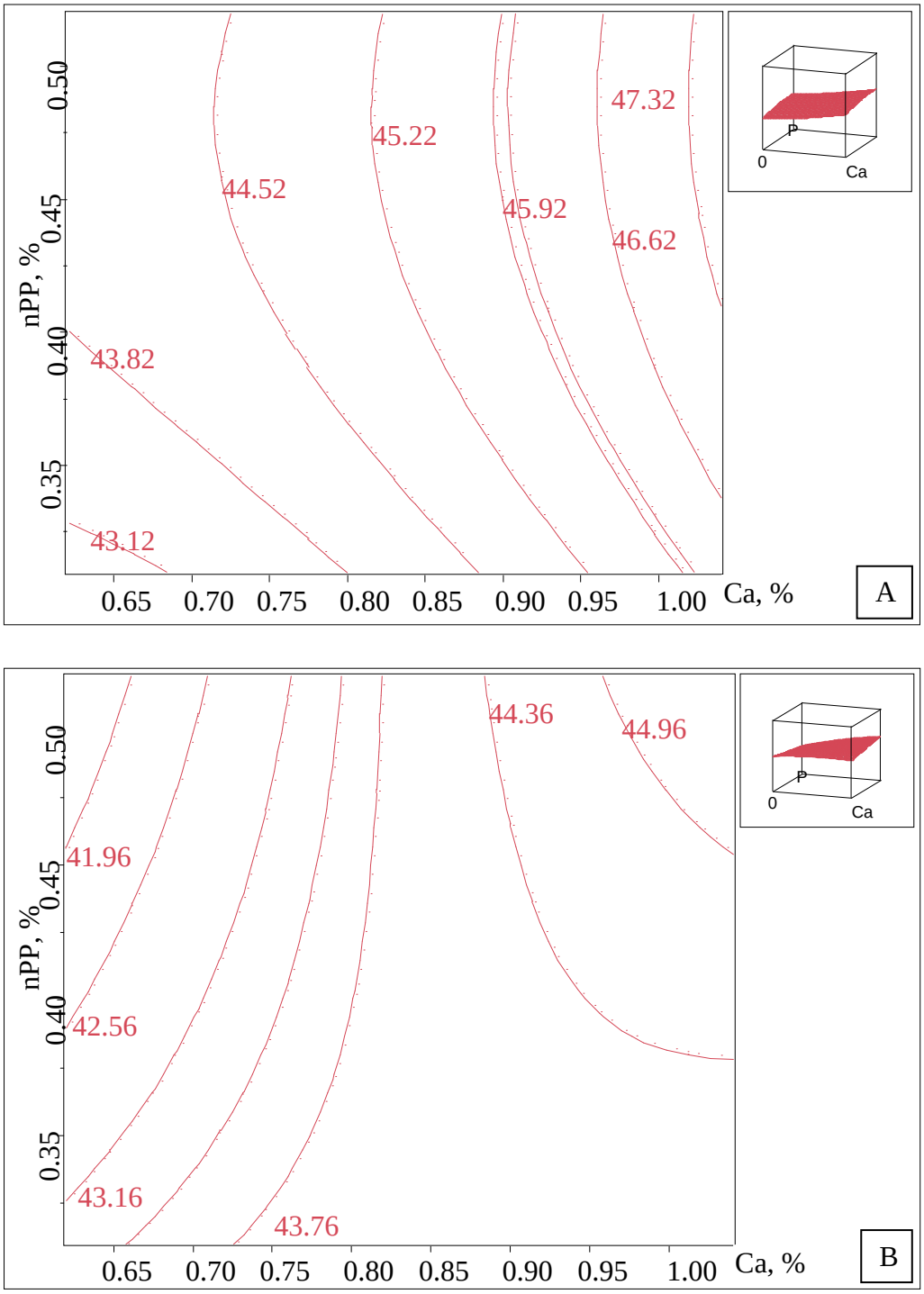

Figure II-6. Response contours of tibia ash (%) at 17 days of females (A) and males (B) with varying dietary levels of Ca and nPP during the starter phase (0-17 days).

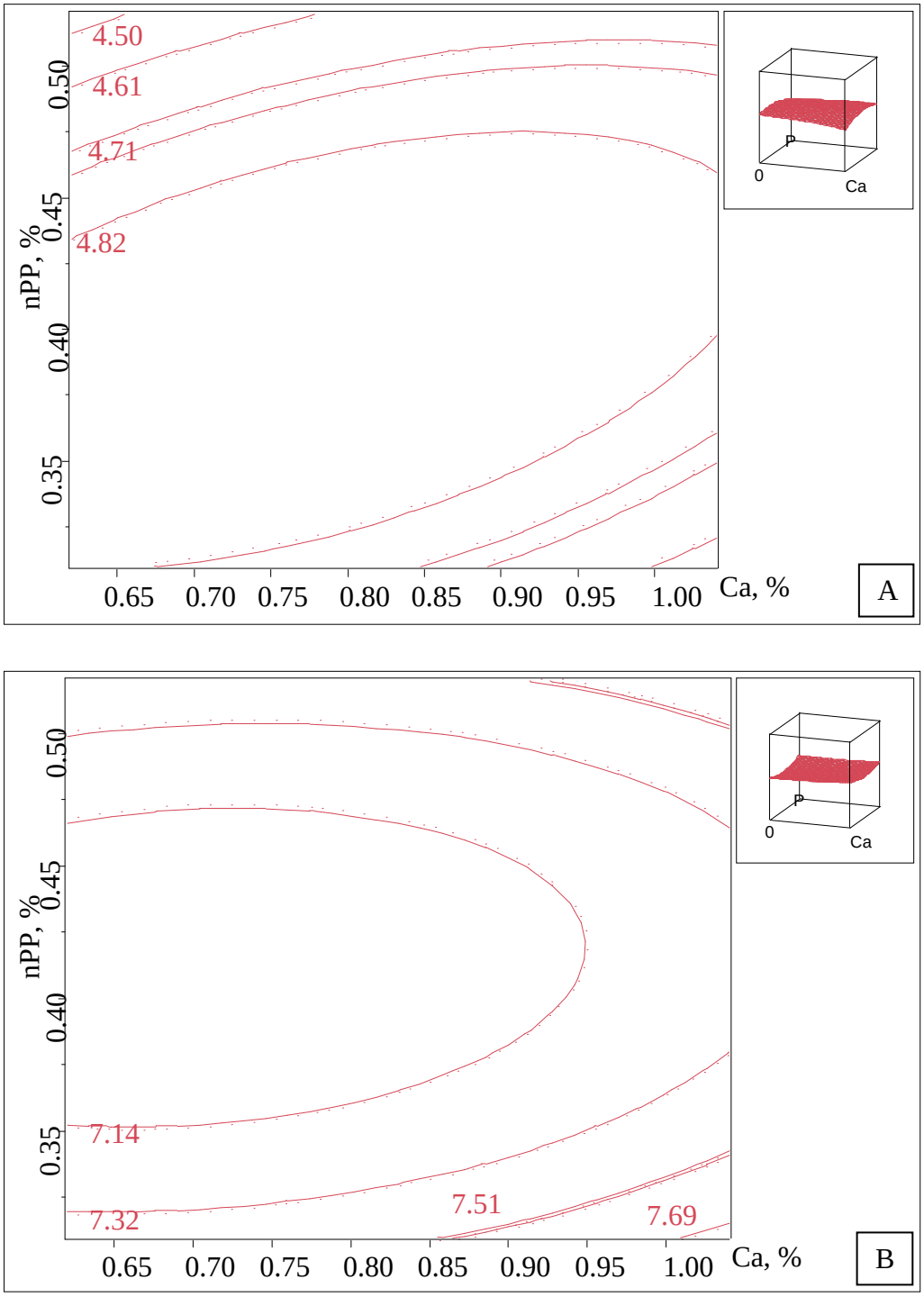

Figure II-7. Response contours of bone mineral content (g) of shanks at 49 days of females (A) and males (B) with varying dietary levels of Ca and nPP during the starter phase (0-17 days).

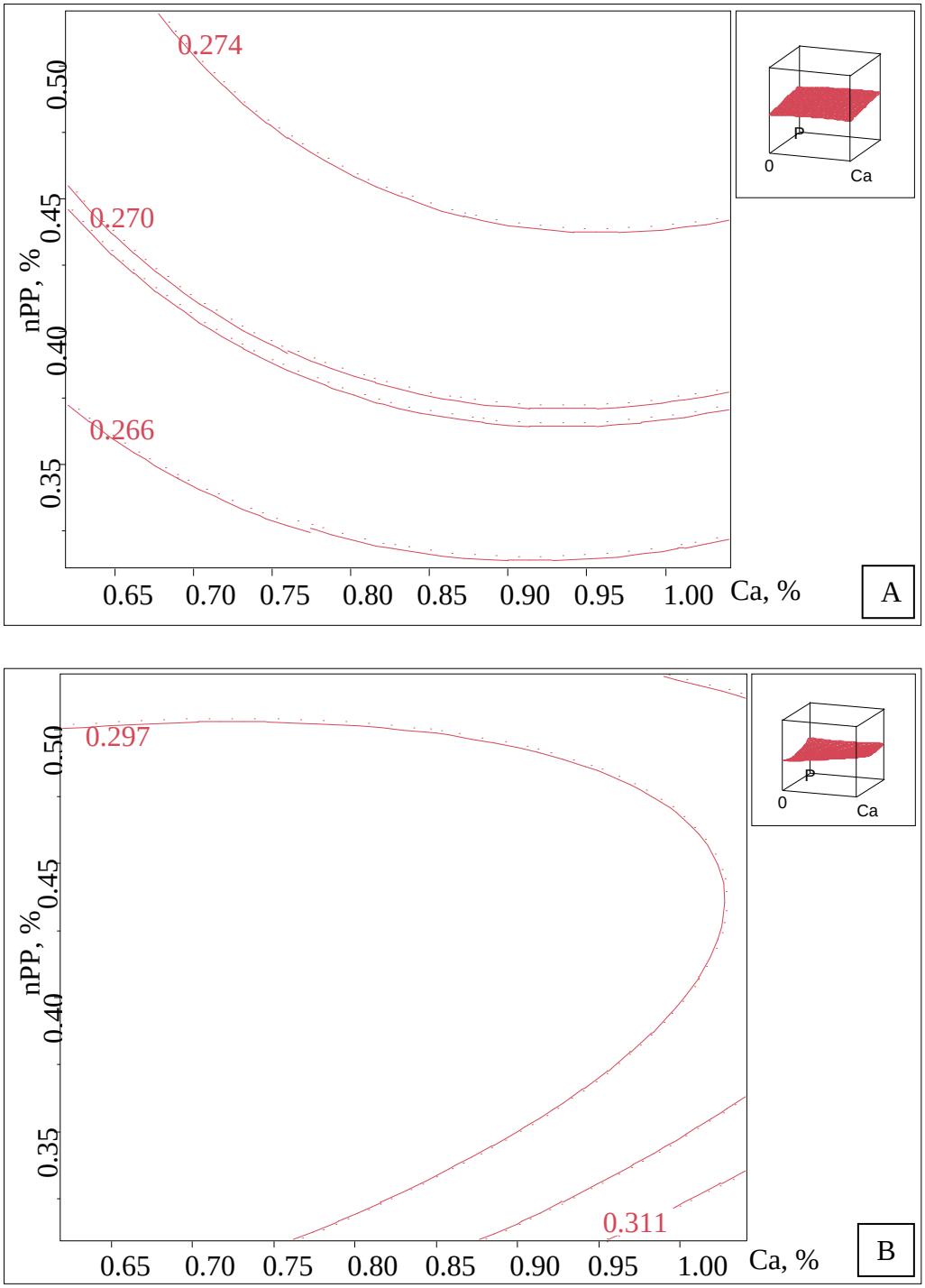

Figure II-8. Response contours of bone mineral density (g/cm^2) of shanks at 49 days of females (A) and males (B) with varying dietary levels of Ca and nPP during the starter phase (0-17 days).

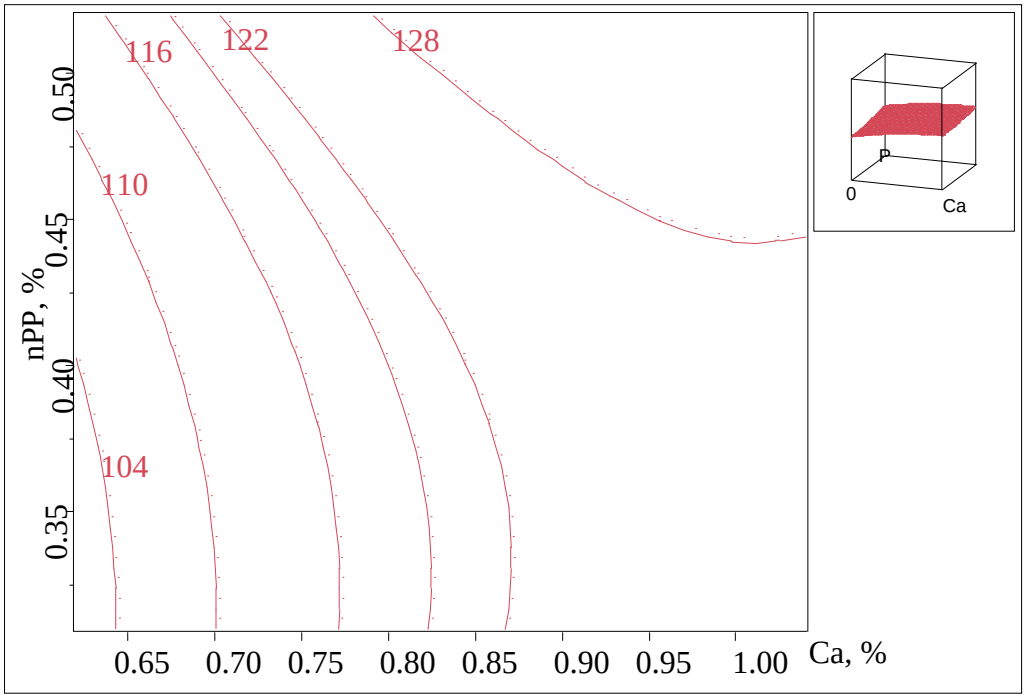

Figure II-9. Response contours of tibia breaking strength (N) at 49 days of males with varying dietary levels of Ca and nPP fed during the starter phase (0-17 days).

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CHAPTER III

Effects of Calcium and Phosphorus Levels in Grower Diets on Heritage Broiler Performance, Mineral Retention, Bone Characteristics, Leg Abnormalities, and Walking Ability

ABSTRACT

The objective of this experiment was to determine the impact of using different Ca and nPP dietary levels during the grower phase (18 to 35 days) on broiler performance, mineral retention, bone mineralization and strength, walking ability, and the incidence of leg abnormalities. 1,920 male and female Heritage broiler chicks were distributed in 64 pens with 15 chicks of each sex per pen. Common corn-soybean meal based starter and finisher diets were fed from 1 to 17 and 36 to 49 days, respectively. The 16 grower treatments were fed from 18 to 35 days, containing combinations of 4 levels of Ca (0.46, 0.62, 0.78, 0.94%) and 4 levels of nPP (0.23, 0.30, 0.37, 0.44%). BW gain, FI, and FCR were assessed at the end of each dietary phase. At 31 days, Ca and P retention was evaluated. Tibia strength, TD scores, and tibia ash were determined from legs collected at 35 days. Gait scores, leg abnormalities, and individual BW were obtained at 49 days. At 49 days, legs were collected, shanks used for bone densitometry (BMC and BMD), and thighs used for determining bone breakage during mechanical deboning. Data were analyzed within a randomized complete block design by response surface methodology. Male BW gain was affected ($P \leq 0.05$) by the interaction of Ca and nPP levels during the grower and also the whole grow out period. Female BW gain was affected by Ca levels, quadratically ($P \leq 0.05$) during the grower phase, and linearly ($P \leq 0.05$) in the whole grow out. FCR in the grower period was affected quadratically ($P \leq 0.01$) by Ca and linearly ($P \leq 0.01$) by nPP. Tibia ash at 35 days increased linearly ($P \leq 0.01$) as Ca and nPP increased. The nPP levels had a linear effect ($P \leq 0.01$) on male BMC and BMD. P retention at 31 days was affected quadratically ($P \leq 0.01$) by nPP

and linearly by Ca, although at 44 days only a quadratic effect ($P \leq 0.05$) of Ca levels during the grower phase was observed on P retention. Tibia strength represented as the force needed to break the bones was affected by Ca levels, quadratically ($P \leq 0.05$) in females and linearly ($P \leq 0.01$) in males. An interaction effect ($P \leq 0.05$) of Ca and nPP was observed on crooked toe incidence, and the occurrence of severe valgus condition was quadratically affected ($P \leq 0.05$) by nPP. TD incidence at 35 days was affected quadratically ($P \leq 0.05$) by nPP. The incidence of femur epiphyseal breakage during deboning was affected by the interaction ($P \leq 0.05$) of Ca and nPP. No significant effects of treatments were observed on TD incidence and severity and gait scores at 49 days. In conclusion, Ca levels between 0.75 and 0.90% and nPP levels between 0.41 to 0.44% resulted in best performance during the grower phase, and bone mineralization and strength were maximized when the highest levels of Ca and nPP were used (0.94 and 0.44%); however, these high levels were related to increased incidence of bone and leg problems at 49 days. Furthermore, Ca and P retention was improved when chickens were fed diets containing Ca levels between 0.90 and 0.94% combined with 0.33 to 0.35% nPP.

Key words: Calcium, phosphorus, grower, bones, mineral retention

INTRODUCTION

Calcium (Ca) and phosphorus (P) levels in broiler diets have been largely discussed, with focus on reducing phosphate inclusion due to its high cost and environmental impact. However, most of literature reports were done by evaluating the ideal levels of Ca and P for young broilers or for the finisher/withdrawal period. This leaves a gap in knowledge about

Ca and P requirements for the grower diets, which are extremely important for both performance, bone development, and the volume of feed consumed during this dietary phase.

The levels of Ca and P used by the broiler industry in USA during the grower phase (approximately 22 to 35 days) are around 0.91% Ca and 0.44% nPP (Agristats, 2011), which are similar to the NRC (1994) recommendations of 0.90% Ca and 0.40% aP (from 3 to 6 weeks of age). During the past five decades, however, genetic selection has significantly changed broiler performance, carcass composition, and birds' ability to utilize nutrients (Havenstein et al., 2003). Several researchers have demonstrated that Ca and P levels could be reduced in broiler diets without compromising growth performance (Yan et al., 2001; Fritts and Waldroup, 2006), reduce the environmental impact of P emission (Ziaei et al., 2008), and lowering the cost of the feed. Moreover, the requirement of these minerals for optimum growth performance is lower than for optimum bone characteristics (Angel, 2011). Accordingly, Waldroup (1999) suggested that the amount of P needed to maximize various criteria is ordered as follows: bone calcification > body weight > feed efficiency > mortality.

The objective of this project was to comprehensively evaluate the effects of different levels of Ca and P in grower diets (18 to 35 days) on growth performance, mineral retention, bone characteristics, and leg health during the grower period and for the whole grow-out period of Heritage broilers.

MATERIAL AND METHODS

Birds and Management

All practices regarding bird care were approved by the Institutional Animal Care and Use Committee of North Carolina State University. The experiment was conducted at the North Carolina State University Poultry Research Unit (Raleigh, NC) between March 30th and May 18th 2010. Heritage broiler chicks were hatched from fertile eggs provided by a local hatchery (Perdue Farms, Inc., Candor, NC). The experimental house was curtain-sided, with exhaust fans and forced air heating system combined with upward blowing ventilation fans to assure even temperature distribution throughout the house. There were a total of 64 pens, divided into 4 blocks within the house. Pens were 1.22 x 3.96 m (approximately 6 birds/m^2, or a final density of 17.5 kg/m^2), and covered with a 15 cm layer of new pine wood shavings to avoid Ca and P intake from used litter. Each pen was equipped with 2 tube feeders and 2 bell-shaped drinkers. Chicks were feather-sexed at hatching, and a total of 1920 birds (960 males and 960 females) were individually identified with neck tags. They were weighed in groups of 15 males and 15 females, and 30 birds (one group of each sex) were randomly allocated to each of the 64 pens. Brooding temperatures at placement were set at 35-36 °C and kept like this for the first night. Temperatures were gradually reduced to 32.2-33.5 °C from 1 to 7 days, 29.4 °C from 8 to 14 days, 26.7 °C from 15 to 21 days, and to ambient temperature thereafter. Observed house temperatures were recorded twice a day in 8 different points within the house, and are shown in Appendix A.

The lighting program consisted of 23 hours of light per day during the first week, 4 hours of darkness per day during the second week, and 16 hours of light per day from 22 to

49 days. During this last light period, supplemental light was provided from 10:00 pm to 2:30 am, in order to obtain the 16 hours of light. Feeders were shaken once a day until 21 days of age and twice a day thereafter.

Dietary Treatments

Broiler chickens were fed a common pelleted-crumbled starter diet containing 0.93% Ca and 0.45% nPP from placement until 17 days of age. For the grower phase (18 to 35 days), a corn-soybean meal basal diet was mixed, in which limestone, dicalcium phosphate, and washed sand were added to obtain the 16 treatments. The treatment diets were fed as pellets, and were formulated to contain 16 combinations of 4 levels of Ca (0.46, 0.62, 0.78, and 0.94%) and 4 levels of nPP (0.23, 0.30, 0.37, and 0.44%). Celite was added at 1% to the grower and finisher diets as an acid insoluble ash marker, for further calculation of mineral retention. To simulate commercial conditions, phytase was added to the starter and finisher diets; however, grower diets did not contain the enzyme to avoid mixing or pelleting variability and effects on enzyme activity among the different treatments. Each treatment was randomly assigned to one pen within each block, with a total of 4 replicate pens per treatment. After the experimental period, a common finisher diet containing 0.61% Ca and 0.30% nPP was offered for all broilers as pellets from 36 to 49 days of age,. The composition of the diets utilized throughout the trial is shown in Table III-1. Feed and water were provided for *ad libitum* consumption during the whole grow-out; however, the amount of feed per dietary phase was limited to approximately 900 g for the starter and 2,700 g for the grower phase, in order to mimic the feeding scheme normally used by poultry companies. In

the days before each new dietary period (16 and 34 days) the amount of feed issued per pen was adjusted after accounting for the mortality, so all birds within pens had access to approximately the same amount of feed for each dietary phase.

Data Collection

Feed samples from each diet were collected after the pelleting process and analyzed for Ca, tP, crude protein and acid insoluble ash. Group body weights (BW) per sex were obtained at hatch, 18, and 35 days, and individual BW at 49 days to assess flock uniformity as the coefficient of variation (CV%). FI was documented at the end of each dietary phase (18, 35, and 49 days), and mortality BW was recorded daily for the adjustment of feed conversion ratio (FCR). BW gain (BWG), FI, and FCR were calculated for the grower phase (18 to 35 days) and for the whole grow-out period (1 to 49 days). At 49 days of age, all birds were evaluated for the prevalence of leg abnormalities (crooked toes, valgus, varus, and twisted legs), and walking ability was assessed using the gait score system of Kestin et al. (1992). The 6 walking ability scores assessed by the Kestin method were then grouped in 3 gait score categories (Webster et al., 2008) for statistical analysis, with score 1 representing birds not affected, 2 representing birds with some degree of lameness, and 3 representing birds with severe lameness. At 35 and 49 days, two chickens of each sex per pen were randomly selected, euthanized, and legs were collected. Approximately 100 g of fresh fecal samples per pen were collected at 31 and 45 days of age, frozen overnight, and then freeze-dried (Virtis Freezemobile - Model 12XL, Warminster, PA) for further acid insoluble ash (AIA) determination and Ca and P analysis.

Analytical Methods

The AIA analysis was performed by weighing 5 g of feed or 3 g of freeze-dried feces in pre-weighed porcelain crucibles, and digesting the samples in 50 mL of acid (4 *N* HCl) on a hot plate at approximately 95°C for 45 minutes. The solution was filtered using a 125 mm hardened ashless filter paper (Whatman, International Ltd, Maidstone, England) dried at 70°C for 4 hours, and ashed at 600°C overnight in a muffle furnace. The ash weight was then used to calculate the percent AIA of the samples (procedure adapted from Scott and Boldaji, 1997). For Ca and P analysis, 2.5 g of feed or fecal samples were weighed into pre-weighed porcelain crucibles and ashed at 600°C overnight. Ash content were digested in 4 mL of acid (6 N HCl) on a hot plate at approximately 100°C until fully evaporated. Samples were then resuspended in 8mL 6 N HCl, rinsed into 100mL volumetric flasks, and filled to volume with deionized water. Flasks were covered with parafilm, vigorously agitated, and 13 mL of the suspension was filtered into 15mL conical centrifuge tubes using 125 mm hardened ashless filter paper (Whatman, International Ltd, Maidstone, England) to remove particulates (procedure adapted from Leske and Coon, 2002). Inductively-coupled plasma optical emission spectroscopy (Perkin Elmer 2000 DV ICP-OES, Waltham, MA) was used to determine Ca and P concentrations from each sample, and corrected to a corn-based standard and a negative control.Ca and P retention were calculated using the formula exemplified by Brenes et al. (2003), as follows: 1 - [(AIA concentration in feed / AIA concentration in excreta) × (mineral concentration in excreta / mineral concentration in feed)].

The drumsticks collected at 35 days were manually deboned, remaining tibiae measured (length and diameters), wrapped in cheese-cloth, and then kept frozen in bags at -

20°C to maintain bone moisture for further analyses. Tibiae were thawed by leaving them inside the plastic bags at room temperature for 6h, and the 3-point bending test was then performed to evaluate bone strength. Bones were sheared midshaft using a crosshead speed of 30 mm/min (Crenshaw et al., 1981). The resistance of bones to automatic deboning forces was tested with the 49 days thighs by using a mechanical deboning machine (Meyn-D40, Oostzaan, The Netherlands). Tibiae collected at 35 and 49 days of age were evaluated for the incidence and severity of tibial dyschondroplasia (TD) according to the TD scoring system developed by Edwards and Veltmann (1983). Bone ash percentage was determined on tibiae collected at 35 days (Hall et al., 2003). Bone mineral content (BMC) and bone mineral density (BMD) were determined in the shanks collected at 49 days by Dual Energy X-ray Absorptiometry (DEXA) at the USDA-ARS in Beltsville, MD.

Data Analysis

Data were analyzed using the response surface methodology within a randomized complete block design, containing 16 treatments and 4 replicates per treatment. Blocks represented the pen distribution within the house and it was considered as a random effect. In order to satisfy the normality assumption, all percentage data were converted by an arcsine-square root transformation prior to the analyses. Each pen of 30 broilers was an experimental unit for performance and mineral retention data, and JMP 9 (SAS Inst. Inc., Cary, NC) was used for the statistical analyses. The categorical data (mechanical deboning, leg abnormalities, gait scores, and TD evaluations) was statistically analyzed using each broiler chicken as an experimental unit. For these parameters, data were treated as binomial, for each

condition the response take the value 0 (absence) or 1 (presence). The GLIMMIX Procedure of SAS (SAS Inst. Inc., Cary, NC) was used, having the linear and quadratic effects of levels of Ca and P in the diet and their interaction effects as fixed, and considering the pens as random effects nested within treatment combinations. The results, as log odds of a certain condition, were modeled within the effects to obtain the probability of observing each condition individually.

Statistical model used for the response surface methodology:

$$Y_{ijkl} = \mu + Ca_i + nPP_j + Ca*nPP_{ij} + Ca^2_i + nPP^2_j + P_l\,(T_{ij}) + B_k + \varepsilon_{ijkl}$$

Where:

Y_{ijkl}: Variable response

μ: Overall mean

Ca_i: Linear effect of the i^{th} Ca level (i = 1-4)

nPP_j: Linear effect of the j^{th} nPP level (j = 1-4)

$Ca*nPP_{ij}$: Effect of the first order interaction between Ca level i and nPP level j

Ca^2_i: Quadratic effect of the i^{th} Ca level

nPP^2_j: Quadratic effect of the j^{th} nPP level

$P_l\,(T_{ij})$: Random effect of dietary treatment combinations nested within pens (l = effect of pen, l = 1-4)

B_k: Random effect of the block (k = 1-4)

ε_{ijkl}: The experimental error associated to each observation.

Table III-1. Composition of broiler diets (%) and formulated nutrient contents

Ingredients	Starter 0-17 days	Grower basal 18-35 days	Finisher 36-49 days
	----------%----------		
Corn	51.02	56.30	60.44
Soybean meal, 48%	30.71	23.72	19.89
Distillers dried grains with solubles	10.00	10.00	12.00
Poultry fat	4.03	4.72	3.92
Salt (NaCl)	0.41	0.38	0.36
Limestone	1.50	0.06	1.19
Dicalcium phosphate, 18.5%	1.12	0.18	0.19
DL-methionine, 99%	0.33	0.27	0.21
L-lysine-HCl, 78,8%	0.23	0.27	0.25
Choline chloride, 60%	0.20	0.20	0.20
Sodium bicarbonate	0.15	0.12	0.09
L-threonine, 98%	0.12	0.14	0.13
Coccidiostat[1]	0.06	0.05	0.05
Mineral premix[2]	0.05	0.05	0.03
Vitamin premix[3]	0.05	0.04	0.03
Phytase[4]	0.02	-	0.02
Filler[5]	-	3.50	1.00
Total	100.00	100.00	100.00
Nutrient composition			
Metabolizable energy, kcal/kg	3,065	3,140	3,175
Crude protein, %	22.23	19.30	18.26
Calcium, %	0.93	0.18	0.61
Total phosphorus, %	0.61	0.43	0.43
Non phytate phosphorus, %	0.45	0.20	0.30
Digestible lysine, %	1.20	1.05	0.96
Digestible total sulfur amino acids, %	0.93	0.81	0.74
Digestible threonine, %	0.75	0.68	0.63
Sodium, %	0.25	0.23	0.22
Potassium, %	0.91	0.78	0.74
Chloride, %	0.32	0.31	0.30
Dietary electrolyte balance, mEq/100 g	261	224	210

[1]Monteban® 45 (Narasin), Elanco Animal Health, Greenfield, IN, at 60 g/ton in the starter and 54 g/ton in the grower diet.
[2]Trace minerals premix provided per kilogram of premix: manganese (MnO_2), 220 g; zinc (ZnO and $ZnSO_4$), 250 g; iron ($FeCO_3$), 75 g; copper (CuSO4 and $CuCl_2$), 10 g; iodine ($Ca(IO_3)_2$), 5 g; selenium (Na_2SeO_3), 1 g.
[3]Vitamins premix provided per kilogram of premix: vitamin A, 18,739,292 IU; vitamin D3, 6,613,868 IU; vitamin E, 66,139 IU; vitamin B12, 33 mg; riboflavin, 22,046 mg; niacin, 88,185 mg; d-pantothenic acid, 30,865mg; menadione, 3,968 mg; folic acid, 2,646 mg; vitamin B6, 7,716 mg; thiamine, 5,512 mg; biotin, 176 mg.
[4]Ronozyme® P CT at 185 g/ton to provide 930 FYT (DSM Nutritional Products, Parsippany, NJ).
[5]Celite, Celite Corp., Lompoc, CA, at 1 g/kg of feed in the finisher diet. Filler also contained the amounts of dicalcium phosphate, limestone, and washed sand used to obtain the 16 treatments.

RESULTS AND DISCUSSION

The formulated Ca and P concentration of the diets was in accordance to the lab analysis results. The correlation coefficient between formulated and analyzed values for Ca and P were 0.98 and 0.94, respectively.

Performance during the grower phase (18-35 days)

Broilers had similar ($P > 0.05$) BW (520 and 576 g for females and males, respectively) among treatments at 18 days. The effects of Ca and nPP on performance parameters are described in Tables III-2 and III-3. Female BWG was quadratically affected ($P \leq 0.05$) by Ca levels. At the lowest nPP concentration (0.23%), the BWG improved as levels of Ca increased up to 0.70%, and further increments in Ca levels negatively affected BWG. When a concomitant increase in nPP to levels higher than 0.42% took place, Ca levels between 0.80 and 0.85% resulted in the optimum BWG (Figure III-1 A). The male BWG showed an interaction effect ($P \leq 0.05$) of Ca and nPP levels, with a similar response to the females. Male chickens gained the most weight during the grower phase when diets with nPP levels higher than 0.42% and Ca between 0.80 and 0.90% were provided (Figure III-1 B). Similar effects of P were reported by Gomes et al. (2004), who tested levels of 0.15, 0.24, 0.33, 0.42, 0.51, and 0.60% aP (with 0.93% Ca) in the diet of Hubbard broilers from 22 to 42 days of age. The researchers demonstrated that male and female BWG improved as levels of nPP increased up to 0.42%, and that further increments impaired BWG. In a trial conducted by Sá et al. (2004), levels of Ca ranging from 0.16 to 1.41% (with 0.41% aP) were fed to broiler chickens from 22 to 42 days, with results demonstrating that BWG improved as

dietary Ca increased up to 0.91%. Yan et al. (2001) tested levels of nPP ranging from 0.10 to 45% (0.90% Ca) in broiler diets from 21 to 42 days, and noted no differences in BWG and FCR by feeding levels as low as 0.15% as compared to 0.45% nPP. However, these birds received starter diets containing 0.45% nPP and 1.00% Ca up to 21 days, which could have influenced the needs in the subsequent phase. In another trial, Yan et al. (2005) found no negative effects of feeding grower diets with lower levels of nPP (0.30% nPP and 0.60% Ca) than a standard diet (0.40% nPP and 0.80% Ca), provided that birds were fed adequate levels of Ca and P in the previous phase (0.45% nPP and 0.90% Ca).

The dietary treatments did not affect FI during the grower phase; however, a tendency for an interaction effect (P = 0.08) of Ca and nPP levels occurred. The maximum FI was related to levels of nPP higher than 0.42% and Ca higher than 0.85%, suggesting that the BWG responses could be a result of the alterations in FI. Similarly, Gomes et al. (2004) reported no significant differences in FI from 22 to 42 days at various aP levels (from 0.15 to 0.60%), and Sá et al. (2004) reported no significant differences in FI from 22 to 42 days at various Ca levels (from 0.16 to 1.41%). Ziaei et al. (2008) noted that FI from 14 to 39 days was higher in broiler chickens that received a diet containing 0.83% Ca and 0.66% tP or 0.73% Ca and 0.55% tP as compared to those offered diets with 0.63% Ca and 0.55%, and the reduction in FI was associated with a tendency for lower BW at 39 days, although no effects were observed in FCR. These findings contrast with those of Schoulten et al. (2003), who reported that for younger chickens (1 to 21 days) the increments in dietary Ca from 0.46 to 1.30% led to a linear reduction in FI and BWG.

FCR during the grower phase was affected quadratically ($P \leq 0.01$) by Ca and linearly ($P \leq 0.01$) by nPP dietary levels. As demonstrated in Figure III-3, there was an improvement in FCR as levels of nPP were increased up to 0.41%, and Ca increased up to 0.74%, at which point the optimum FCR occurred (1.69 g:g). Even though Gomes et al. (2004) noted no significant differences in FI when testing several levels of nPP, female FCR improved with increments in nPP up to 0.42%.

Levels of Ca and P close to the ones that resulted in maximum BWG in the present study were used as a control diet (0.85% Ca and 0.425% aP) by Cardoso Jr. et al. (2010) to make comparisons with different combinations of Ca (0.55, 0.65, 0.75, and 0.85%) and aP (0.275, 0.325, and 0.375%) in an experiment using male Cobb broilers from 8 to 35 days. In contrast to the results reported herein, those authors showed that FCR was not affected by treatments. However, they reported that broilers fed diets with 0.65% Ca combined with 0.325% aP, as well as 0.55% Ca with 0.275% aP, gained more weight and had higher FI when compared to the control diet. However, birds in that experiment started consuming the treatment diets at 8 days of age, and phytase was included (except in the control), while in the present study birds were offered the treatments only at 18 days of age, and the treatments did not contain phytase.

The carryover effect of previous dietary phases should be taken in consideration when analyzing the performance parameters and defining Ca and nPP levels for the grower period. Driver et al. (2005) tested two combinations of Ca and tP (0.60% Ca with 0.47% tP vs. 0.90% Ca with 0.68% tP) from hatch to 18 days, and two combinations (0.30% Ca with 0.37% aP vs. 0.80% Ca with 0.67%) during the grower phase (19 to 35 days). It was

observed that if broiler chickens were fed the higher levels of Ca and tP during the starter phase, the levels of these minerals could be reduced to the lower levels during the grower phase without significant differences in BWG, FI, and FCR.

The treatments affected male mortality during the grower period, where an interaction effect ($P \leq 0.05$) of Ca and nPP levels was observed. The lowest mortality was observed when diets with the highest level of Ca and lowest level of nPP or the lowest Ca combined with the highest nPP were fed. The mortality was the highest in two main situations: when the lowest levels of both minerals were fed; and when levels of Ca higher than 0.70% were combined with levels of nPP higher than 0.30%. It seems that the mortality is related to the growth performance of the broiler chickens. Birds that did not receive enough minerals (lowest levels of Ca and nPP) did not grow well and had the highest mortality rate. However, broilers that had the highest BWG also had higher mortality rate, which could be a result of the metabolism being pushed to the maximum growth.

Performance during the Whole Grow-out (0-49 days)

When evaluating the whole grow-out, female BWG was linearly affected ($P \leq 0.05$) by Ca, and male BWG had an interaction effect ($P \leq 0.05$) of Ca and nPP levels (Tables III-2 and III-3). For both sexes, broilers fed diets containing the highest levels of Ca (0.94%) and nPP (0.44%) during the grower phase (18-35 days) showed the maximum BWG from hatch to 49 days of age (Figures III-2 A and B). According to Waldroup (1999), the gap between mineral needs to reach maximum tibia ash and maximum BW is much narrower today, due to the much more rapid growth of modern broilers. Although the bulk of the P needs are related

to skeletal development, P is also actively involved in energy metabolism in the body; therefore, one might expect that the P demands of the modern bird to support BWG should be much greater in the current genetic strains.

The male BW uniformity (CV%) at 49 days tended to be quadratically affected (P = 0.09) by nPP levels in the grower diet. The minimum CV% (6.49%), which represents the best uniformity, was estimated at 0.82% Ca and 0.34% nPP. Both higher and lower levels of nPP increased CV%, and the worst uniformity (about 8.5% CV) was observed when the highest Ca and nPP levels were fed.

Mortality rate from 0 to 49 days had a linear effect (0.0313) of nPP levels fed during the grower diet. The lowest mortality rate was observed when high levels of Ca (> 0.70%) combined with the lowest level of nPP (0.23%) were fed during the grower phase, and when the lowest level of Ca (0.46%) was combined with nPP levels between 0.30 and 0.40%. As nPP levels increased in the grower diet, the mortality during the whole grow-out increased up to its maximum levels when the highest Ca (0.94%) and nPP (0.44%) dietary levels were fed during the grower phase. The maximum levels of minerals (0.94% Ca and 0.44% nPP) in the grower diet were responsible for both the optimum BWG, and the highest mortality rate during the whole grow-out, a similar response to that observed during the grower period.

Table III-2. Response surface regression coefficients and predicted optimal values for the effects of dietary Ca and nPP levels during the grower phase on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of male and female Heritage broilers during the grower period (18-35 days) and on BWG from 0 to 49 days.

	BWG 18-35 d		FI	FCR	BWG 0-49 d	
	Females	Males	18-35 d	18-35 d	Females	Males
Intercept	1123.84**	1237.95**	2.46**	2.25**	2711.75**	3390.20**
Ca	472.40*	484.53	0.06	-0.91	-218.95*	-508.53**
nPP	-872.41	175.49*	-1.11	-1.09**	-777.88	-591.23
Ca x Ca	-412.57*	-509.80	-0.19	0.62**	139.33	122.62
Ca x nPP	469.37	858.22*	0.76	-0.01	297.70	1500.69*
nPP x nPP	913.42	-913.69	0.85	1.35	883.08	-439.81
Predicted minimum				1.69		
Ca at predicted				0.74		
nPP at predicted				0.41		

*$P \leq 0.05$, **$P \leq 0.01$

Table III-3. Effects of dietary Ca and nPP levels in grower diets on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of male and female Heritage broilers during the grower period (18-35 days) and on BWG from 0 to 49 days.

Ca	nPP	BWG 18-35 d*		FI 18-35 d	FCR 18-35 d	BWG 0-49 d**	
		Females	Males			Female	Male
-----%-----		----------g----------			---g/g---	----------g----------	
0.46		1144[b]	1438[b]	2296	1.755[a]	2534	3165[b]
0.62		1165[ab]	1465[ab]	2299	1.714[ab]	2525	3179[ab]
0.78		1187[a]	1488[a]	2326	1.711[b]	2558	3216[ab]
0.94		1165[ab]	1462[ab]	2310	1.732[ab]	2563	3244[a]
Pooled SEM		10	12	11	0.012	16	16
	0.23	1162	1436	2311	1.755	2547	3179
	0.30	1158	1475	2312	1.728	2539	3200
	0.37	1164	1462	2295	1.714	2542	3207
	0.44	1178	1479	2313	1.714	2552	3216
Pooled SEM		10	12	11	0.012	16	16
0.46	0.23	1172	1460	2341	1.774	2584	3211
0.46	0.30	1132	1450	2290	1.747	2508	3158
0.46	0.37	1123	1423	2277	1.756	2511	3126
0.46	0.44	1151	1419	2276	1.741	2533	3166
0.62	0.23	1163	1431	2313	1.761	2497	3154
0.62	0.30	1149	1453	2288	1.719	2503	3203
0.62	0.37	1177	1483	2286	1.683	2571	3158
0.62	0.44	1170	1493	2311	1.692	2531	3201
0.78	0.23	1157	1424	2272	1.727	2535	3199
0.78	0.30	1193	1514	2351	1.709	2589	3181
0.78	0.37	1186	1510	2356	1.704	2550	3252
0.78	0.44	1212	1504	2324	1.703	2559	3230
0.94	0.23	1157	1429	2317	1.759	2574	3154
0.94	0.30	1158	1485	2319	1.737	2557	3260
0.94	0.37	1168	1432	2263	1.712	2538	3295
0.94	0.44	1177	1502	2341	1.720	2585	3267
Pooled SEM		18	24	23	0.023	27	35
Source of Variation		----------*P*-values----------					
Ca		0.421	0.052	0.242	0.030	0.116	0.013
nPP		0.434	0.063	0.654	0.040	0.906	0.497
Ca*nPP		0.013	0.126	0.035	0.972	0.134	0.240

[a,b] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

*Average BW at hatch was 44 g for both males and females

**Average BW at 18 days were 520 and 576 g for males and females, respectively

Table III-4. Response surface coefficients for the effects of dietary Ca and nPP levels in grower diets on Heritage broilers: male mortality rate during the grower phase (18-35 days) and female mortality rate during the whole grow-out (0-49 days).

	Mortality 18-35 d	Mortality 0-49 d
	Male	Female
Intercept	3.17	7.16
Ca	-9.49	15.93
nPP	16.73	-75.65*
Ca x Ca	-11.69	-21.51
Ca x nPP	79.50*	43.80
nPP x nPP	-105.47	84.13

*$P \leq 0.05$, ** $P \leq 0.01$

Table III-5. Effects of dietary Ca and nPP levels in grower diets on Heritage broilers: male mortality rate during the grower phase (18-35 days) and female mortality rate during the whole grow-out (0-49 days) of Heritage broilers.

Ca	nPP	Mortality 18-35 d	Mortality 0-49 d
		Male	Female
-----%-----		----------%----------	
0.46		1.67	1.47
0.62		2.50	2.08
0.78		2.49	2.99
0.94		2.08	1.42
Pooled SEM		1.02	0.70
	0.23	1.67	1.05
	0.30	2.08	1.67
	0.37	3.33	1.49
	0.44	1.66	3.75
Pooled SEM		1.02	0.70
0.46	0.23	3.35	2.53
0.46	0.30	1.67	1.67
0.46	0.37	1.67	0.00
0.46	0.44	0.00	1.67
0.62	0.23	3.34	1.67
0.62	0.30	1.67	1.67
0.62	0.37	5.00	1.67
0.62	0.44	0.00	3.34
0.78	0.23	0.00	0.00
0.78	0.30	3.33	1.67
0.78	0.37	4.99	1.95
0.78	0.44	1.67	8.34
0.94	0.23	0.00	0.00
0.94	0.30	1.67	1.67
0.94	0.37	1.68	2.35
0.94	0.44	5.00	1.67
Pooled SEM		2.03	1.54
Source of Variation		----------*P*-values----------	
Ca		0.927	0.497
nPP		0.617	0.084
Ca*nPP		0.416	0.245

Mineral Retention during the Grower Phase (31 days of age)

The retention of Ca at 31 days was quadratically affected by Ca ($P \leq 0.05$) and nPP ($P \leq 0.01$) levels fed during the grower phase (Tables III-6 and III-7). Increments in dietary Ca up to its highest levels (0.94%) and in nPP up to 0.35-0.40% resulted in maximum Ca retention (approximately 87%) in Heritage broilers (Figure III-4 A). However, it was demonstrater that even though Ca retention is improved as Ca levels increase, the excretion of Ca in the grower period (Table III-7) was higher as dietary Ca levels are increased. Plumstead et al. (2008) demonstrated that male Ross chickens fed diets with 0.47, 0.70, 0.93, and 1.16% Ca (0.31% nPP) from 16 to 21 days retained less Ca at 19 days (24-h period) as the levels of this mineral increased in the diet. It is important to note that in this case, the level of nPP was fixed at 0.31%, while in the present experiment the improvement in Ca retention was observed when Ca levels in the diet increased with concomitant increments in nPP up to 0.35 to 0.40%. It is possible that the controversial results are due to differences in an ideal nPP level for Ca retention, as well as the ratio between these minerals, besides the Ca level itself and genetics.

In the present study, the retention of P at 31 days was linearly affected ($P \leq 0.01$) by Ca and quadratically ($P \leq 0.01$) by nPP dietary levels (Tables III-6 and III-7). The optimum P retention (approximately 84%) was observed in broiler chickens fed diets containing levels of Ca and nPP at about 0.90% and 0.33% (Ca:nPP ratio of 2.7:1), respectively (Figure III-5 A), which were similar to the levels that resulted in the highest Ca retention. The excretion of P during the grower phase (Table III-7) increased linearly as dietary nPP was increased, and reduced linearly as dietary Ca increased. These data agree partially with the findings of

Plumstead et al. (2008), who reported a quadratic increase in P retention during a 24-h period starting at 19 days of age when dietary levels of Ca varying from 0.47 to 1.16% (0.31% nPP) were fed to Ross broiler chickens from 16 to 21 days. According to the authors, the response in P retention to increasing Ca levels differed among the sources of soybean meal used, and a plateau in P retention was reached at 0.74% Ca. In that experiment, the Ca:nPP ratio in which the maximum P retention was observed was lower (2.4:1) than the ratio reported herein. These authors stated that although lower dietary Ca levels increased phytate hydrolysis and P absorption in the intestines, the concurrent increase in P excretion indicate insufficient Ca for bone mineralization, and any excess in circulating P would have been excreted in the urine, which was reflected in the higher total P in the excreta, and reduced P retention at low dietary Ca concentrations.

Mineral Retention during the Finisher Phase (44 days of age)

The retention of Ca at 44 days was affected quadratically ($P \leq 0.01$) by Ca levels fed from 18 to 35 days (Tables III-6 and III-7). Broilers retained the most Ca at 44 days when a diet low in Ca was provided during the grower phase (Figure III-4 B). The best retention (approximately 65%) was estimated at levels of Ca in the grower diet lower than 0.52%. This data suggest that birds reacted to an early deficiency in Ca by improving the retention, which was observed in the ulterior dietary phase. This agrees with Bar et al. (2003), who concluded that modern broilers exhibit higher capacity of adaptation to P or Ca deficiency and this capacity remains high for the whole growth period. Results are also in agreement with Yan et al. (2005), who demonstrated that broilers exposed to Ca and P deficient diets during the

starter phase had ability to adapt to the deficiency in a later growth phase (18 to 32 d). As shown in Tables III-6 and III-7, P retention at 44 days was quadratically affected ($P \leq 0.05$) by Ca levels used in the grower diet. By using the response surface methodology, it was possible to estimate a minimum retention (about 45%) when diets containing 0.71% Ca and 0.39% nPP were provided in the grower phase. When broilers were fed diets containing Ca levels lower than 0.60%, or higher than 0.80% during the grower phase, P retention at 44 days improved (Figure III-5 B). It was shown herein that high Ca and nPP levels were related to the best BWG during the grower phase and the whole grow-out. Chickens fed diets low in Ca in the grower phase probably were compensating the growth during the finisher phase (when feces were collected), and could have elevated the needs of minerals during this period, resulting in better P retention. On the other hand, broilers fed high Ca in the grower phase gained more weight and may have higher requirements of Ca and nPP during the finisher phase, also retaining more P.

Table III-6. Response surface coefficients and predicted optimal values for the effects of dietary Ca and nPP levels in grower diets (18-35 days) on Ca and P retention (%) at 31 and 44 days, and on Ca and P excretion (g) from 18 to 35 days for Heritage broilers.

	Ca retention		P retention		Excretion 18-35 d	
	31 d	44 d	31 d	44 d	Ca	P
Intercept	0.71**	1.32**	0.37**	1.02**	-10.90**	32.46**
Ca	-0.52**	-1.99	0.07**	-0.96	172.80**	5.51**
nPP	1.26	-0.28	1.71	-0.80	-138.91	4.00**
Ca x Ca	0.35*	1.35**	0.07	0.74**	-82.40*	-5.19
Ca x nPP	0.51	0.09	0.28	-0.23	-102.11*	-94.76**
nPP x nPP	-2.33**	0.20	-2.89**	1.22	300.53*	179.15*
Predicted minimum				0.45		
Ca at predicted				0.71		
nPP at predicted				0.39		

$^{*}P \leq 0.05$, $^{**}P \leq 0.01$

Table III-7. Effects of dietary Ca and nPP levels in grower diets (18-35 days) on Ca and P retention (%) at 31 and 44 days, and on Ca and P excretion (g) from 18 to 35 days for Heritage broilers.

Ca	nPP	Ca retention		P retention		Excretion 18-35 d	
		31 d	44 d	31 d	44 d	Ca	P
-----%-----		----------%----------				--------mg--------	
0.46		76.90^{c}	64.95^{a}	69.67^{d}	58.59^{a}	24.27^{b}	41.75^{a}
0.62		76.26^{c}	52.75^{b}	72.51^{c}	51.08^{b}	33.43^{a}	36.37^{b}
0.78		80.68^{b}	57.98^{ab}	78.10^{b}	55.83^{ab}	35.02^{a}	31.74^{c}
0.94		83.37^{a}	59.01^{ab}	81.69^{a}	56.16^{ab}	36.12^{a}	25.63^{d}
Pooled SEM		0.063	3.51	0.63	3.74	1.03	0.93
	0.23	77.55^{b}	59.71	74.58^{bc}	57.12	34.60^{a}	28.53^{d}
	0.30	80.43^{a}	59.13	76.41^{ab}	56.94	31.37^{ab}	31.47^{c}
	0.37	80.46^{a}	58.33	77.40^{a}	52.93	30.08^{b}	34.44^{b}
	0.44	78.77^{ab}	57.52	73.58^{c}	54.67	32.78^{ab}	41.06^{a}
Pooled SEM		0.63	3.51	0.63	3.73	1.03	0.93
0.46	0.23	74.37^{ef}	64.36	66.85^{h}	59.24	27.04^{cdef}	36.71^{bcde}
0.46	0.30	80.70^{abcde}	72.92	71.83^{ef}	62.43	20.36^{f}	34.84^{cdefg}
0.46	0.37	75.57^{def}	60.84	71.56^{ef}	53.57	25.59^{def}	41.52^{bc}
0.46	0.44	76.98^{cdef}	61.68	68.45^{fh}	59.12	24.11^{ef}	53.92^{a}
0.62	0.23	76.27^{cdef}	52.24	75.03^{cde}	50.61	32.28^{abcde}	28.96^{fgh}
0.62	0.30	77.99^{abcdef}	51.56	73.44^{def}	53.51	31.20^{abcdef}	34.71^{cdef}
0.62	0.37	79.52^{abcde}	52.67	75.69^{bcde}	50.50	29.04^{bcdef}	38.30^{bcd}
0.62	0.44	71.25^{f}	54.55	65.87^{h}	49.72	41.19^{a}	43.52^{b}
0.78	0.23	77.53^{bcdef}	59.58	75.82^{bcde}	60.91	39.83^{ab}	27.64^{fghi}
0.78	0.30	80.47^{abcde}	56.24	78.52^{abcd}	54.97	35.83^{abcd}	31.14^{defgh}
0.78	0.37	82.64^{abc}	54.87	80.01^{abcd}	49.21	31.90^{abcdef}	30.99^{defgh}
0.78	0.44	82.07^{abcd}	61.22	78.05^{abcd}	58.23	32.53^{abcde}	37.21^{bcde}
0.94	0.23	82.04^{abcd}	62.66	80.61^{ab}	57.71	39.24^{ab}	20.81^{i}
0.94	0.30	82.54^{abcd}	55.79	81.84^{a}	56.86	38.10^{abc}	25.19^{hi}
0.94	0.37	84.10^{ab}	64.95	82.34^{a}	58.45	33.81^{abcde}	26.94^{ghi}
0.94	0.44	84.80^{a}	52.64	81.98^{a}	51.62	33.31^{abcde}	29.58^{efgh}
Pooled SEM		1.34	5.10	1.11	4.82	2.23	1.56
Source of variation				----------*P*-values----------			
Ca		<0.001	0.003	<0.001	0.025	<0.001	<0.001
nPP		0.009	0.924	<0.001	0.289	0.045	<0.001
Ca*nPP		0.002	0.318	<0.001	0.502	0.009	0.001

[a,b,c,d,e,f,g,h,i] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

Bone Mineralization

The evaluation of tibia ash percentage at 35 days showed a linear effect ($P \leq 0.01$) of Ca and nPP dietary levels in the grower phase for both males and females (Tables III-8 and III-9). The maximum tibia ash percentage was approximately 46% for females, when diets contained levels of Ca higher than 0.80% and nPP levels higher than 0.35% (Figure III-6 A). For males (Figure III-6 B), the highest tibia ash percentage (about 45%) was obtained at levels of Ca and nPP higher than 0.90% and 0.40%, respectively. This is in agreement with Gomes et al. (2004), who demonstrated that tibia ash percentage at 42 days increased as levels of aP increased from 0.15 to 0.42% in Hubbard broiler diets from 22 to 42 days, and further increments in aP kept increasing tibia ash percentage only in females. Tibia ash percentage was also affected by Ca levels in broiler diets for the same period (22 to 42 days), as stated by Sá et al. (2004). These researchers showed that the concentration of ash increased quadratically as levels of Ca increased, and the maximum ash percentage was estimated at 1.01% Ca (with 0.41% aP). In contrast, Cardoso Jr. et al. (2010) reported that the levels of Ca and aP could be reduced to as low as 0.65% Ca and 0.325% aP without significant negative effects on tibia ash percentage at 35 days, when compared to the results of the control diet (0.85% Ca and 0.425% aP). Yan et al. (2005) showed that tibia ash increased quadratically as levels of nPP were increased from 0.10 to 0.45% (0.90% Ca) in grower diets (21 to 42 days), and that the point of inflection was observed at 0.33% nPP.

Driver et al. (2005) reported that the levels of Ca and P fed in the starter diet (1 to 18 days) did not affect tibia ash at the end of the grower period (35 days). In their experiment, tibia ash percentage decreased when levels used in the grower diet were reduced from 0.80%

Ca and 0.67% tP to 0.30% Ca and 0.37% tP, independently of the Ca and tP levels fed previously. Within the same treatment during the grower phase, there were no changes in tibia ash percentage whether the dietary levels of Ca and tP were increased or not.

Bone densitometry of shanks (BMC and BMD) at 49 days were only affected by treatments on males, as shown in Tables III-8 and III-9. There was a linear effect ($P \leq 0.01$) of nPP levels fed in the grower diet. Both BMC and BMD increased as dietary nPP increased (Figures III-7 A and B). Onyango et al. (2003) reported that BMC, BMD, and tibia ash percentage increased linearly as the level of Ca and tP increased from 0.45 to 0.91% and 0.37 to 0.72%, respectively. However, the researchers noted no differences in bone breaking strength, measured by the shear force.

Table III-8. Response surface coefficients and predicted optimal values for the effects of dietary Ca and nPP levels in grower diets (18-35 days) on tibia ash at 35 days, and bone densitometry (BMC and BMD) of shanks at 49 days for male and female Heritage broilers.

	Tibia ash		BMC		BMD	
	Females	Males	Females	Males	Females	Males
Intercept	33.41**	39.07**	0.17**	0.21**	0.166**	0.214**
Ca	7.71**	2.46**	0.09	-0.09	0.090	-0.091
nPP	37.78**	7.84**	0.17	-0.11**	0.173	-0.106**
Ca x Ca	-6.99	-3.72	-0.06	0.03	-0.059	0.034
Ca x nPP	17.21	16.90	-0.04	0.12	-0.036	0.117
nPP x nPP	-63.73	-19.53	-0.24	0.09	-0.238	0.092
BW	-	-	0.012**	0.032**	0.012**	0.032**

*$P \leq 0.05$, **$P \leq 0.01$

Table III-9. Effects of dietary Ca and nPP levels in grower diets (18-35 days) on tibia ash at 35 days, and bone densitometry (BMC and BMD) of shanks at 49 days for male and female Heritage broilers.

Ca	nPP	Tibia ash		BMC		BMD	
		Females	Males	Females	Males	Females	Males
-----%-----		--------%--------		--------g--------		--------g/cm^2--------	
0.46		43.25^{b}	42.19^{b}	4.747	6.85	0.251	0.281
0.62		44.17^{ab}	43.42^{ab}	4.683	6.69	0.251	0.277
0.78		44.80^{a}	43.02^{ab}	4.751	6.70	0.254	0.278
0.94		45.00^{a}	43.87^{a}	4.622	6.73	0.248	0.278
Pooled SEM		0.44	0.49	0.056	0.08	0.001	0.002
	0.23	43.12^{b}	42.14^{b}	4.696	6.57^{b}	0.251	0.275
	0.30	44.74^{a}	43.61^{a}	4.725	6.72^{ab}	0.252	0.277
	0.37	44.49^{a}	42.84^{ab}	4.739	6.78^{ab}	0.253	0.279
	0.44	44.86^{a}	43.92^{a}	4.643	6.89^{ab}	0.248	0.283
Pooled SEM		0.44	0.49	0.056	0.08	0.001	0.002
0.46	0.23	43.64	42.39	4.694	6.67	0.248	0.281
0.46	0.30	42.87	41.80	4.808	6.72	0.256	0.276
0.46	0.37	42.77	42.03	4.774	6.98	0.251	0.284
0.46	0.44	43.72	42.56	4.713	7.01	0.249	0.281
0.62	0.23	42.38	42.79	4.677	6.37	0.253	0.274
0.62	0.30	44.78	43.34	4.673	6.72	0.249	0.275
0.62	0.37	45.58	42.34	4.760	6.76	0.256	0.277
0.62	0.44	43.92	45.23	4.622	6.92	0.247	0.283
0.78	0.23	42.79	41.62	4.815	6.63	0.255	0.274
0.78	0.30	45.48	44.20	4.633	6.65	0.250	0.280
0.78	0.37	45.01	43.24	4.848	6.85	0.259	0.281
0.78	0.44	45.93	43.02	4.709	6.67	0.251	0.274
0.94	0.23	43.68	41.75	4.599	6.62	0.249	0.271
0.94	0.30	45.82	45.09	4.785	6.79	0.252	0.276
0.94	0.37	44.63	43.75	4.574	6.54	0.245	0.274
0.94	0.44	45.88	44.87	4.529	6.98	0.245	0.292
Pooled SEM		0.76	0.77	0.105	0.16	0.003	0.004
Source of variation		----------*P*-values----------					
Ca		0.005	0.012	0.229	0.518	0.074	0.729
nPP		0.004	0.004	0.560	0.053	0.230	0.071
Ca*nPP		0.108	0.090	0.718	0.625	0.284	0.162
BW		-	-	<0.001	<0.001	<0.010	<0.001

[a,b] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

Bone Breaking Strength

In the bone strength evaluation by 3-point bending test using tibiae collected at 35 days, the maximum force needed to break the tibias (N) was linearly affected ($P \leq 0.01$) by Ca in males and quadratically ($P \leq 0.05$) in females (Tables III-10 and III-11). Figures III-8 A and B demonstrates that for both sexes, force increased with increments in dietary Ca up to its maximum level (0.94%). In earlier studies testing Ca levels from 1.00 to 1.40% and aP from 0.32 to 0.51% from 22 to 42 days, Hulan et al. (1985) reported a positive response in tibia strength at 42 days with increments in the levels of Ca and aP, and suggested that it was due to dietary Ca rather than aP. The results of the present experiment are in accordance with the findings of Sá et al. (2004), who demonstrated that the maximum resistance of tibia to breakage at 42 days was estimated at 1.28% Ca (with 0.41% aP), when levels of Ca varying from 0.16 to 1.41% were tested in broiler diets from 22 to 42 days of age. In a trial testing combinations of dietary Ca and tP levels for broiler chickens from 14 to 39 days, Ziaei et al. (2008) reported that reducing mineral content below 0.73% Ca and 0.55% tP impaired bone strength at 21 and 39 days. Moreover, the authors did not detect deleterious effects on bone strength when the levels were reduced from 0.83% Ca and 0.66% tP to 0.70% Ca and 0.55% tP.

In addition to the force needed to break the bones, another measurement was performed: shear stress (MPa), which is a measure of the average force per unit area of a surface within the body on which internal forces act. In male tibiae, the stress was increased linearly ($P \leq 0.01$) as Ca levels increased in the diet (Tables III-10 and III-11), and the lowest shear stress value was observed with the lowest Ca levels combined with the highest nPP

(Figure III-9 A). In females, the shear stress was affected by an interaction ($P \leq 0.01$) of Ca and nPP, and the maximum shear stress was observed at the highest Ca levels combined with nPP levels between 0.35 and 0.40% (Figure III-9 B). According to Gomes et al. (2004), the resistance of bones to breakage was quadratically affected by aP levels in the grower diet (22 to 42 days), improving as aP increased from 0.15 to 0.42% and being negatively affected by further increments (0.51 and 0.60%) in aP.

The evaluation of bone breakage during mechanical deboning using thighs collected at 49 days showed that the incidence of femur epiphyseal breaks was affected by the interaction ($P \leq 0.05$) of Ca and nPP fed during the grower period (Tables III-12 and III-13). The probability of incidence of epiphyseal breaks was the highest (around 35%) in two situations: when Ca was lower than 0.50% combined with nPP higher than 0.40%; or when Ca was higher than 0.90% combined with nPP lower than 0.25%. The lowest probability (around 5%) of incidence of femur epiphyseal breaks was observed when Ca was higher than 0.80% and nPP higher than 0.40% in the grower diet. Driver et al. (2006) experimented different levels of Ca and tP in the starter (1 to 18 days) and grower (19 to 35 days) diets, reporting that broiler chickens fed low Ca and P diets during the starter (0.60% Ca and 0.47% tP) and grower (0.30% Ca and 0.37% tP) phases had higher broken tibia incidence than birds that received high Ca and P diets during both phases (0.90% Ca with 0.68% tP and 0.80% Ca with 0.67% tP, respectively). However, when diets were low in Ca and tP only in one of the dietary phases, no negative effects were observed on tibia breaking strength. When the parameter evaluated was the incidence of broken clavicles and bloody pectoralis minor, the levels fed during the grower phase had the most important impact.

Table III-10. Response surface regression coefficients for the effects of dietary Ca and nPP levels in grower diets (18-35 days) on tibia breaking strength (force and shear stress) at 35 days.

	Force		Shear stress	
	Females	Males	Females	Males
Intercept	-12.60**	-11.23**	18.95**	41.19**
Ca	333.70**	104.82**	7.54**	-9.46**
nPP	231.90	1123.02	189.91	89.22
Ca x Ca	-217.58*	-30.17	-33.52	12.68
Ca x nPP	302.13	198.95	201.06**	15.85
nPP x nPP	-702.44	-1889.02	-506.90**	-170.90

*$P \leq 0.05$, **$P \leq 0.01$

Table III-11. Effects of dietary Ca and nPP levels in grower diets (18-35 days) on tibia breaking strength (force and stress) at 35 days.

Ca	nPP	Force		Shear stress	
		Females	Males	Females	Males
-----%-----		----------N----------		----------MPa----------	
0.46		135[c]	212[b]	50.30[c]	52.25
0.62		170[b]	240[ab]	54.93[bc]	52.57
0.78		186[ab]	254[a]	61.60[ab]	55.74
0.94		200[a]	277[a]	63.02[a]	58.16
Pooled SEM		6	11	2.03	1.85
	0.23	171	237	55.64	55.46
	0.30	181	252	61.05	54.92
	0.37	171	256	58.90	56.43
	0.44	168	237	54.27	51.89
Pooled SEM		6	11	2.03	1.86
0.46	0.23	152	224	55.93	54.56
0.46	0.30	131	201	53.79	49.85
0.46	0.37	138	215	49.98	60.85
0.46	0.44	119	207	41.48	43.75
0.62	0.23	163	228	50.82	52.37
0.62	0.30	185	248	61.17	55.34
0.62	0.37	166	230	58.14	48.22
0.62	0.44	166	253	49.60	54.33
0.78	0.23	173	253	60.03	55.89
0.78	0.30	207	268	61.39	54.01
0.78	0.37	175	268	64.89	56.19
0.78	0.44	189	226	60.09	56.86
0.94	0.23	194	242	55.78	59.03
0.94	0.30	202	292	67.82	60.49
0.94	0.37	203	311	62.58	60.48
0.94	0.44	199	261	65.90	52.63
Pooled SEM		11	21	4.03	3.78
Source of variation				----------*P*-values----------	
Ca		<0.001	0.001	<0.001	0.104
nPP		0.278	0.455	0.076	0.387
Ca*nPP		0.293	0.572	0.225	0.251

[a,b,c] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

Leg Abnormalities, Tibial Dyschondroplasia, and Walking Ability

The prevalence of crooked toes at 49 days was affected by an interaction ($P \leq 0.05$) of Ca and nPP levels fed during the grower phase, and was higher ($P \leq 0.01$) for males than for females (Tables III-12 and III-13). At low nPP levels, the probability of incidence of crooked toes increased with increments in Ca levels in the grower feed. As levels of nPP increased, the probability of incidence of crooked toes was augmented, and less influence of Ca was observed. When using levels of Ca below 0.50% and nPP below 0.25%, there was a reduction in the probability of incidence of crooked toes.

The prevalence of severe valgus was quadratically affected ($P \leq 0.05$) by nPP levels, and was higher ($P \leq 0.01$) in males (Tables III-12 and III-13). The probability of incidence of this condition increased as levels of nPP increased up to 0.33%, and was maximized in combination with Ca between 0.60 to 0.80%. It was observed that with wider Ca:nPP ratios, by both using high levels of Ca combined with low nPP or high nPP and low Ca, the probability of incidence of severe valgus was reduced. It may have occurred due to the effects of BW, since birds with wider Ca:nPP ratios had lower BWG, and consequently less stress on their legs. Driver et al. (2005) reported no effects of treatments on valgus incidence at 35 days when chickens were fed Ca and tP deficient or adequate diets in the starter phase and during the grower phase levels of 0.30% Ca with 0.37% tP or 0.80% Ca with 0.37% tP.

The TD incidence at 35 days was quadratically affected ($P \leq 0.05$) by nPP levels (Tables III-12 and III-13), reaching its maximum (50-60%) when levels of nPP between 0.30 to 0.35% were fed in grower diets, regardless of the levels of Ca used. Broilers fed diets containing the lowest level of nPP had less probability of developing TD at 35 days. At 49

days, TD incidence tended to be affected ($P = 0.07$) by an interaction of Ca and nPP levels in the grower feed. The highest probability of incidence of TD was estimated at the maximum Ca and nPP levels used in the grower diets, and it was reduced by using levels of Ca higher than 0.70% and nPP lower than 0.30%. Hulan et al. (1985) described that from 22 to 42 days of age, the optimum BWG, FCR and tibia strength were obtained when the highest levels of Ca plus P were fed, but these levels were associated with the highest incidence of TD and total leg abnormalities. No significant effects of treatments were observed on TD severity (score 3) at 35 and 49 days and walking ability at 49 days.

Table III-12. Response surface regression coefficients for the effects of dietary Ca and nPP levels in grower diets (18-35 days) on the probability of incidence of leg abnormalities and femur breakage during mechanical deboning at 49 days, and TD at 35 days.

	Type of leg abnormality		TD Incidence	Mechanical deboning
	Severe valgus	Crooked toes		Epiphyseal breakage
Intercept	-8.69	-6.57*	-9.05	-1.66
Ca	6.69	8.54*	-7.42	-0.39
nPP	25.50	14.57	70.08*	6.96
Ca x Ca	13.98	-10.34*	15.93	-23.21*
Ca x nPP	-7.62	-3.20	1.15	4.93
nPP x nPP	-54.95*	-8.19	-118.85**	8.88
Sex (female)	-0.78**	-0.95**	0.21	-0.43

*$P \leq 0.05$,**$P \leq 0.01$

Table III-13. Effects of dietary Ca and nPP levels in grower diets (18-35 days) on the probability of incidence of leg abnormalities and femur breakage during mechanical deboning at 49 days, and TD at 35 days.

Ca	nPP	Type of leg abnormality		TD Incidence
		Severe valgus	Crooked toes	
-----%-----		----------Probability----------		
0.46		2.52	16.72	42.78
0.62		5.80	26.27	43.01
0.78		4.31	21.25	36.67
0.94		4.56	24.23	39.85
Pooled SEM		1.51	3.49	11.20
	0.23	3.44	17.36	24.32
	0.30	7.93	22.57	53.77
	0.37	2.86	22.98	58.94
	0.44	3.67	25.25	28.75
Pooled SEM		1.54	3.50	10.22
0.46	0.23	3.79	7.42	23.80
0.46	0.30	5.56	16.15	75.50
0.46	0.37	0.80	19.20	50.00
0.46	0.44	2.35	30.66	24.50
0.62	0.23	3.96	25.27	24.50
0.62	0.30	11.64	27.92	50.00
0.62	0.37	5.88	26.02	87.90
0.62	0.44	4.07	25.93	12.10
0.78	0.23	4.04	24.13	24.50
0.78	0.30	9.35	19.52	50.00
0.78	0.37	2.34	22.29	36.88
0.78	0.44	3.82	19.30	37.24
0.94	0.23	2.32	18.44	24.50
0.94	0.30	6.43	28.49	37.24
0.94	0.37	5.81	24.85	50.00
0.94	0.44	4.91	26.00	50.00
Pooled SEM		2.28	4.91	18.51
Source of variation		----------*P*-values----------		
Ca		0.165	0.007	0.975
nPP		0.002	0.051	0.063
Ca*nPP		0.660	0.027	0.654
Sex		0.001	<0.001	0.555

Conclusions

The data presented herein indicated that optimum growth performance of Heritage broilers was achieved with dietary Ca between 0.75 and 0.90% combined with 0.41 to 0.44% nPP during the grower phase. Bone mineralization and strength were maximized with the highest Ca and nPP levels used in grower diets (0.94% and 0.44%, respectively); however, these high levels were related to increased incidence of bone and leg problems at 49 days. The highest retention of Ca and P during the grower phase occurred when chickens were fed diets containing Ca levels between 0.90 and 0.94% combined with 0.33 to 0.35% nPP. No effects of Ca and nPP levels fed in grower diets were observed on broiler walking ability at market age (49 days).

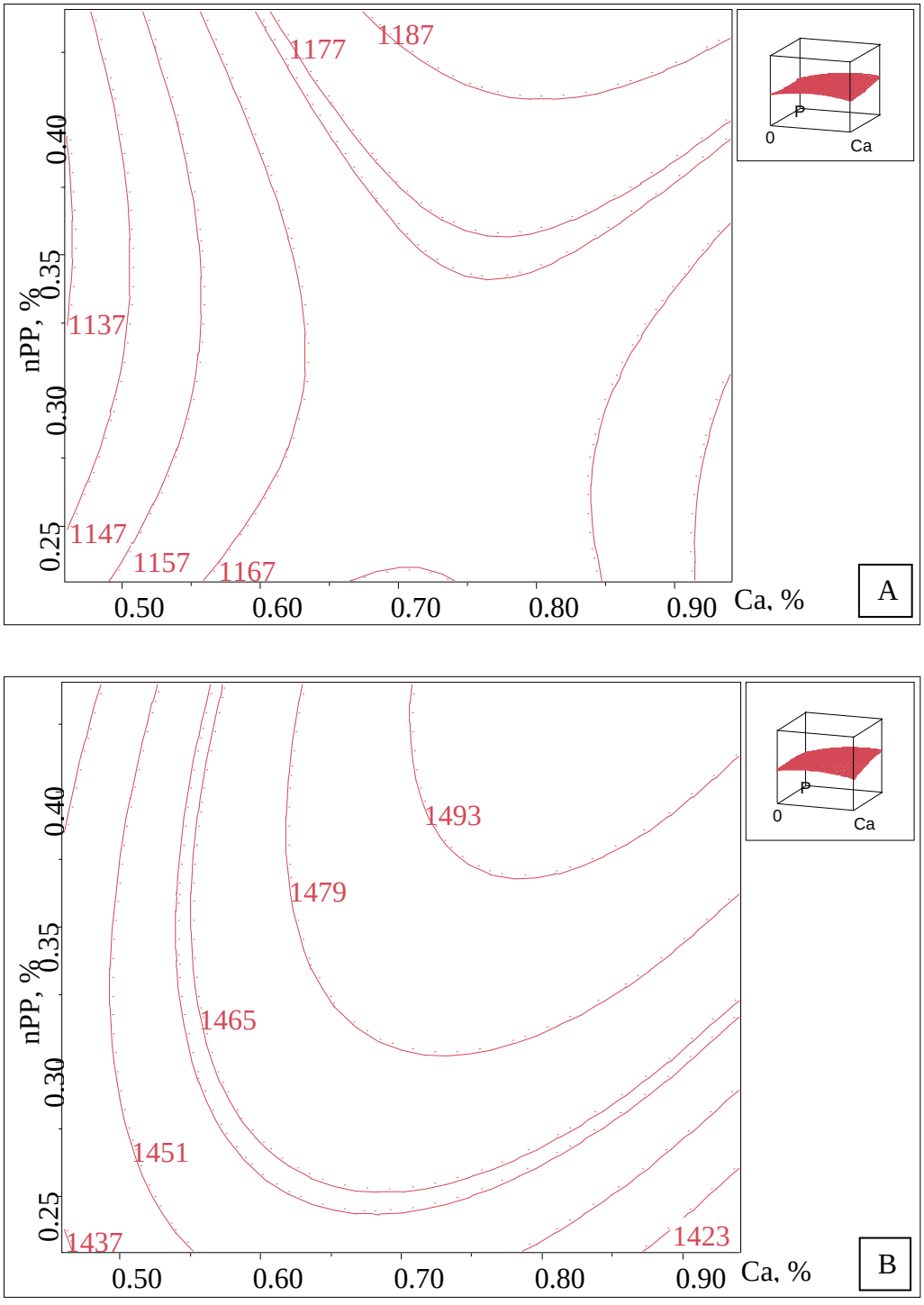


Figure III-1. Response contours of female (A) and male (B) body weight gain (g) from 18 to 35 days with varying dietary levels of Ca and nPP during the grower phase (18-35 days).

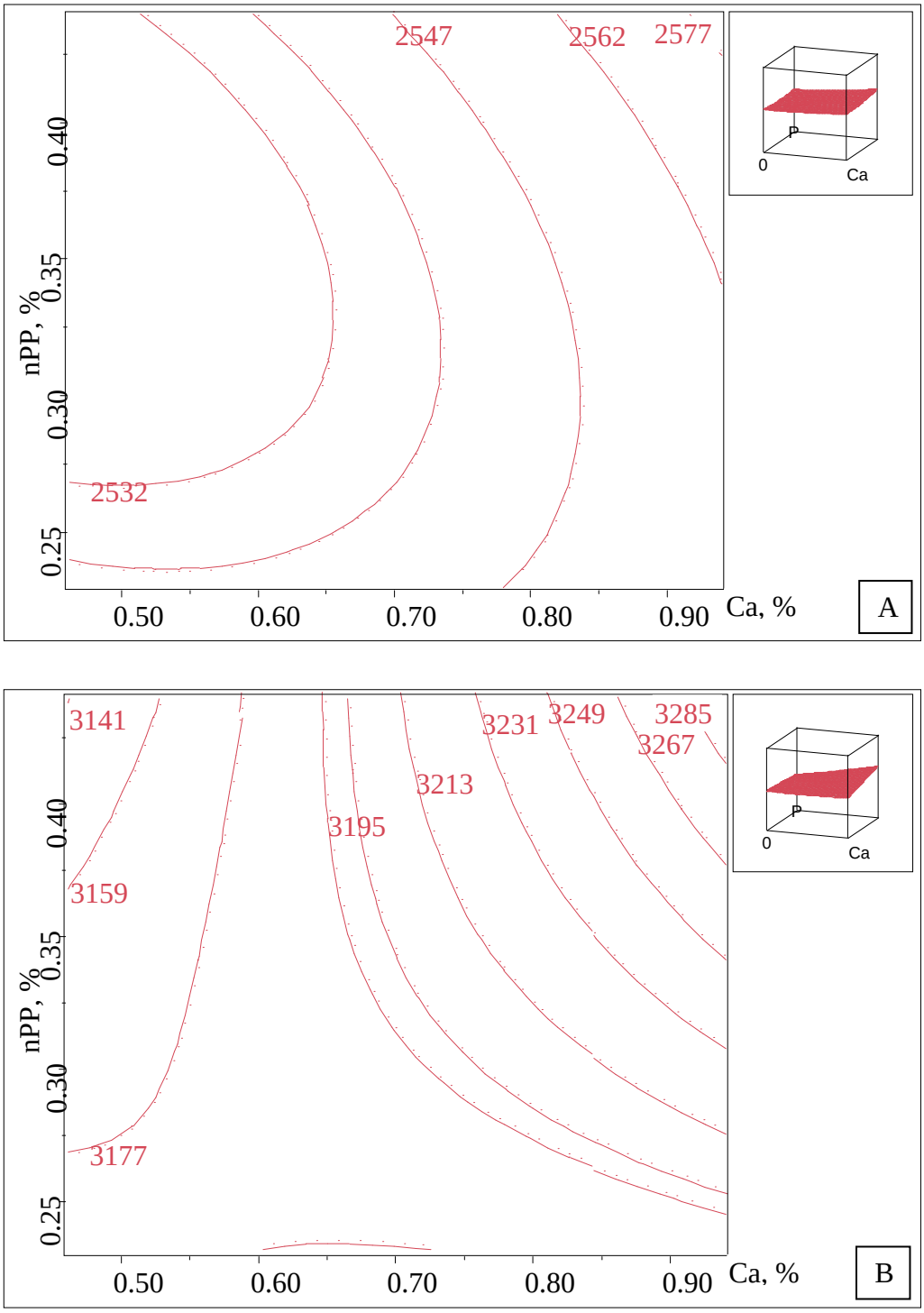


Figure III-2. Response contours of female (A) and male (B) body weight gain (g) from 0 to 49 days with varying dietary levels of Ca and nPP during the grower phase (18-35 days).

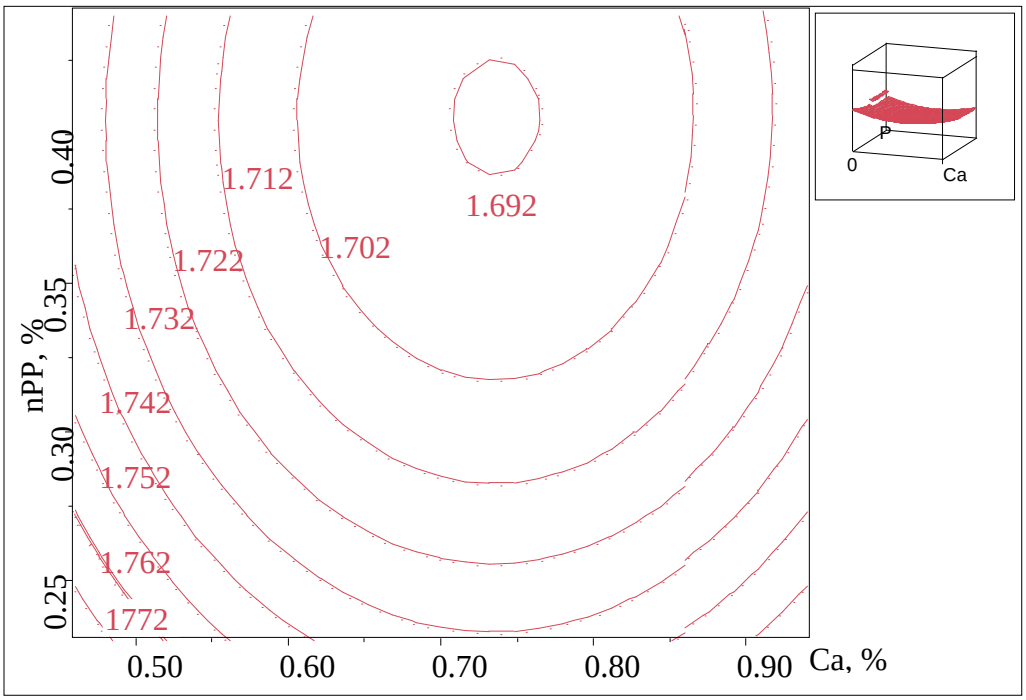
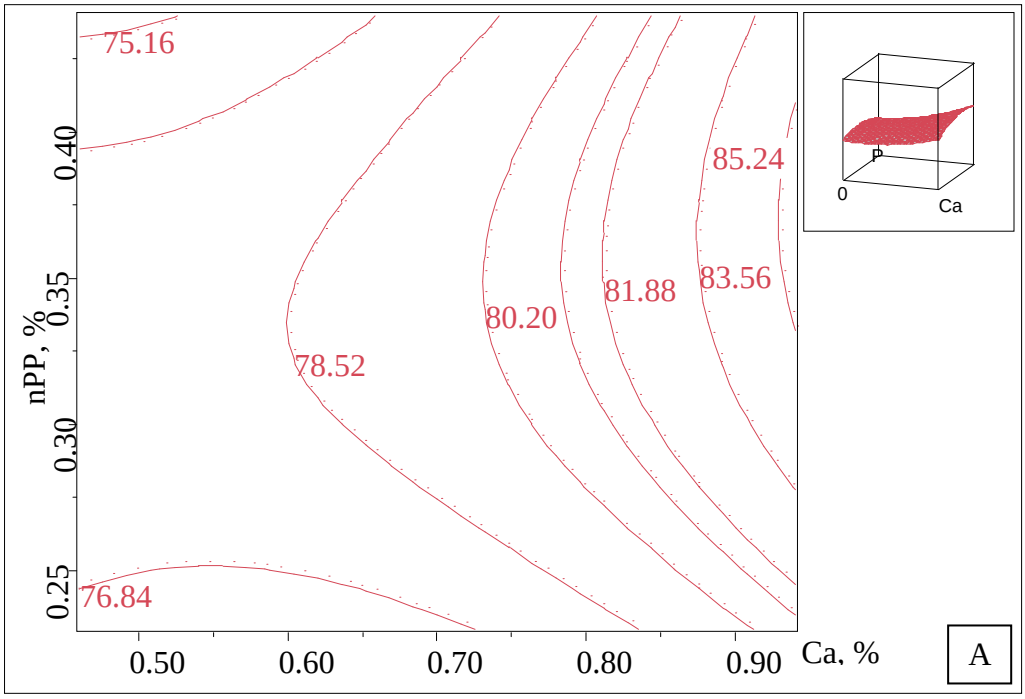


Figure III-3. Response contours of feed conversion ratio from 18 to 35 days with varying dietary levels of Ca and nPP (%) fed during the grower phase (18-35 days).



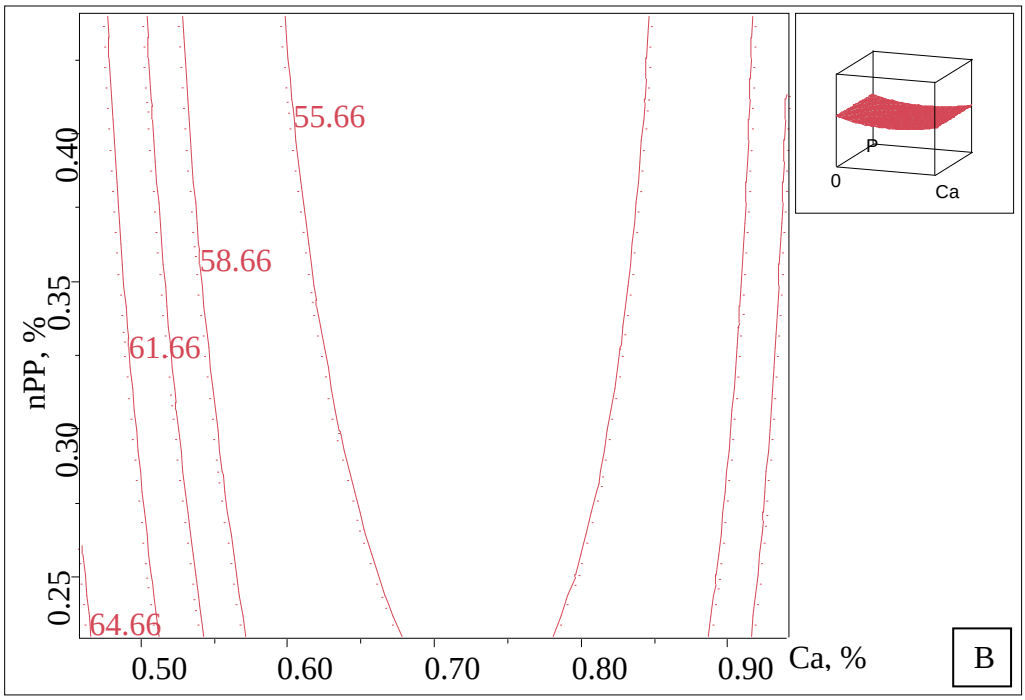


Figure III-4. Response contours of calcium retention (%) at 31 (A) and 44 days (B) with varying dietary levels of Ca and nPP during the grower phase (18-35 days).

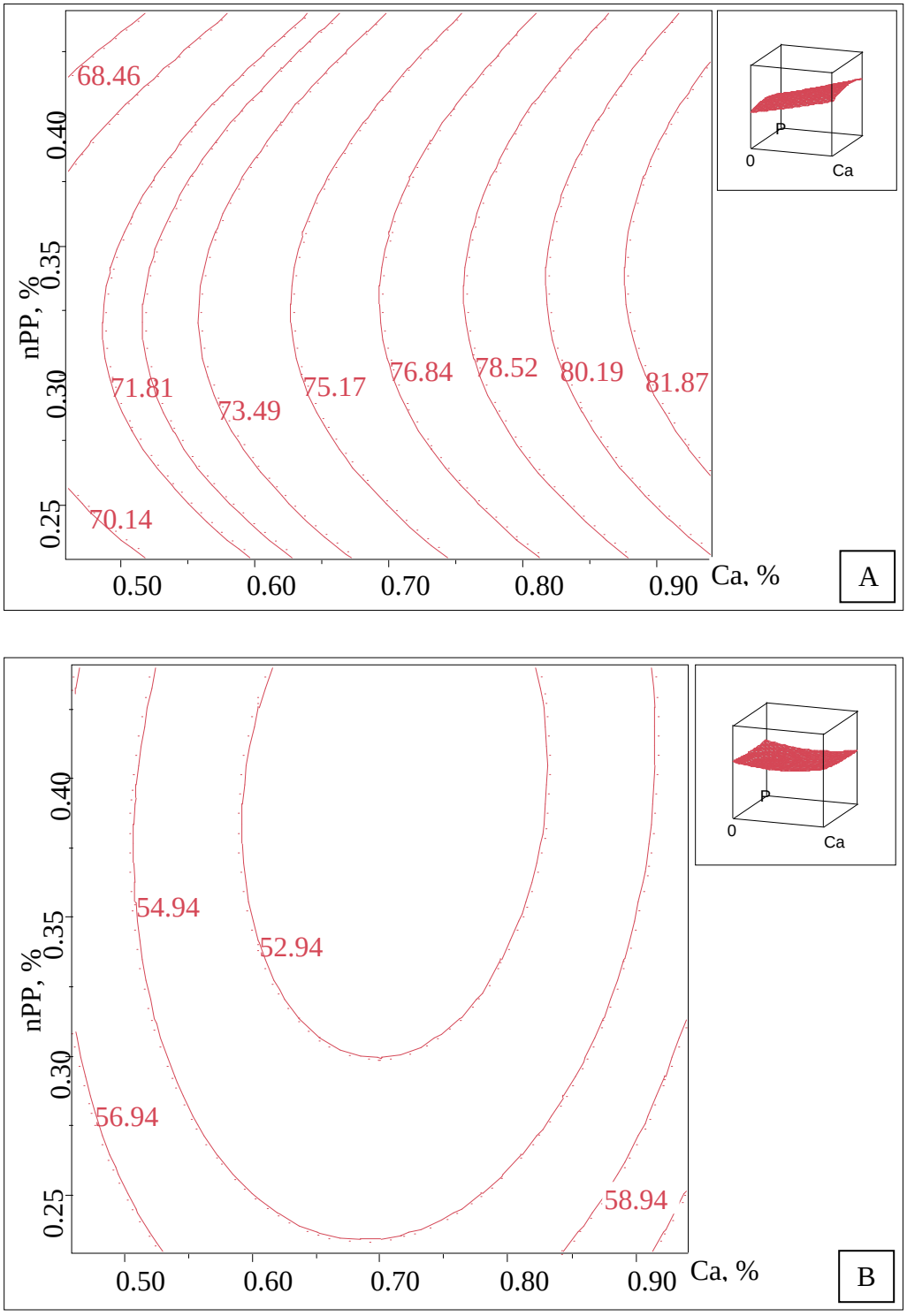


Figure III-5. Response contours of phosphorus retention (%) at 31 (A) and 44 days (B) with varying dietary levels of Ca and nPP during the grower phase (18-35 days).

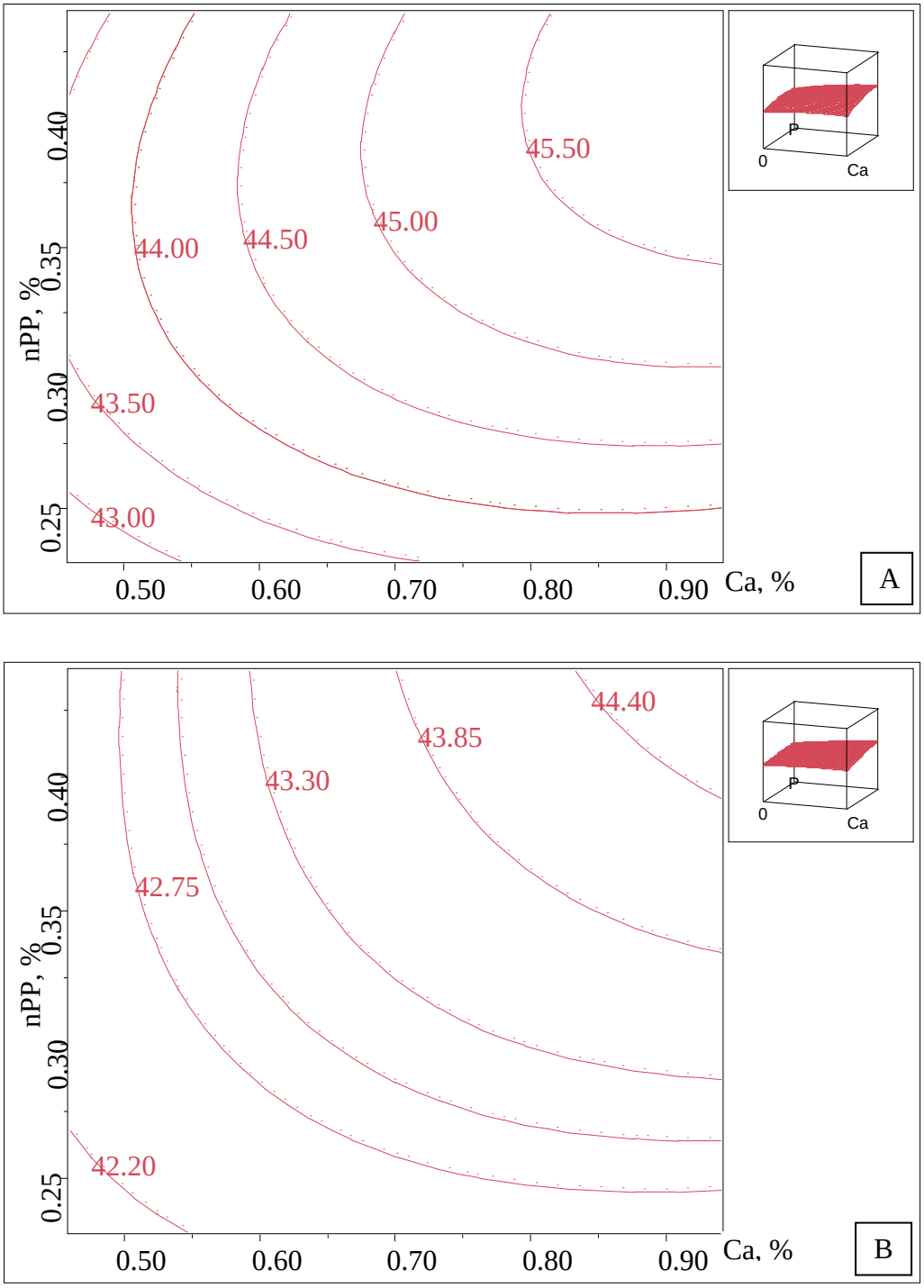


Figure III-6. Response contours of tibia ash (%) at 35 days for females (A) and males (B) with varying dietary levels of Ca and nPP during the grower phase (18-35 days).

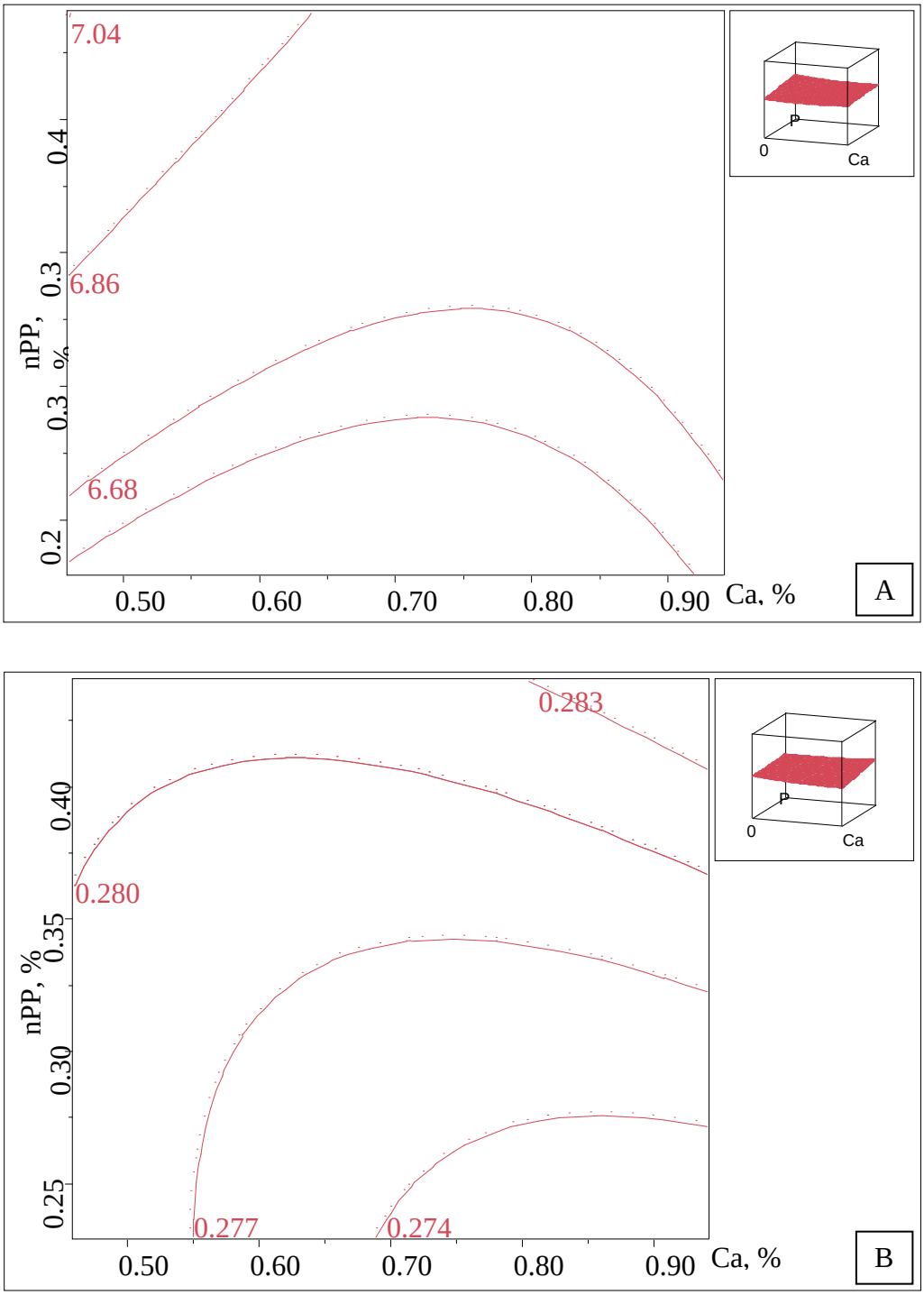


Figure III-7. Response contours of (A) bone mineral content (g) and (B) bone mineral density (g/cm2) of male shanks at 49 days with varying dietary levels of Ca and nPP during the grower phase (18-35 days).

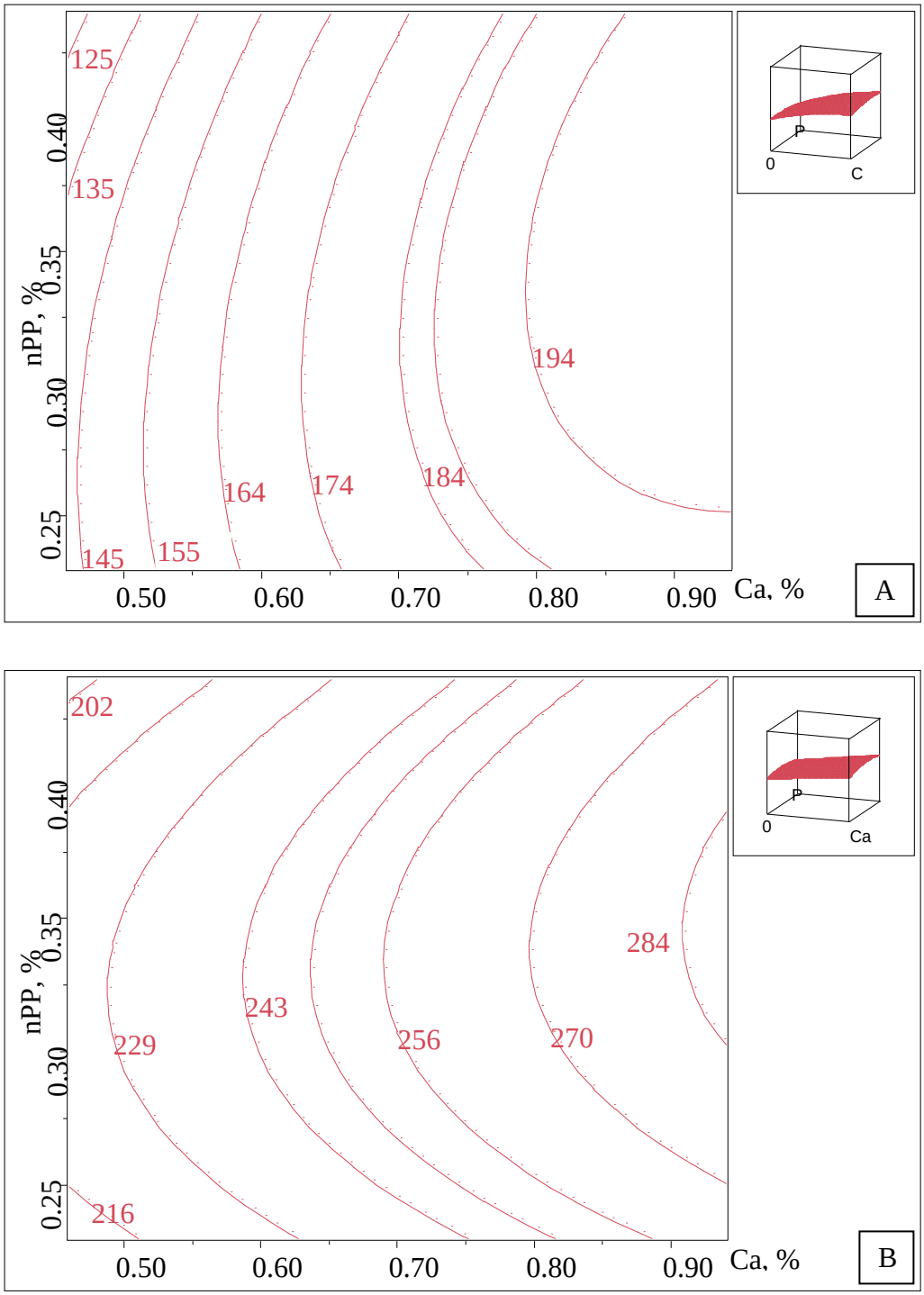


Figure III-8. Response contours of tibia breaking strength – maximum force (N) for females (A) and males (B) with varying dietary levels of Ca and nPP during the grower phase (18-35 days).

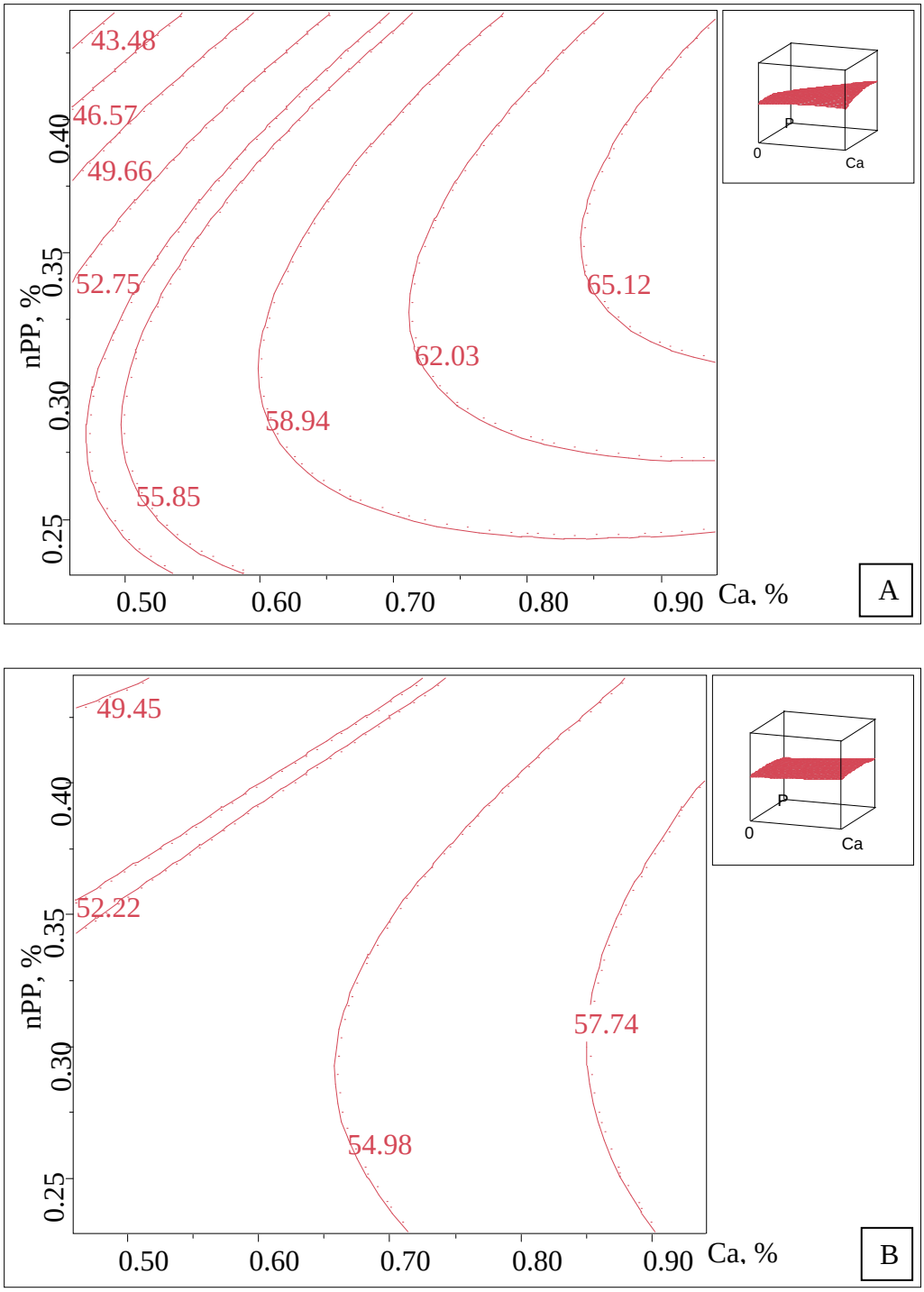


Figure III-9. Response contours of tibia breaking strength – shear stress (MPa) for females (A) and males (B) with varying dietary levels of Ca and nPP during the grower phase (18-35 days).

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CHAPTER IV

Effects of Calcium and Phosphorus Levels in Finisher Diets on Heritage Broiler Performance, Mineral Retention, Bone Characteristics, and Leg Abnormalities

ABSTRACT

The objective of this experiment was to determine the impact of different Ca and nPP dietary levels during the finisher phase (36 to 49 days) on Heritage broiler performance, mineral retention, bone mineralization and strength, and the incidence of leg abnormalities. There were 6 replicate pens per treatment, each pen containing 8 male and 8 female day-old chicks individually identified and randomly assigned. All diets were corn-soybean meal based, and celite was added as a marker in the finisher diet. Common starter and grower diets were fed from 1 to 17 and 18 to 35 days, respectively. Broilers consumed the treatment diets from 36 to 49 days of age, formulated to contain combinations of 4 levels of Ca (0.38, 0.54, 0.70, and 0.86%) and 4 levels of nPP (0.18, 0.26, 0.34, and 0.42%). BW gain, FI, and FCR were assessed at the end of each dietary phase, and all chickens were individually weighed at 49 days to determine flock uniformity. At 44 days, fresh fecal samples were collected for Ca and P retention analysis. Data were analyzed within a RCBD by response surface methodology. During the finisher phase, BW gain and mortality were not affected by the treatments, but FI decreased linearly ($P \leq 0.01$) as nPP dietary levels increased. FCR from 36 to 49 days had an interaction effect ($P \leq 0.05$) of Ca and nPP. The FCR for the whole grow-out had a tendency to be quadratically affected ($P = 0.089$) by nPP levels used during the finisher phase, and the optimum FCR was estimated at 0.65% Ca and 0.29% nPP. Female uniformity at 49 days was affected by an interaction ($P = 0.056$) of Ca and nPP levels. A linear increment ($P \leq 0.05$) in tibia ash was observed as dietary Ca increased in males and as nPP increased in females. BMC was affected quadratically ($P \leq 0.05$) by Ca in females and linearly ($P \leq 0.05$) in the

males. Male BMD increased linearly ($P \leq 0.01$) as Ca levels increased in the finisher diet. In the females, BMD had an interaction effect ($P \leq 0.05$) of Ca and nPP, increasing as dietary Ca and nPP levels increased. Tibia breaking strength in males had a linear increase ($P \leq 0.01$) as Ca was increased in the diet. The probability of TD incidence and severity increased linearly ($P \leq 0.05$) with reduction in dietary nPP levels. Ca levels fed during the finisher phase quadratically affected ($P \leq 0.01$) the probability of incidence of leg problems. The probability of incidence of epiphyseal breakages during mechanical deboning tended to be affected quadratically ($P = 0.068$) by nPP levels. In conclusion, the present data demonstrated that Heritage broiler performance, mineral retention, bone mineralization and strength, and leg abnormalities were affected by the levels of Ca and nPP fed during the finisher phase.

Key words: Calcium, phosphorus, finisher, bones, mineral retention

INTRODUCTION

Calcium (Ca) and phosphorus (P) are essential minerals for most metabolic processes, directly affecting broiler performance and bone formation. Disorders in these metabolic functions caused by Ca and P deficiency, excess, or imbalanced ratios have effects on the efficiency of the live production as well as of the processing plant, ultimately impairing the overall profitability of poultry companies. The high cost of inorganic phosphate supplementation and the environmental concerns related to the use of commercial poultry litter for land application as fertilizer impose additional constraints to P utilization in broiler diets (Sharpley, 1999; Takemasa and Takagi, 2001; Cody, 2003). The ability of broilers to

utilize dietary Ca and P depends on their source (Henry and Pesti, 2002; Lima et al., 1995), the interaction with other components of the diet (Wodzinski and Ullah, 1996; Applegate et al., 2003), and the presence of exogenous enzymes in the feed (Qian et al., 1997; Manangi and Coon, 2008). Moreover, it is known that birds are less efficient in retaining minerals as they age (Bar et al., 2003). Since the finisher phase represents approximately 40 to 50% of the feed consumed during the whole grow-out and broilers are less efficient to absorb nutrients during that period, the decisions on Ca and P levels to be used in finisher diets play an important role in reducing the cost of the feed and the P excretion to the environment.

The levels of Ca and P recommended by the main genetic companies for the finisher period, as well as the levels normally used in the US broiler industry, are quite similar to those proposed by the last NRC (1994). However, broilers used currently have improved performance and carcass composition, and are more efficient utilizing nutrients (Havenstein, 2003); thus, the requirements should be reviewed. Angel (2011), in a summary of research reports, suggested that the levels required for both mineralization and performance parameters are lower than those recommended by the genetic companies and the NRC (1994), and used by the industry (Table IV-1).

Table IV-1. Comparison of Ca and P levels for broiler finisher diets as recommended by the NRC and genetic companies, normally used by the US poultry industry, and the requirements determined in scientific reports for performance and mineralization.

Dietary phase	Criteria	NRC[1]	Genetic companies[2]	Average US companies[3]	Top 5 US companies[3]	Research summary[4]
	Perf.					0.83% Ca 0.30% nPP
Finisher 35-42 d		0.90% Ca 0.40% aP	0.85-0.95% Ca 0.40-0.45% aP	0.84% Ca 0.39% aP	0.95% Ca 0.39% aP	
	Miner.					0.82% Ca 0.31% nPP

[1]Adapted from NRC (1994)
[2]Adapted from Hubbard (2007), Cobb 500 (2008), and Ross 708 (2009) nutritional guidelines
[3]Adapted from Agristats (2011)
[4]Adapted from Angel (2011), based on male broiler chickens only

The carryover effect of previous nutrition also influences the levels of Ca and P needed during the finisher phase. There are many reports showing that it is possible to reduce Ca and P concentrations during later stages of broiler life, provided that adequate levels of these minerals were fed in previous dietary phases (Nelson et al., 1990; Skinner et al., 1992a,b; Skinner and Waldroup, 1992; Angel et al., 2000; Fritts and Waldroup, 2006). In addition, Powell (2011) showed that increasing the P levels in starter diets improved performance and bone characteristics in the subsequent dietary phases.

For mitigating the environmental impact of P excretion, as well as reducing the cost of the feed, several researchers and companies have focused on reducing supplemental P in broiler diets. The challenge relies on determining what the ideal levels of Ca and P are, in order to reduce P excretion without harming broiler performance, bone parameters, and leg health. Therefore, the objective of this project was to evaluate the effects of Ca and P dietary

levels used during the finisher phase on the parameters mentioned above for Heritage broilers.

MATERIAL AND METHODS

Birds and Management

The Institutional Animal Care and Use Committee of North Carolina State University approved all practices regarding the animal work, which were conducted at the Poultry Research Unit (Raleigh, NC) during the period from August 10th to September 27th 2010. The experimental house was divided into 6 blocks with 16 pens each, referring to the location within the house. Pens were 1.22 x 1.83 m with a stocking density of approximately 7 birds/m^2 or a final density 21 kg/m^2. Pens were covered with a 15-cm layer of new pine wood shavings to avoid any Ca and P intake from used litter, and equipped with 1 tube feeder and 1 bell-shaped drinker. The house was curtain-sided, with exhaust fans and forced air heating system combined with upward blowing ventilation fans. Broiler chicks, hatched from fertile eggs provided by a local hatchery (Perdue Farms Inc., Candor, NC), were immediately feather-sexed and individually identified with neck tags. A total of 1,536 birds were then weighed in groups of 8 males and 8 females, and 16 birds (one group of each sex) were randomly allocated to the 96 pens. Brooding temperatures at placement were set at 35-36 °C and kept like this for the first night. Temperatures were reduced gradually to 32.2-33.5 °C from 1 to 7 days, 29.4 °C from 8 to 14 days, 26.7 °C from 15 to 21 days, and to ambient temperature thereafter. Observed house temperatures were recorded twice a day in 8 different points within the house, and are shown in Appendix A.

The lighting program consisted of 23 hours of light per day during the first week, 4 hours of darkness per day during the second week, and 16 hours of light per day from 22 to 49 days. During this last lighting period, supplemental light was provided from 10:00 pm to 2:30 am, in order to provide the 16 hours of light. Feeders were shaken once a day until 21 days of age and twice a day thereafter.

Dietary Treatments

All broiler chickens were fed a common starter diet as crumbles from placement to 17 days (0.93% Ca and 0.45% nPP) and a common grower diet as pellets from 18 to 35 days (0.75% Ca and 0.37% nPP). Dietary ingredients and nutrient composition used throughout the experiment are described in Table IV-2. The finisher treatment diets were provided *ad libitum* as pellets from 36 to 49 days of age, manufactured from a corn-soybean meal basal diet in which limestone, dicalcium phosphate, and washed sand were added to obtain the dietary treatments. Celite was added as an acid insoluble ash marker to the treatment diets at 1%, for further calculation of mineral retention. The 16 treatments were formulated to contain combinations of 4 levels of Ca (0.38, 0.54, 0.70, and 0.86%) and 4 levels of nPP (0.18, 0.26, 0.34, and 0.42%). To simulate commercial conditions, phytase was added to the starter and grower diets; however, the finisher diet did not contain this enzyme to avoid mixing or pelleting variability and effects on enzyme activity among the treatments. Each treatment had 1 pen randomly assigned within each block, with a total of 6 replicate pens per treatment. Feed and water were provided for *ad libitum* consumption during the whole grow-out; however, the amount of feed per dietary phase was limited to approximately 900 g for

the starter and 2,700 g for the grower phase, in order to mimic the feeding scheme normally used by poultry companies. In the days before each new period (16, and 34 days of age), feed was adjusted for the estimated feed intake of the mortality, so all birds within pens had access to approximately the same amount of feed calculated for each dietary phase.

Data Collection

Feed samples from each diet were collected after pelleting/crumbling-cooling process and analyzed for Ca, tP, crude protein and acid insoluble ash (AIA). Group body weights (BW) per sex were obtained at hatch and 18 days, and birds were individually weighed at 35 and 49 days. The FI per pen was documented at the end of the dietary phases (18, 35, and 49 days), and mortality BW was registered daily to adjust the feed conversion ratio (FCR). BW gain (BWG), FI, and FCR were calculated for the finisher period (36 to 49 days) and for the whole grow-out (1 to 49 days). The individual BW at 35 days were used to perform a statistical analysis in order to confirm that there were no significant differences among pens and treatments. When needed, birds were transferred between pens, and finally all pens were adjusted to have only 7 males and 7 females before the dietary treatments were offered. At 49 days the prevalence of leg abnormalities (crooked toes, valgus, varus, and twisted legs) was evaluated along with the individual BW, which were used to assess flock uniformity as the coefficient of variation (CV%). Approximately 100 g of fresh fecal samples per pen were collected at 44 days, frozen overnight, and then freeze-dried (Virtis Freezemobile - Model 12XL, Warminster, PA) for further acid insoluble ash determination and Ca and P analysis.

Moreover, at 49 days, 2 males and 2 females per pen were randomly selected, euthanized, and legs were collected.

Analytical Methods

Legs collected at 49 days were used to perform several evaluations. The drumsticks were manually deboned, remaining tibiae measured (length and diameters), wrapped in cheese-cloth, and then kept frozen in bags at -20°C to keep bones moist for further analyses. Tibiae were thawed by leaving them inside the plastic bags at room temperature for 6 h, and the 3-point bending test was then performed to evaluate bone strength as the maximum force needed to break the bones (N) and the shear stress (MPa). Bones were sheared midshaft using a crosshead speed of 30 mm/min (Crenshaw et al., 1981). Afterwards, tibiae were evaluated for the incidence and severity of tibial dyschondroplasia (TD) according to the TD scoring system developed by Edwards and Veltmann (1983), and bone ash percentage determined (Hall et al., 2003). The resistance of bones to automatic deboning forces was tested with the thighs by using a mechanical deboning machine (Meyn-D40, Oostzaan, The Netherlands). Shanks were used for the determination of bone mineral content (BMC) and bone mineral density (BMD) by Dual Energy X-ray Absorptiometry (DEXA) at the USDA-ARS in Beltsville, MD. The AIA analysis was performed by weighing 5 g of feed or 3 g of freeze-dried feces in pre-weighed porcelain crucibles, and digesting the samples in 50 mL of acid (4 *N* HCl) on a hot plate at approximately 95°C for 45 minutes. The solution was filtered using a 125 mm hardened ashless filter paper (Whatman, International Ltd, Maidstone, England) dried at 70°C for 4 hours, and ashed at 600°C overnight in a muffle furnace. The ash weight

was then used to calculate the percent AIA of the samples (procedure adapted from Scott and Boldaji, 1997). For Ca and P analysis, 2.5 g of feed or fecal samples were weighed into pre-weighed porcelain crucibles and ashed at 600°C overnight. Ash contents were digested in 4 mL of acid (6 N HCl) on a hot plate at approximately 100°C until fully evaporated. Samples were then resuspended in 8mL 6 N HCl, rinsed into 100mL volumetric flasks, and filled to volume with deionized water. Flasks were covered with parafilm, vigorously agitated, and 13 mL of the suspension was filtered into 15mL conical centrifuge tubes using 125 mm hardened ashless filter paper (Whatman, International Ltd, Maidstone, England) to remove particulates (procedure adapted from Leske and Coon, 2002). Inductively-Coupled Plasma Optical Emission Spectroscopy (Perkin Elmer 2000 DV ICP-OES, Waltham, MA) was used to determine Ca and P concentrations from each sample, and corrected to a corn-based standard and a negative control. Ca and P retention were calculated using the formula exemplified by Brenes et al. (2003), as follows: 1 - [(AIA concentration in feed / AIA concentration in excreta) × (Mineral concentration in excreta / Mineral concentration in feed)].

Data Analysis

Data were analyzed using the response surface methodology within a randomized complete block design, containing 16 treatments and 6 replicates per treatment. Blocks represented the pen distribution within the house, and block was considered as a random effect. In order to satisfy the normality assumption, all percentage data were converted by an arcsine-square root transformation prior to the analyses.

Pens were the experimental unit for performance and mineral retention data, and JMP 9 (SAS Inst. Inc., Cary, NC) was used for the statistical analyses. The categorical data (mechanical deboning, leg abnormalities, and TD evaluations), was statistically analyzed using each broiler chicken as an experimental unit. For these parameters, data were treated as binomial, for each condition the response take the value 0 (absence) or 1 (presence). The GLIMMIX Procedure of SAS (SAS Inst. Inc., Cary, NC) was used, having the linear and quadratic effects of levels of Ca and P in the diet and their interaction effects as fixed, and considering the pens as random effects nested within treatment combinations. The results, as log odds of a certain condition, were modeled within the effects to obtain the probability of observing each condition individually.

Statistical model used for the response surface methodology:

$$Y_{ijkl} = \mu + Ca_i + nPP_j + Ca*nPP_{ij} + Ca^2_i + nPP^2_j + P_l\,(T_{ij}) + B_k + \varepsilon_{ijkl}$$

Where:

Y_{ijkl}: Variable response

μ: Overall mean

Ca_i: Linear effect of the i^{th} Ca level (i = 1-4)

nPP_j: Linear effect of the j^{th} nPP level (j = 1-4)

$Ca*nPP_{ij}$: Effect of the first order interaction between Ca level i and nPP level j

Ca^2_i: Quadratic effect of the i^{th} Ca level

nPP^2_j: Quadratic effect of the j^{th} nPP level

P_l (T_{ij}): Random effect of dietary treatment combinations nested within pens (l = effect of pen, l = 1-6)

B_k: Random effect of the block (k = 1-6)

ε_{ijkl}: The experimental error associated to each observation.

Table IV-2. Composition of broiler diets (%) and formulated nutrient contents

Ingredients	Starter 1-17 days	Grower 18-35 days	Finisher basal 36-49 days
	----------%----------		
Corn	51.31	58.39	57.12
Soybean meal, 48%	30.67	24.72	21.53
Distillers dried grains with solubles	10.00	10.00	12.00
Poultry fat	3.93	3.61	4.75
Salt (NaCl)	0.40	0.37	0.36
Limestone	1.58	1.37	-
Dicalcium phosphate, 18.5%	0.90	0.48	-
DL-methionine, 99%	0.33	0.22	0.18
L-lysine-HCl, 78,8%	0.23	0.27	0.25
Choline chloride, 60%	0.20	0.20	0.20
Sodium bicarbonate	0.15	0.12	0.08
L-threonine, 98%	0.12	0.09	0.07
Coccidiostat[1]	0.06	0.05	-
Mineral premix[2]	0.05	0.05	0.03
Vitamin premix[3]	0.05	0.04	0.03
Phytase[4]	0.02	0.02	-
Filler[5]	-	-	3.40
Total	100.00	100.00	100.00
Nutrient composition			
Metabolizable energy, kcal/kg	3,065	3,140	3,175
Crude protein, %	22.23	19.40	18.25
Calcium, %	0.93	0.75	0.11
Total phosphorus, %	0.59	0.51	0.41
Non phytate phosphorus, %	0.45	0.37	0.18
Digestible lysine, %	1.20	1.05	0.95
Digestible total sulfur amino acids, %	0.93	0.81	0.73
Digestible threonine, %	0.75	0.68	0.63
Sodium, %	0.25	0.23	0.22
Potassium, %	0.91	0.81	0.76
Chloride, %	0.32	0.31	0.30
Dietary electrolyte balance, mEq/100 g	261	232	216

[1]Monteban® 45 (Narasin), Elanco Animal Health, Greenfield, IN, at 60 g/ton in the starter and 54 g/ton in the grower diet.
[2]Trace minerals provided per kilogram of premix: manganese (MnO_2), 220 g; zinc (ZnO and $ZnSO_4$), 250 g; iron ($FeCO_3$), 75 g; copper (CuSO4 and $CuCl_2$), 10 g; iodine ($Ca(IO_3)_2$), 5 g; selenium (Na_2SeO_3), 1 g.
[3]Vitamins provided per kilogram of premix: vitamin A, 18,739,292 IU; vitamin D3, 6,613,868 IU; vitamin E, 66,139 IU; vitamin B12, 33 mg; riboflavin, 22,046 mg; niacin, 88,185 mg; d-pantothenic acid, 30,865mg; menadione, 3,968 mg; folic acid, 2,646 mg; vitamin B6, 7,716 mg; thiamine, 5,512 mg; biotin, 176 mg.
[4]Ronozyme® P CT at 185 g/ton to provide 930 FYT (DSM Nutritional Products, Parsippany, NJ).
[5]Celite, Celite Corp., Lompoc, CA, at 1 g/kg of feed in the finisher diet. Filler also contained the amounts of dicalcium phosphate, limestone, and washed sand used to obtain the 16 treatments.

RESULTS AND DISCUSSION

Ca and P analyses of feed samples indicated that formulated values were in agreement with obtained lab results. The correlation coefficient between formulated and analyzed values for Ca and P were 0.99 and 0.99, respectively.

Performance during the Finisher Phase (36-49 days)

Due to its economical and environmental importance, many researchers have focused on testing reduced levels of P in the finisher phase, keeping the levels of Ca fixed. Most of these studies have reported no statistically significant differences in broiler performance during the finisher/withdrawal phase, even when the levels of nPP were as low as 0.10% (Skinner et al., 1992a,b; Chen and Moran, 1994; Yan et al., 2001; Dhandu and Angel, 2003; Sá et al., 2004). One explanation for not observing effects on performance could be that during the finisher phase broiler ability to utilize phytate P is higher, and if the requirements of Ca and P are not met, minerals from bone can be resorbed to supply the needs for optimum performance when adequate diets were provided previously.

In the present study, several combinations of Ca and nPP levels were tested, and the performance results are described in Tables IV-3 and IV-4. Even though no effects of treatments were detected on BWG during the finisher phase, a linear effect ($P \leq 0.01$) of nPP levels and an interaction effect ($P \leq 0.05$) of Ca and nPP levels were observed on FI and FCR, respectively. No significant effects of treatments were observed on mortality during the finisher phase.

Broilers had similar ($P > 0.05$) BW (1827 g and 2228 g for females and males, respectively) among treatments at 35 days. The average final BW at 49 days was 2667 g for females and 3343 g for males. The BWG during the finisher phase in both sexes was not affected by Ca and nPP levels, which ranged from 0.38 to 0.86% Ca and 0.18 to 0.42% nPP. This is in agreement with Dhandu and Angel (2003), who reported no significant differences in BWG when male Ross broilers were fed diets varying from 0.15 to 0.31% nPP (0.69% Ca) from 32 to 42 days, nor when fed levels ranging from 0.10 to 0.31% nPP (0.72% Ca) from 42 to 49 days of age. Similar results were described by Sá et al. (2004), finding no major differences in BWG from 22 to 42, and 43 to 53 days when Ca ranged from 0.41 to 1.40% (0.41% aP). Additionally, Gomes et al. (2004) showed that BWG from 43 to 53 days of age in Hubbard broilers fed aP levels ranging from 0.15 to 0.50% combined with 0.93% Ca was not affected by the treatments. However, at a younger age (22 to 42 days), male and female BWG were quadratically affected by aP, increasing up to its maximum values at 0.42% aP. Bar et al. (2003) tested diets ranging in nPP from 0.13 to 0.32% combined with two Ca levels (1.04 or 0.81%) in broilers from 29 to 43 days. Results demonstrated that BWG was reduced when the lowest level of nPP was used, and although no main effect of Ca was observed, BWG was superior with 1.04% Ca when levels of nPP were between 0.28 and 0.33%.

The FI during the finisher phase decreased linearly as the nPP levels in the feed were increased from 0.17 to 0.41%, independently of the Ca levels used (Figure IV-1 B). Sá et al. (2004) also did not find effects of Ca on FI of broilers fed diets containing levels varying from 0.16 to 1.40% Ca combined with 0.41% aP, from 22 to 42 and 43 to 53 days of age. In contrast to the results reported herein, Gomes et al. (2004) demonstrated that the increase in

nPP levels from 0.15 to 0.60% (22 to 42 days), and 0.15 to 0.50% (43 to 53 days) for Hubbard broilers did not affect FI. As mentioned before, Dhandu and Angel (2003) reported no differences in FI when levels of nPP were reduced from 0.30 down to 0.15%.

The best FCR during the finisher phase (2.40 g:g) was obtained at levels of nPP between 0.22 to 0.27% combined with Ca levels higher than 0.80% (Figure IV-2 A). As the levels of nPP were increased from 0.18% up to approximately 0.27%, there was a reduction in FI with a concomitant improvement in FCR. Further increments in nPP (>0.27%) continued causing reductions in FI; however, FCR was negatively affected, showing that the absorption or utilization of dietary nutrients might have been impaired by higher levels of nPP. Bar et al. (2003) conducted an experiment to test dietary levels of nPP ranging from 0.13 to 0.32% in broilers from 29 to 43 days, and noted that the lowest level of nPP showed the worst FCR; however, further increments from 0.16 to 0.32% nPP did not change FCR significantly. Gomes et al. (2004) reported that female FCR from 22 to 42 days in diets with 0.93% Ca improved 7.4% (2.32 to 2.16 g:g) as the levels of aP went from 0.15 to 0.42%, and after this level further increments in nPP (to 0.60%) negatively impacted FCR. When a similar experiment was conducted from 43 to 53 days of age, no effects on FCR were detected among treatments (0.15 up to 0.50% aP combined with 0.89% Ca). In the present experiment, it is important to note that the lowest level of nPP used (0.18%) did not contain supplemental inorganic P, and that none of the treatment diets contained phytase. Phytate might have had a negative effect on FI and FCR. The next level of nPP (0.26%) already contained inorganic P supplementation, which could be one of the reasons that the best FCR was not obtained when using lower levels of nPP. The effects of Ca on FCR were described

by Sá et al. (2004), who observed no effects of Ca levels (varying from 0.16 to 1.40% with 0.41% aP) in chickens from 22 to 42 and 43 to 53 days, agreeing with Chen and Moran (1994) and Dhandu and Angel (2003).

Male flock uniformity at 49 days was not affected by the dietary treatments. In the females, there was an interaction effect ($P \leq 0.05$) of Ca and nPP levels used during the finisher phase. The best uniformity (lowest CV%) was observed at Ca levels lower than 0.40% and nPP levels higher than 0.38%. However, when using these levels of nPP with Ca levels higher than 0.80%, the lowest uniformity (higher CV%) occurred, with a difference of around 5 points in the CV% from the highest to the lowest uniformity (Figure IV-1 A).

The carryover effect of the previous nutrition should be considered when comparing the results of the experiment reported herein. In the present experiment, birds received a starter diet with 0.93% Ca and 0.45% nPP, and a grower diet with 0.75% Ca and 0.37% nPP, which are lower than the NRC (1994) recommendations of 1.00 Ca and 0.45% aP for the starter and 0.90% Ca and 0.40% aP for the grower phase. The Ca and P levels used were proven to be adequate for growth, feed conversion, and bone development traits in previous experiments with Heritage broilers (data not published, Chapters II and III). Most experiments involving Ca and P during the finisher phase make use of Ca and P levels that meet or exceed the NRC (1994) recommendations in the previous phases. Therefore, the performance obtained in the present trial is also a result of the levels tested and the carryover effect of the previous nutrition.

Overall Performance (0-49 days)

When evaluating the grow-out period as a whole, no effects of treatments were observed in BWG and FI. The FCR from 0 to 49 days tended to be quadratically affected (*P* = 0.09) by the nPP levels fed during the finisher phase(Tables IV-3 and IV-4), and the optimum FCR (1.94 g:g) was estimated at 0.65% Ca and 0.29% nPP (Figure IV-2 B).

Table IV-3. Response surface regression coefficients and predicted optimal values for the effects of dietary Ca and nPP levels during the finisher phase on feed intake (FI), and feed conversion ratio (FCR) during the finisher period (36-49 days), FCR for the whole grow-out (0-49 days), and body weight coefficient of variation (BW CV%) at 49days for Heritage broilers.

	FI	FCR	FCR	BW CV% 49 d	
	36-49 d	36-49 d	0-49 d	Females	Males
Intercept	2438.61	2.85	2.09	25.75	-1.79
Ca	-3.55	-0.20	-0.14	-22.77	29.18
nPP	-83.20**	-2.39	-0.67	-58.87	27.03
Ca x Ca	15.61	-0.14	0.11	9.53	-13.05
Ca x nPP	-76.04	1.17*	0.02	48.46*	-25.52
nPP x nPP	-131.86	2.73	1.12	47.60	-8.62
Predicted minimum			1.94		
Ca at predicted			0.65		
nPP at predicted			0.29		

*$P \leq 0.05$, ** $P \leq 0.01$

Table IV-4. Effects of dietary Ca and nPP levels in finisher diets on feed intake (FI), and feed conversion ratio (FCR) during the finisher period (36-49 days), FCR for the whole grow-out (0-49 days), and body weight coefficient of variation (BW CV%) at 49days for Heritage broilers.

Ca	nPP	FI	FCR	FCR	BW CV% 49 d*	
		36-49 d	36-49 d	0-49 d	Female	Male
-----%-----		-----g-----	----------g:g----------		----------%----------	
0.38		2393	2.440	1.960	11.17	12.08
0.54		2391	2.444	1.968	10.75	12.58
0.70		2391	2.436	1.945	11.96	14.79
0.86		2391	2.429	1.965	12.55	14.28
Pooled SEM		16	0.022	0.009	0.75	1.32
	0.18	2413	2.467	1.967	12.04	12.71
	0.26	2408	2.408	1.950	11.25	13.36
	0.34	2377	2.430	1.955	11.38	13.63
	0.42	2368	2.445	1.967	11.77	14.04
Pooled SEM		16	0.022	0.009	0.75	1.32
0.38	0.18	2424	2.544	1.980	14.00	13.82
0.38	0.26	2419	2.416	1.948	10.33	8.17
0.38	0.34	2419	2.422	1.950	10.50	12.50
0.38	0.42	2419	2.380	1.962	9.83	13.83
0.54	0.18	2413	2.442	1.965	10.33	11.17
0.54	0.26	2402	2.404	1.947	12.00	10.50
0.54	0.34	2396	2.442	1.970	10.67	16.17
0.54	0.42	2393	2.490	1.992	10.02	12.50
0.70	0.18	2384	2.452	1.963	11.33	13.17
0.70	0.26	2384	2.410	1.922	11.17	16.17
0.70	0.34	2378	2.417	1.957	12.33	13.00
0.70	0.42	2375	2.467	1.940	13.00	16.83
0.86	0.18	2370	2.430	1.960	12.50	12.67
0.86	0.26	2365	2.403	1.983	11.50	18.61
0.86	0.34	2364	2.438	1.942	12.00	12.83
0.86	0.42	2361	2.443	1.973	14.21	13.00
Pooled SEM		22	0.040	0.017	1.52	2.80
Source of Variation		----------*P*-values----------				
Ca		0.999	0.945	0.204	0.315	0.431
nPP		0.091	0.191	0.346	0.855	0.882
Ca*nPP		0.997	0.359	0.308	0.740	0.540

*The average BW at 49 days was 2667 g for females and 3343 g for males

Bone Mineralization (49 days)

As demonstrated in the Tables IV-5 and IV-6, tibia ash percentage increased linearly ($P \leq 0.01$) as nPP levels in the finisher diet increased in the females, and this response was more pronounced with a concomitant increase in Ca levels (Figure IV-3 A). Dhandu and Angel (2003) reported that tibia ash at 42 days was reduced by using 0.15% nPP as compared to 0.19, 0.26, and 0.31% nPP in broilers fed the experimental diets for 10 days. At 49 days, tibia ash was reduced when broilers were fed diets with 0.10 and 0.13% as compared to 0.22 and 0.27% for 7 days. In an experiment with broilers fed experimental diets from 29 to 43 days, Bar et al. (2003) demonstrated that tibia ash percentage at 43 days increased progressively as levels of nPP increased from 0.13 to 0.32%. Although Ca had no major effect, when using levels of nPP between 0.20 and 0.28% a Ca content of 1.04% increased bone ash as compared with 0.81%. In the same experiment, the authors observed that at the lowest level of nPP (0.13%), higher dietary Ca (1.04%) markedly reduced BW and tibia ash. Gomes et al. (2004), testing levels of nPP ranging from 0.15 to 0.60% in broiler diets from 22 to 42 days demonstrated that in males, tibia ash percentage increased with increments in the levels of nPP up to 0.42% and then kept constant. Among the females, the ash percentage increased with increments in nPP up to 0.51%. Those authors reported no differences in tibia ash percentage when the same levels of nPP were tested in broiler diets from 43 to 53 days. In the experiment reported herein, male tibia ash percentage increased linearly ($P \leq 0.05$) as the levels of Ca increased in the finisher diet (Figure IV-3 B). It contrasts with the findings of Bar et al. (2003), who suggested that males appear to be more sensitive to dietary P than females for both growth performance and bone formation. According to Sá et al. (2004), tibia

ash percentage was lower when broilers from 22 to 42 and 43 to 53 days were fed diets containing 0.16 and 0.41% Ca as compared to treatments with Ca ranging 0.66 to 1.41%; however, within this range no significant differences occurred.

The BMC was influenced mainly by the levels of Ca fed in the finisher period (Tables IV-5 and IV-6). Female BMC was quadratically affected ($P \leq 0.01$) by Ca, and the highest BMC occurred when levels of Ca were around 0.75% (Figure IV-4 A). The female BMC was also affected by the chicken BW, showing a linear increase ($P \leq 0.01$) in BMC as the BW increased. For males, the effect of Ca was linear ($P \leq 0.05$) and higher BMC was obtained with increments in dietary Ca during the finisher phase (Figure IV-4 B). The male BMC reduced drastically when using the minimum level of Ca together with a wide Ca:nPP ratio. As the dietary level of Ca increased, less effect of nPP levels was observed.

The female BMD had an interaction effect of Ca and nPP ($P \leq 0.05$), and in the males a linear effect ($P \leq 0.01$) of Ca was detected (Tables IV-5 and IV-6). In both sexes the highest BMD was observed at the highest Ca and nPP levels used (Figures IV-5 A and B); however, only in the females there was a significant linear increase ($P \leq 0.05$) in BMD as the BW of the birds increased.

Table IV-5. Response surface coefficients for the effects of dietary Ca and nPP levels in finisher diets on tibia ash at 49 days and bone densitometry (BMC and BMD) of shanks at 49 days for male and female Heritage broilers.

	Tibia ash		BMC		BMD	
	Females	Males	Females	Males	Females	Males
Intercept	43.61**	37.39**	0.48**	4.18**	0.211**	0.232**
Ca	-2.10	7.79*	4.26**	1.54*	0.011**	-0.018**
nPP	1.94**	14.30	-0.49	-0.05	-0.131	-0.013
Ca x Ca	1.95	-1.51	-2.80**	-1.36	-0.023	0.017
Ca x nPP	2.23	-14.37	-0.93	2.68	0.104*	0.062
nPP x nPP	0.41	-7.16	1.23	-3.09	0.128	-0.013
BW	-	-	0.001**	0.001	0.001*	0.001

*$P \leq 0.05$, ** $P \leq 0.01$

Table IV-6. Effects of dietary Ca and nPP levels in finisher diets on tibia ash at 49 days and bone densitometry (BMC and BMD) of shanks at 49 days for male and female Heritage broilers.

Ca	nPP	Tibia ash		BMC		BMD	
		Females	Males	Females	Males	Females	Males
-----%-----		--------%--------		--------g--------		--------g/cm^2--------	
0.38		43.93	42.03	4.124^{b}	5.914^{b}	0.233^{b}	0.259^{b}
0.54		44.08	42.48	4.455^{a}	6.365^{a}	0.237^{ab}	0.267^{ab}
0.70		44.09	42.59	4.383^{a}	6.061^{ab}	0.237^{ab}	0.262^{b}
0.86		44.44	42.86	4.425^{a}	6.366^{a}	0.239^{ab}	0.272^{ab}
Pooled SEM		2.00	1.72	0.059	0.109	0.003	0.003
	0.18	43.68	42.28	4.386	6.192	0.236	0.263
	0.26	44.10	42.56	4.378	6.173	0.236	0.265
	0.34	44.17	42.49	4.302	6.217	0.236	0.266
	0.42	44.59	42.63	4.320	6.126	0.239	0.268
Pooled SEM		2.00	1.72	0.059	0.108	0.002	0.002
0.38	0.18	43.89	41.10	4.111	6.013	0.236	0.258
0.38	0.26	43.77	41.94	4.120	5.602	0.231	0.257
0.38	0.34	43.87	41.83	4.159	6.061	0.227	0.256
0.38	0.42	44.19	43.26	4.107	5.981	0.236	0.265
0.54	0.18	43.26	42.56	4.504	6.499	0.238	0.265
0.54	0.26	44.10	42.74	4.476	6.637	0.237	0.276
0.54	0.34	43.85	42.16	4.413	6.423	0.239	0.268
0.54	0.42	45.11	42.47	4.425	5.903	0.235	0.262
0.70	0.18	43.88	42.56	4.499	5.939	0.235	0.258
0.70	0.26	43.78	43.15	4.382	6.096	0.236	0.261
0.70	0.34	44.40	42.77	4.293	6.192	0.240	0.264
0.70	0.42	44.31	41.90	4.358	6.018	0.237	0.265
0.86	0.18	43.68	42.89	4.432	6.317	0.235	0.271
0.86	0.26	44.76	42.42	4.533	6.357	0.238	0.267
0.86	0.34	44.57	43.22	4.344	6.190	0.24	0.273
0.86	0.42	44.77	42.89	4.391	6.601	0.25	0.279
Pooled SEM		2.04	1.77	0.117	0.217	0.003	0.005
Source of variation		----------*P*-values----------					
Ca		0.528	0.164	<0.001	0.006	0.040	0.002
nPP		0.095	0.784	0.678	0.944	0.587	0.648
Ca*nPP		0.825	0.179	0.991	0.245	0.297	0.544
BW		-	-	<0.001	0.194	0.031	0.293

[a,b] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

Mineral Retention during the Finisher Phase

The Ca retention at 44 days was affected by an interaction ($P \leq 0.01$) of Ca and nPP levels fed during the finisher diet (Tables IV-7 and IV-8). Broilers retained the most Ca (50-60%) when diets containing Ca concentration lower than 0.50% and nPP higher than 0.38% were provided (Figure IV-6 A). When dietary nPP was lower than 0.25%, keeping the same Ca (lower than 0.50%), the retention of Ca dropped to levels between 20-30%. Ca excretion during the finisher phase (Table IV-8) increased as dietary Ca augmented, and reduced as nPP levels in the diet increased. Most of the published research on P levels in broiler feeds demonstrates that as the levels of dietary P increase, the retention of this mineral is reduced. Evidently, broiler chickens have the ability to better absorb P when the concentration in the diet is restricted. The data presented here demonstrated that in this experiment P retention did not follow this pattern. P retention at 44 days was affected by the interaction ($P \leq 0.01$) of Ca and nPP levels fed in the finisher phase (Tables IV-7 and IV-8). Figure IV-6 B demonstrates that at the lowest dietary Ca (0.38%) the retention of P improved as the nPP levels in the diet increased, from approximately 23% (at 0.18% nPP) to around 39% (at 0.42% nPP). The P retention increased as Ca levels in the diet increased up to 0.70% Ca and then kept constant. The best retention of P (around 41.39%) was obtained when Ca levels higher than 0.70% were combined with levels of nPP ranging from 0.26 to 0.37%. Levels of nPP higher than 0.37% caused a reduction in P retention. The excretion of P during the finisher phase (Table IV-8) increased as dietary Ca reduced, and increased as dietary nPP increased. There is a known negative impact that dietary Ca has on P absorption and utilization, due to its effects on phytase activity and on forming insoluble complexes with the phytate molecule (Shafey et

al., 1991; Tamin and Angel, 2004; Selle et al., 2009). However, Plumstead et al. (2008) reported a quadratic effect of Ca levels on P retention in broilers from 19 to 20 days. Ca levels ranging from 0.40 to 1.20% were tested in their experiment, and the breakpoint for P retention was found when using 0.74 to 0.99% Ca (depending on the variety of soybeans used) in the diets. These authors suggested that at low dietary Ca (before breakpoint), P appeared to be absorbed but was later excreted in the urine, presumably due to insufficient Ca for P retention to occur. Three factors that could have influenced the improvements in P retention as the levels of dietary nPP increased are listed as follows: 1) no phytase was added to the treatment diets and chickens were fed common diets containing phytase in starter and grower phases; 2) the diet with the lowest nPP level (0.18%) has no supplemental inorganic P; and 3) the increasing levels of dietary nPP were formulated by adding dicalcium phosphate to the basal diet. Since only a small part of the phytate P present in the basal diet is utilized and that the P in dicalcium phosphate is considered almost 100% bioavailable, it is logical that as the ratio of inorganic P to total P in the diet was increased, an improvement in the retention of P would be observed.

Table IV-7. Response surface coefficients for the effects of dietary Ca and nPP levels in finisher diets on Ca and P retention (%) at 44 days, and on Ca and P excretion (g) from 36 to 49 days for Heritage broilers.

	Mineral retention 44 d		Excretion from 36 to 49 d	
	Ca	P	Ca	P
Intercept	-0.04**	-0.37	13.60**	85.89**
Ca	-0.19	0.79**	240.58**	-4.36
nPP	1.79**	2.73**	-100.57**	-29.30**
Ca x Ca	0.84	-0.18	-88.40	-69.97
Ca x nPP	-2.54**	-1.38**	42.63	253.74**
nPP x nPP	1.08	-2.44*	-84.64	-127.92

*$P \leq 0.05$, ** $P \leq 0.01$

Table IV-8. Effects of dietary Ca and nPP levels in finisher diets on Ca and P retention at 44 days, and on Ca and P excretion from 36 to 49 days for Heritage broilers.

Ca	nPP	Mineral retention 44 d		Excretion 36-49 d	
		Ca	P	Ca	P
-----%-----		----------%----------		--------mg--------	
0.38		35.66	33.36^{b}	57.88^{c}	81.76
0.54		29.69	35.50^{ab}	90.64^{b}	82.80
0.70		37.63	38.98^{a}	104.71^{b}	79.68
0.86		38.50	39.25^{a}	129.88^{a}	74.53
Pooled SEM		2.70	1.75	4.49	2.28
	0.18	25.80^{c}	29.37^{b}	111.28^{a}	73.97^{b}
	0.26	32.41^{bc}	38.15^{a}	97.34^{a}	74.97^{b}
	0.34	36.87^{b}	38.08^{a}	95.01^{b}	85.42^{a}
	0.42	46.40^{a}	41.49^{a}	79.48^{c}	84.41^{a}
Pooled SEM		2.70	1.75	4.49	2.28
0.38	0.18	9.54^{d}	14.01^{g}	82.64^{de}	85.26^{a}
0.38	0.26	33.35^{bcd}	32.07^{ef}	59.17^{ef}	87.40^{a}
0.38	0.34	37.38^{bc}	36.48^{bcdef}	56.05^{ef}	84.14^{ab}
0.38	0.42	62.37^{a}	50.89^{a}	33.64^{f}	70.22^{ab}
0.54	0.18	30.70^{bcd}	41.75^{abcde}	89.74^{bcde}	67.31^{ab}
0.54	0.26	18.48^{cd}	27.75^{f}	106.51^{bcd}	87.64^{ab}
0.54	0.34	37.99^{bc}	39.25^{abcdef}	78.76^{de}	83.80^{ab}
0.54	0.42	31.62^{bcd}	33.22^{def}	87.55^{cde}	92.47^{ab}
0.70	0.18	28.70^{bcd}	32.36^{def}	127.06^{bc}	69.38^{ab}
0.70	0.26	42.18^{abc}	47.27^{ab}	94.98^{bcde}	65.64^{abc}
0.70	0.34	37.92^{bc}	41.26^{abcde}	101.57^{bcd}	89.99^{abc}
0.70	0.42	41.72^{abc}	35.05^{cdef}	95.23^{bcde}	93.71^{abc}
0.86	0.18	34.29^{bc}	29.36^{ef}	145.68^{a}	73.91^{abc}
0.86	0.26	35.63^{bc}	45.50^{abcd}	128.70^{ab}	59.21^{bc}
0.86	0.34	34.17^{bcd}	35.32^{bcdef}	143.65^{a}	83.76^{bc}
0.86	0.42	49.90^{ab}	46.80^{abc}	82.64^{bcd}	81.26^{c}
Pooled SEM		4.98	2.86	8.45	4.85
Source of Variation			----------*P*-values----------		
Ca		0.050	<0.010	<0.001	0.090
nPP		<0.001	<0.001	<0.001	0.001
Ca*nPP		<0.001	<0.001	0.017	<0.001

[a,b,c,d,e,f,g] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

Bone Strength (49 days)

Tibia strength was evaluated by the 3-point bending test. A significant effect of treatments on bone strength was observed in male bones. The bone strength responses are shown in Figures IV-6 A and B. Both the force needed to break the tibias and shear stress followed the same pattern as the other bone parameters, increasing linearly ($P \leq 0.01$) as levels of Ca increased up to its maximum level (0.86%) in the finisher diet (Tables IV-9 and IV-10). This observation is in agreement with Sá et al. (2004), who reported that as levels of Ca increased from 0.16 to 0.91% in broiler diets (22 to 42 days), bone breakage resistance increased, and further increments in Ca up to 1.41% did not affected significantly bone strength. In another experiment conducted by the same researchers, when broilers were fed the Ca levels mentioned above from 43 to 53 days, bone breakage strength increased as Ca levels were incremented from 0.16 up to 1.16%. The effects of Ca on tibia strength were also demonstrated by Skinner et al. (1992b), reporting that an increment in strength happened as dietary levels of Ca were increased from 0.06 to 0.48% (at 0.12 or 0.24% nPP) for 42 to 56 day-old Cobb broilers, and comparing the group fed the diet with the highest Ca concentration with a control treatment containing 0.80% Ca and 0.35% nPP no differences in bone strength were observed. Additional increments in Ca levels up to 0.60% did not increase tibia strength at 0.12% nPP, but at 0.24% an improvement in strength was observed. In the present project, even though female tibia ash and BMD were at their maximum when using the highest Ca and nPP levels in the finisher diet, no significant effects were detected in bone breakage strength. This data suggested no effects of nPP levels fed in the finisher diet on breakage strength. Earlier studies conducted by Hulan et al. (1985) already had shown that

tibia strength at 42 days significantly increased progressively as levels of Ca and aP in the diet of broilers from 21 to 42 days increased (1.00 to 1.40% Ca and 0.32 to 0.51% aP) and that the ratio Ca:aP had no significant effect on bone strength. Based on the data of their experiment, the effect on tibia strength was due to dietary Ca rather than aP. Dhandu and Angel (2003) also showed no effects of nPP levels on bone strength (force and stress) at 42 and 49 days when broilers were fed diets containing levels of nPP ranging from 0.15 to 0.31% and 0.10 to 0.27% during the finisher (32 to 42 days) and withdrawal (42 to 49 days) phases, respectively. Similar results were described by Gomes et al. (2004), obtaining no effects of aP on the resistance of bones to breakage at 53 days, when broilers were fed diets varying in aP levels (0.15 to 0.50%) from 43 to 53 days. However, those researchers demonstrated that bone strength at a younger age (42 days) was affected by aP levels in both male and female broilers fed experimental diets from 22 to 42 days. In this case, a quadratic effect of aP levels was observed, improving bone resistance to breakage when aP increased from 0.15 to 0.42%, and reducing strength when levels of 0.51 and 0.60% aP were used.

During the mechanical deboning evaluation, no effects of treatments were detected on the probability of incidence of total breakages (diaphyseal breakages). The probability of incidence of partial breakages (epiphyseal breakages) tended to be quadratically affected (P = 0.07) by nPP levels fed during the finisher phase (data not shown). The highest probability of incidence of partial breakages was observed at levels of Ca between 0.50 and 0.65% and nPP between 0.33 and 0.37%. The probability of incidence of partial breakages was markedly reduced when the finisher diet contained levels of nPP lower than 0.25%, independently of the levels of Ca.

Angel et al. (2006) did not observe significant differences in the incidence of bruising, broken wings, and broken legs during processing at 49 days between four-phase feeding systems containing NRC (1994) nPP levels (0.45, 0.35, 0.35, and 0.30%) or a reduced nPP diet (0.45, 0.31, 0.23, and 0.18%).

Table IV-9. Response surface regression coefficients for the effects of dietary Ca and nPP levels in finisher diets on tibia breaking strength at 49 days, evaluated by the 3-point bending test and reporting the maximum force and shear stress.

	Force		Shear stress	
	Females	Males	Females	Males
Intercept	144.58**	290.12**	65.69**	47.04**
Ca	208.14	48.41**	-7.75	0.67**
nPP	229.21	-131.81	13.47	18.47
Ca x Ca	-120.38	51.63	9.09	7.82
Ca x nPP	-134.81	-113.21	-19.25	2.47
nPP x nPP	-308.18	363.35	-16.75	-37.06

*$P \leq 0.05$, ** $P \leq 0.01$

Table IV-10. Effects of dietary Ca and nPP levels in finisher diets on tibia breaking strength at 49 days, evaluated by the 3-point bending test and reporting the maximum force and shear stress.

Ca	nPP	Force		Shear stress	
		Females	Males	Females	Males
-----%-----		----------N----------		----------MPa----------	
0.38		228	299[b]	64.02	50.90
0.54		244	309[ab]	64.31	51.28
0.70		236	322[ab]	62.30	54.57
0.86		240	337[ab]	63.47	55.75
Pooled SEM		6	8	1.85	1.17
	0.18	238	317	64.18	52.83
	0.26	245	316	64.94	54.27
	0.34	233	313	62.38	52.42
	0.42	232	321	62.61	52.97
Pooled SEM		6	8	1.85	1.17
0.38	0.18	234	301	66.45	51.22
0.38	0.26	219	287	62.40	52.30
0.38	0.34	228	299	62.14	51.66
0.38	0.42	230	308	65.10	48.43
0.54	0.18	242	283	61.86	45.68
0.54	0.26	252	353	68.39	56.22
0.54	0.34	242	292	65.86	52.36
0.54	0.42	241	308	61.14	50.86
0.70	0.18	238	339	62.02	56.87
0.70	0.26	250	297	64.93	54.68
0.70	0.34	230	315	61.81	50.66
0.70	0.42	226	337	60.43	56.07
0.86	0.18	238	344	66.41	57.57
0.86	0.26	259	326	64.02	53.90
0.86	0.34	232	345	59.69	55.01
0.86	0.42	232	333	63.78	56.52
Pooled SEM		11	16	3.26	2.39
Source of variation		----------*P*-values----------			
Ca		0.123	0.005	0.811	0.009
nPP		0.277	0.886	0.596	0.724
Ca*nPP		0.755	0.039	0.670	0.088

[a,b] Means within a column without a common superscript differ significantly ($P < 0.05$) when tested with Tukey's honestly significant-difference test.

Tibial Dyschondroplasia (49 days)

The effects of Ca and nPP levels on TD incidence and severity are shown in Tables IV-11 and IV-12. Females had significantly lower ($P \leq 0.01$) probability of incidence of TD than males, which agrees with the findings of Hulan et al., (1985). The probability of incidence of TD was affected linearly ($P \leq 0.05$) by nPP levels and showed a tendency to be quadratically ($P = 0.06$) affected by dietary Ca. At high Ca levels (> 0.60%), as levels of nPP were reduced in the finisher diet the probability of incidence of TD increased, and reached its maximum when nPP levels were lower than 0.28% combined with Ca levels higher than 0.80%. However, at low Ca levels (< 0.60%) the response was inverted, and increasing dietary nPP increased the probability of TD incidence, where the minimum incidence was observed at levels of Ca lower than 0.42% combined with the lowest nPP level (0.18%). Hulan et al. (1985) described contrasting results in broilers from 22 to 42 days of age fed diets varying in Ca and aP levels (1.00 to 1.49% Ca and 0.32 to 0.51% aP), in which increasing the Ca:aP ratio reduced the incidence of TD. These authors suggested that the principal factor that predisposes the bird to TD was the increment in dietary aP. In another earlier research, Edwards and Veltmann (1983) reported that TD incidence at 14 days was prevented by using high levels of Ca and was accentuated with high nPP levels in the feed; however the researchers stated that the ratio of Ca to nPP was of primary importance in the expression of the lesion. The probability of incidence of severe TD (score 3) showed a similar response as TD incidence, with a linear effect of nPP levels ($P \leq 0.05$). The probability of incidence of severe TD was higher when levels of nPP were lower than 0.28% in combination with Ca levels higher than 0.70. The probability of incidence of severe TD

was reduced in two scenarios: when high nPP (> 0.39%) combined with high Ca (> 0.80%) or when low nPP (< 0.19%) combined with low Ca (< 0.40%) were used. The results shown here indicated that most likely the Ca:nPP ratio rather than the individual levels played the main role in increasing the probability of incidence and severity of TD.

Leg Abnormalities (49 days)

The probability of incidence of total leg abnormalities, crooked toes, and severe valgus were greater ($P \leq 0.01$) in males than females, and the effects of dietary treatments are shown in Tables IV-11 and IV-12. The probability of incidence of total leg abnormalities was affected quadratically ($P \leq 0.01$) by Ca levels fed during the finisher phase and the probability of incidence of crooked toes showed an interaction effect of Ca and nPP ($P \leq 0.05$), both being reduced when low concentrations of Ca and nPP were fed in the finisher diet. For total leg abnormalities, the probability was reduced when broilers were fed diets containing levels of Ca lower than 0.45% combined with less than 0.20% nPP. Similar results were observed in the probability of incidence of crooked toes, which was at its minimum when the lowest level of Ca (0.38%) and nPP (0.18%) were used. The probability of incidence of valgus/varus deformities had an interaction effect ($P \leq 0.05$) of Ca and nPP levels and followed the same pattern as TD incidence and severity (Tables IV-11 and IV-12). Broilers fed diets containing low Ca and nPP (Ca ≤ 0.45% and nPP ≤ 0.25%) or high Ca and nPP (Ca > 0.80% and nPP > 0.35%) concentrations had less probability of incidence of valgus/varus deformities. Nelson et al. (1990) reported no consistent effects of levels of aP fed to broiler chickens from 21 to 42 days of age on the incidence of varus. No significant

effects of treatments were detected on twisted legs incidence (data not shown), which is in agreement with the findings of Hulan et al. (1984). These authors did not detect differences in twisted legs incidence when testing levels of Ca ranging from 1.0 to 1.40% and aP from 0.32 to 0.51% in broiler chicken diets from 22 to 42 days.

Table IV-11. Response surface regression coefficients for the effects of dietary Ca and nPP levels in finisher diets on the probability of incidence of tibial dyschondroplasia incidence and severity (score 3), total leg abnormalities, crooked toes, and valgus/varus deformities.

	Type of leg abnormality			Tibial dyschondroplasia	
	Total	Crooked toes	Valgus/varus	Incidence	Severity
Intercept	-3.87	-13.77**	-2.44	-4.81	-9.66
Ca	14.79**	27.37**	11.18*	5.70	7.38
nPP	2.80	18.41	-0.69	22.75*	41.09*
Ca x Ca	-8.70	-15.67*	-7.58	-12.90	-19.01
Ca x nPP	-9.21**	-17.73**	-6.72**	-0.54	-1.02
nPP x nPP	3.23	-9.34	7.07	-26.98	-51.09
Sex (female)	-0.50**	-0.48**	-0.36**	-0.96**	-1.38**

*$P \leq 0.05$, ** $P \leq 0.01$

Table IV-12. Effects of dietary Ca and nPP levels in finisher diets on the probability of incidence of tibial dyschondroplasia incidence, total leg abnormalities, crooked toes, and valgus/varus deformities.

Ca	nPP	Type of leg abnormality			TD
		Total	Crooked toes	Valgus/varus	Incidence
-----%-----		----------Probability----------			
0.38		57.76	3.06	55.83	37.67
0.54		73.32	8.57	69.90	42.99
0.70		73.39	9.21	67.10	47.75
0.86		67.66	4.19	64.66	52.18
Pooled SEM		8.39	2.62	8.94	6.55
	0.18	70.43	3.32	68.95	44.26
	0.26	68.48	6.87	63.94	51.21
	0.34	66.98	5.51	62.76	47.63
	0.42	67.38	8.17	62.27	37.49
Pooled SEM		8.51	2.56	9.00	6.56
0.38	0.18	55.55	0.77	55.78	41.06
0.38	0.26	54.75	3.83	53.56	41.08
0.38	0.34	64.39	6.44	59.28	32.25
0.38	0.42	56.12	4.43	54.65	36.60
0.54	0.18	67.91	4.57	68.00	27.89
0.54	0.26	76.59	7.88	72.09	45.52
0.54	0.34	64.57	8.56	60.95	54.47
0.54	0.42	81.90	16.72	77.25	45.54
0.70	0.18	80.31	6.31	77.56	41.08
0.70	0.26	70.91	11.53	64.30	54.47
0.70	0.34	74.47	10.35	68.02	54.55
0.70	0.42	66.64	9.49	56.66	41.08
0.86	0.18	74.90	5.20	72.40	67.93
0.86	0.26	69.78	6.26	64.82	63.52
0.86	0.34	63.81	1.53	62.54	50.01
0.86	0.42	61.18	6.05	58.12	27.75
Pooled SEM		10.71	3.66	11.43	11.22
Source of Variation		----------*P*-values----------			
Ca		0.020	0.005	0.112	0.307
nPP		0.931	0.110	0.673	0.357
Ca*nPP		0.576	0.438	0.759	0.387
Sex		<0.001	0.019	0.003	<0.001

Conclusions

The present study indicated that Heritage broiler performance during the finisher phase (35 to 49 days) was affected by the Ca and nPP combinations tested during this period. When broilers were fed levels of Ca higher than 0.80% and nPP levels about 0.27%, optimum FCR and the highest P retention were observed in the finisher phase. Bone mineralization and strength were affected in different ways for males and females, but in general these parameters were enhanced with increasing dietary levels of Ca and nPP. In the mechanical deboning evaluation, low levels of nPP (< 0.25%) reduced the probability of epiphyseal breakages. Data reported here suggested that valgus/varus deformities, TD incidence, and TD severity were affected by the Ca:nPP ratio rather than the individual levels of these minerals, and altering the ratio (above or below 2:1) resulted in higher probability of incidence of those problems. Low levels of Ca and nPP (< 0.45% and < 0.20%, respectively) reduced the probability incidence of total leg problems and crooked toes.

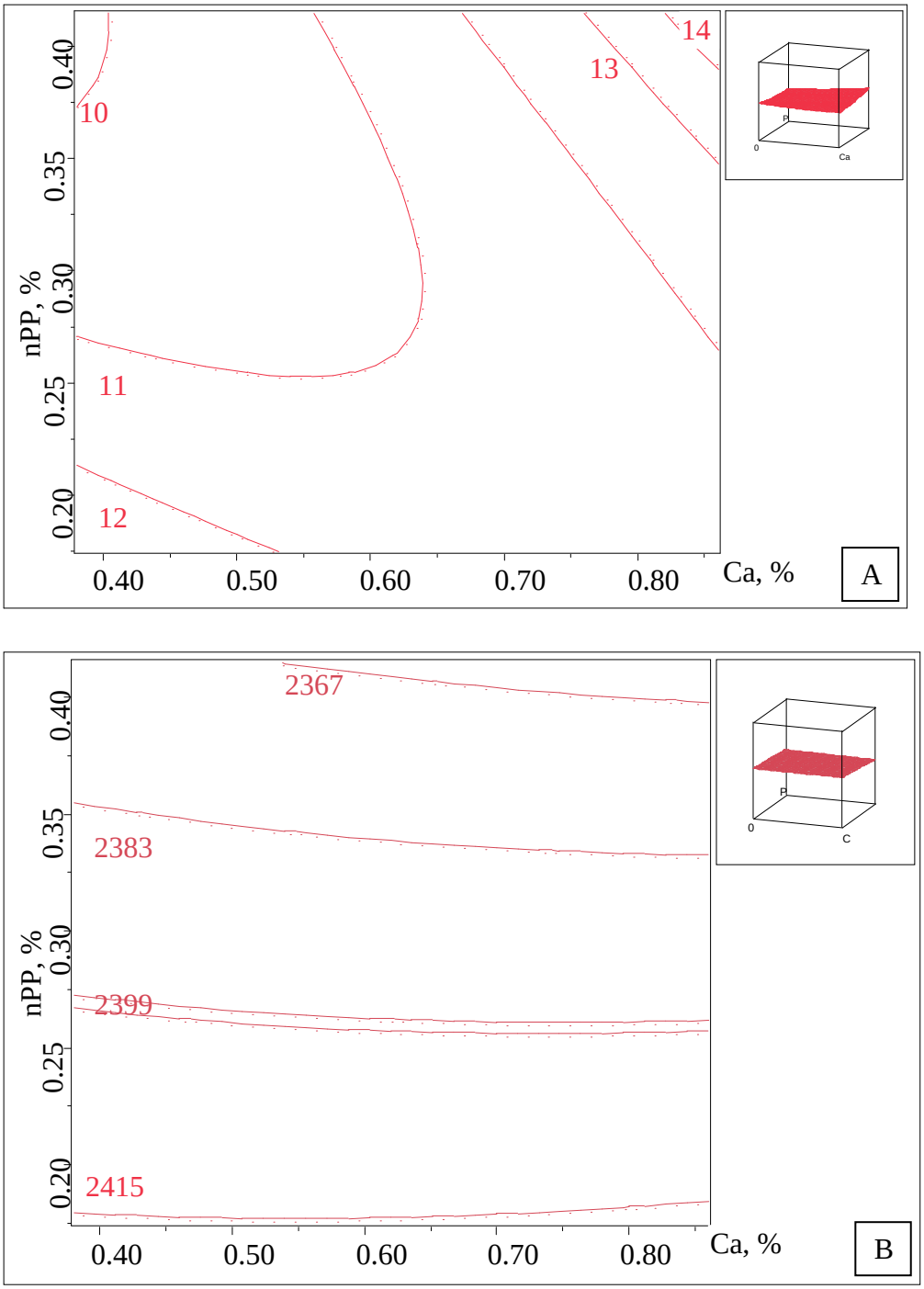

Figure IV-1. Response contours of female flock uniformity (CV%) at 49 days (A) and feed intake (g) from 36 to 49 days (B) with varying dietary levels of Ca and nPP during the finisher phase (36-49 days).

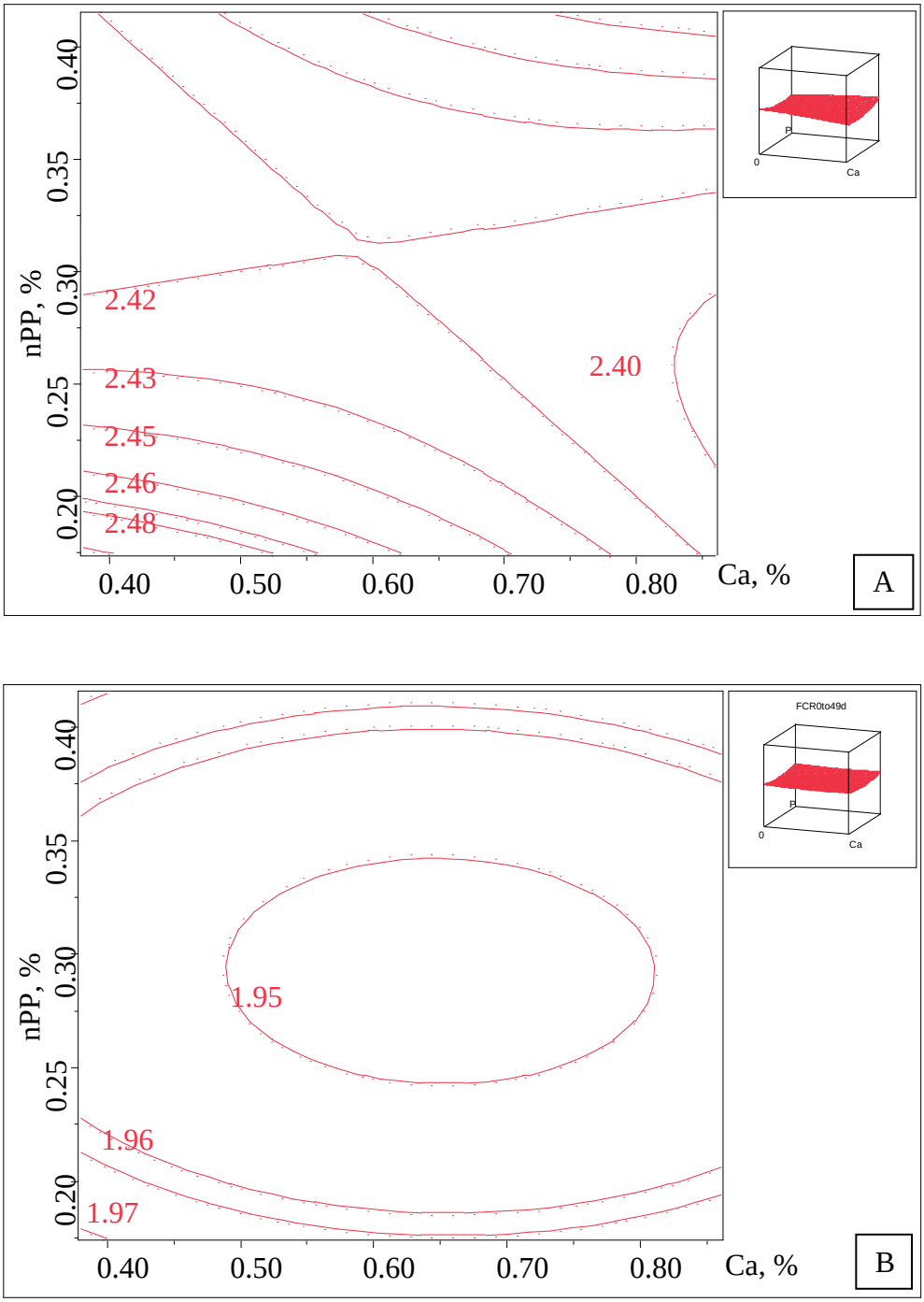

Figure IV-2. Response contours of feed conversion ratio (g:g) from 36-49 days (A) and from 0 to 49 days (B) with varying dietary levels of Ca and nPP during the finisher phase (36-49 days).

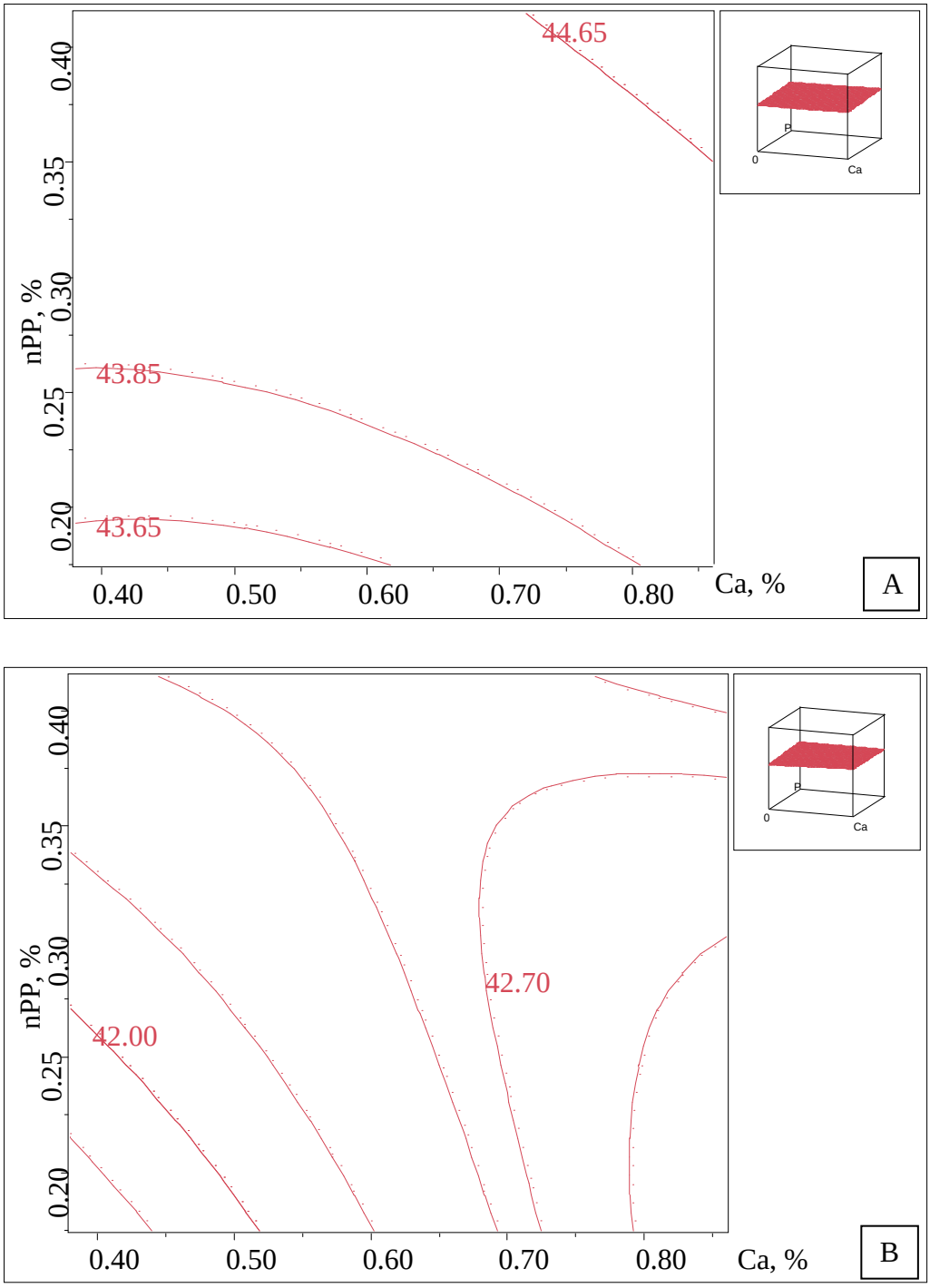

Figure IV-3. Response contours of tibia ash (%) at 49 days for females (A) and for males (B) with varying dietary levels of Ca and nPP during the finisher phase (36-49 days).

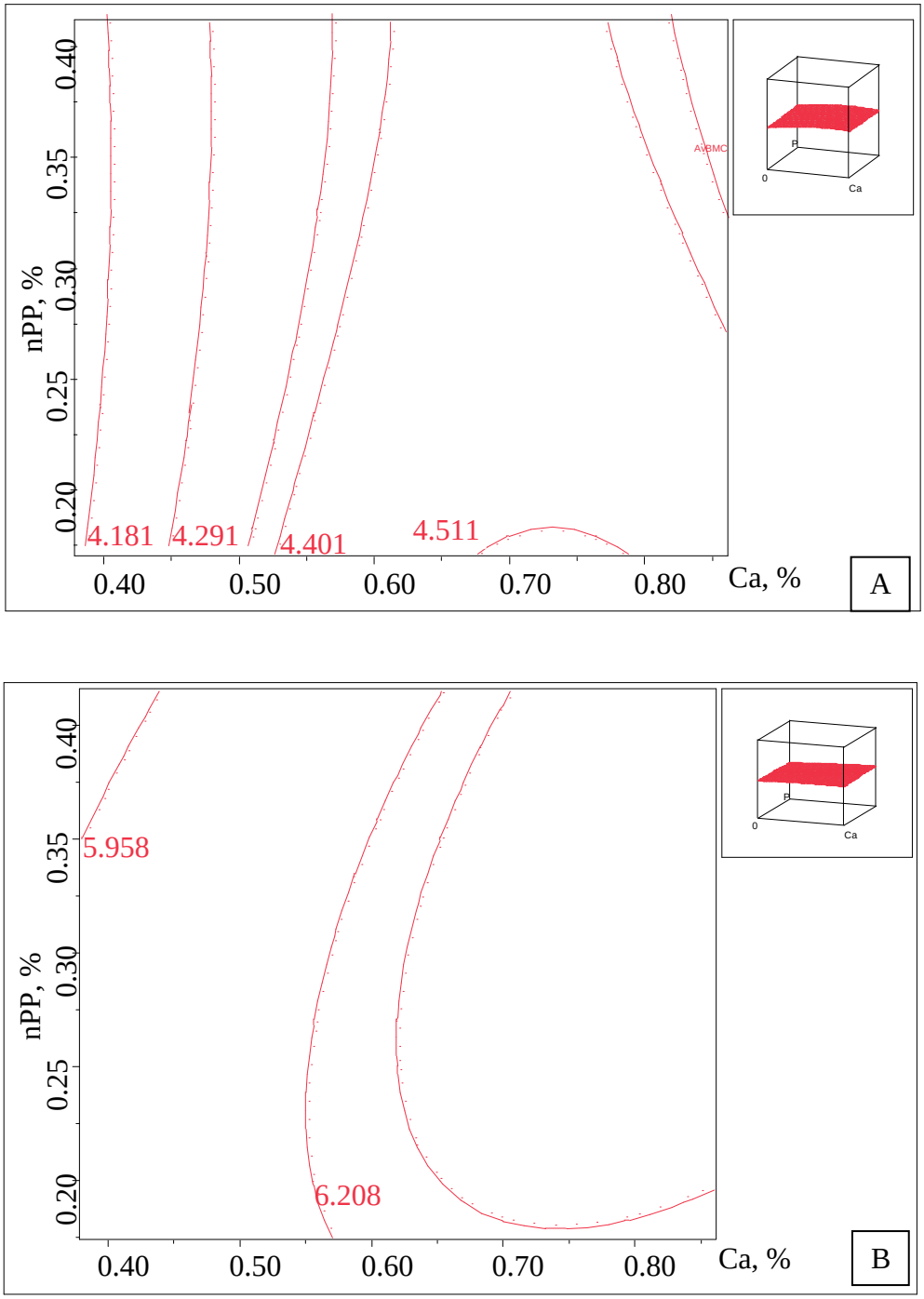

Figure IV-4. Response contours of bone mineral content (g) at 49 days for females (A) and for males (B) with varying dietary levels of Ca and nPP during the finisher phase (36-49 days).

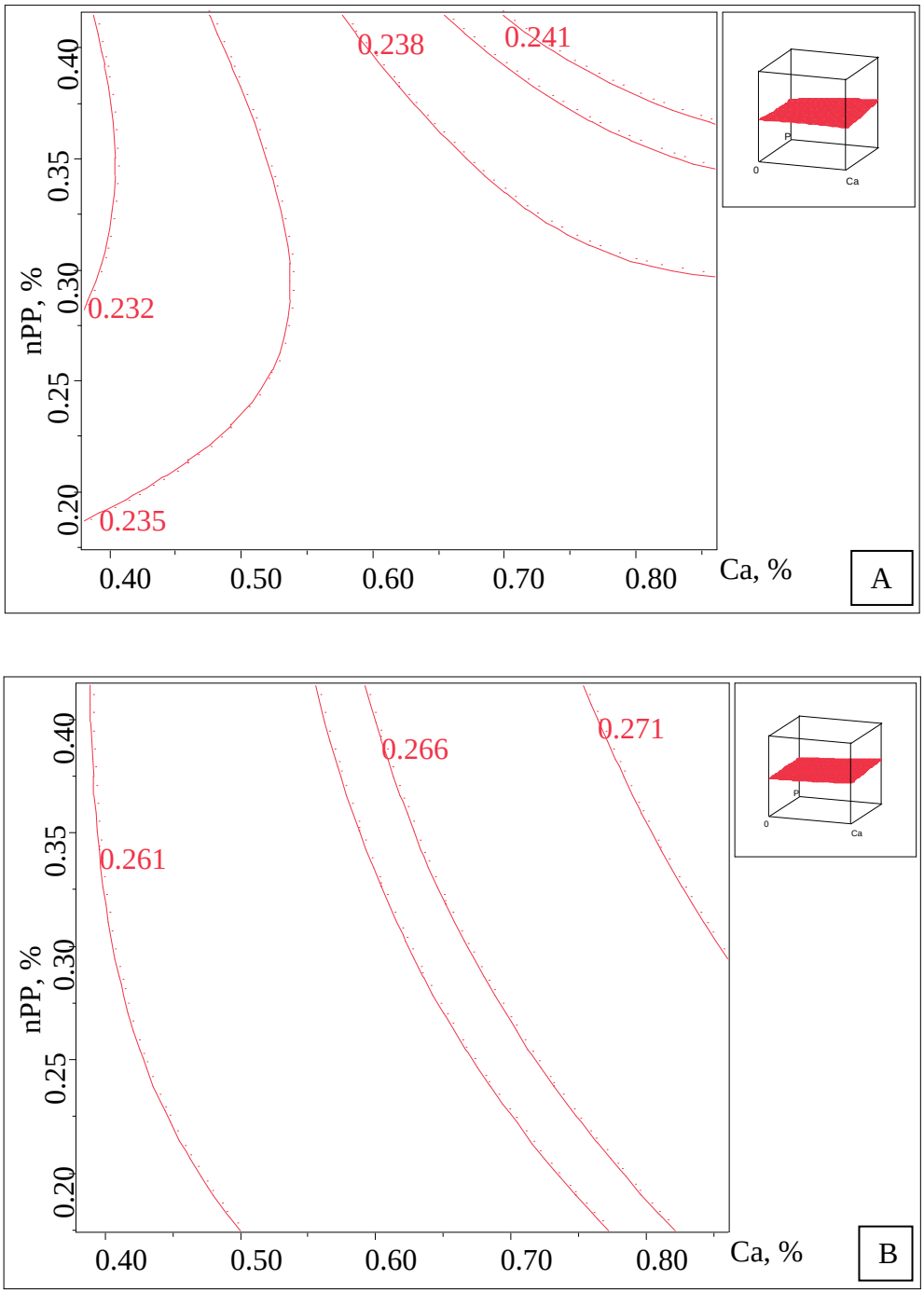

Figure IV-5. Response contours of bone mineral density (g/cm^2) at 49 days for females (A) and for males (B) with varying dietary levels of Ca and nPP during the finisher phase (36-49 days).

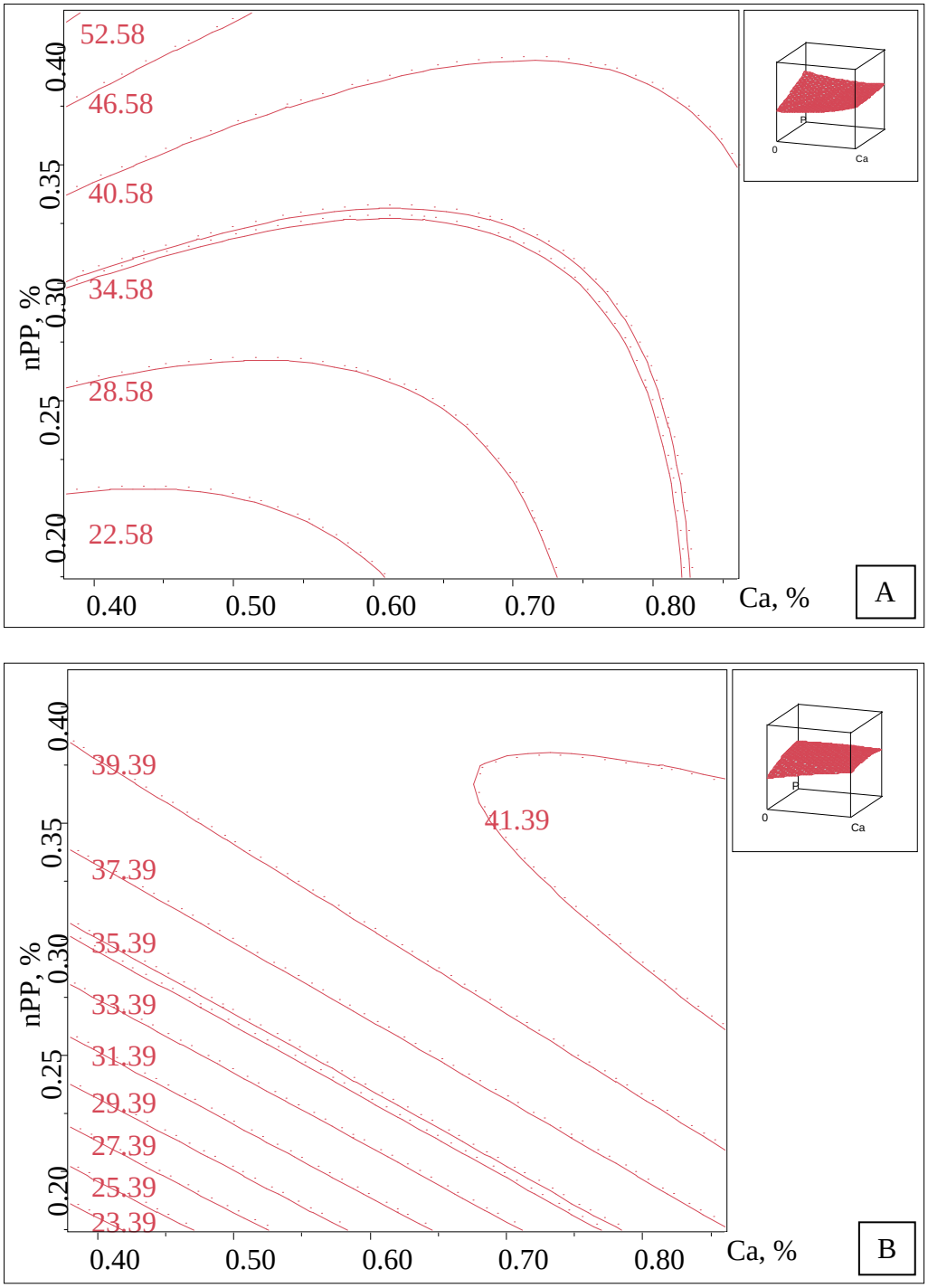

Figure IV-6. Response contours of the retention (%) of Ca (A) and P (B) at 44 days with varying dietary levels of Ca and nPP during the finisher phase (36-49 days).

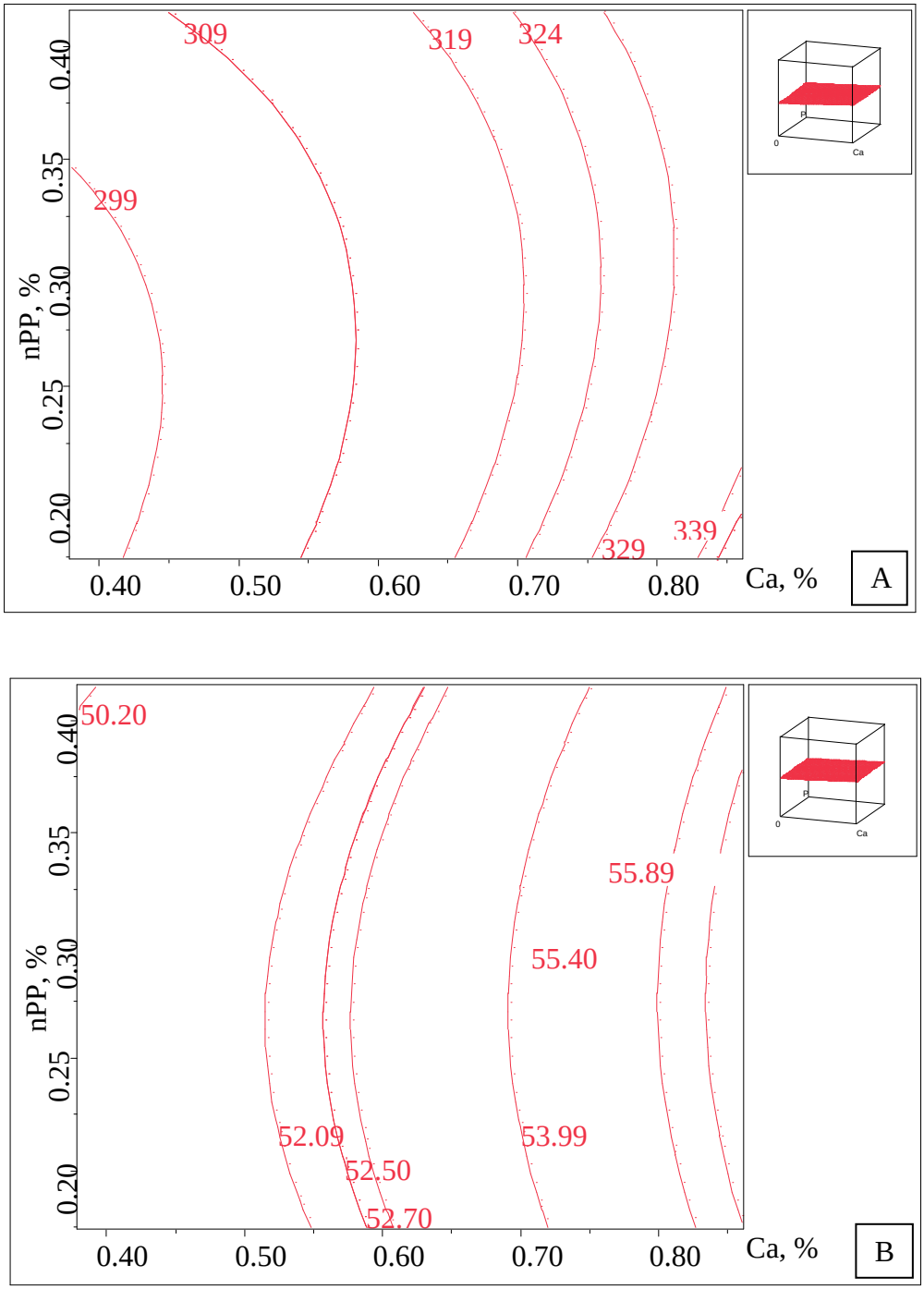

Figure IV-7. Response contours of the male bone strength at 49 days, represented as the maximum force in N (A) and the shear stress in MPa (B), with varying dietary levels of Ca and nPP during the finisher phase (36-49 days).

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CONCLUSION

Calcium (Ca) and phosphorus (P) levels in broiler diets have been largely discussed due to their vital functions, which are interrelated in most metabolic processes, especially in bone formation. The breeding companies have been selecting chickens that are able to reach market weight in less time with each generation, and a proper skeletal development is becoming more constraining for optimum growth. Moreover, consumer concerns about animal welfare have driven broiler producers to consider leg abnormalities and walking ability as requisites in welfare audits. Dietary P levels also has a great impact on feed cost, and due to environmental issues, P excretion is regulated in regions where poultry production is concentrated and litter is used as fertilizer. All these reasons motivate the poultry industry and researchers to find ways to reduce dietary P levels and to improve the ability of broilers in utilizing this mineral. Although Ca is an inexpensive nutrient, it has important effects on broiler production parameters and on P absorption and utilization. The most important strategy to better utilize these minerals is by precise feed formulation, where broilers are fed levels of Ca and P as close as possible to their requirements, always considering genetic variations and growth characteristics.

As discussed in the literature review, many researchers have shown that the dietary Ca and P levels required for optimum performance and bone mineralization seem to be lower than the ones recommended by the NRC (1994) and currently used in the USA industry. However, few studies that integrate growth performance, bone mineralization and strength, leg abnormalities, and welfare for the different dietary phases were published. Thus, the project reported herein was a comprehensive evaluation of different combinations of Ca and

P fed in starter (0-17 days), grower (18-35 days) days, and finisher (36 to 49 days) diets for Heritage broilers (Perdue Farms Inc.). This research provided adequate data that help the industry on deciding the levels to be used based on their specific goals. Additionally, this project also provided the first evaluation of the effects of mineral nutrition on bone breakage during mechanical deboning.

In chapter II, the optimum performance during the starter phase (0 to 17 days) was obtained when Heritage broiler chickens were fed 0.90 to 0.94% Ca and 0.42 to 0.44% non-phytate P (nPP), and that bone mineralization and strength were maximized at the highest levels tested (1.04% Ca and 0.52% nPP). Nevertheless, flock uniformity, leg abnormalities, and walking ability at 42 days, as well as on TD incidence and severity at 17 or 49 days of age were not affected by the starter period treatments. Broiler chicks retained P the most during the starter phase (at 10 days of age) when fed diets containing levels of Ca higher than 0.85% combined with 0.37% nPP. At 45 days, P retention was still influenced by the levels of Ca fed in the starter feed, improving as the levels of this mineral increased from 0.85 to 1.04%.

The chapter III demonstrated that optimum growth performance of Heritage broilers was achieved with dietary Ca between 0.75 and 0.90% combined with 0.41 to 0.44% nPP during the grower phase (18 to 35 days). Bone mineralization and strength were maximized with the highest Ca and nPP levels used in grower diets (0.94% and 0.44%, respectively); however, these high levels were related to increased incidence of bone and leg problems at 49 days. No treatment effects were observed on broiler walking ability at 49 days. The

highest retention of Ca and P during the grower phase occurred when chickens were fed diets containing Ca levels between 0.90 and 0.94% combined with 0.33 to 0.35% nPP.

Chapter IV showed the effects of dietary Ca and nPP levels on production parameters during the finisher phase (36 to 49 days). Optimum FCR and the highest P retention were observed when Heritage broilers were fed diets containing levels of Ca higher than 0.80% and nPP levels about 0.27%. In general, bone mineralization and strength were enhanced with increasing dietary levels of Ca and nPP. Low levels of nPP (< 0.25%) reduced the probability of epiphyseal breakages during mechanical deboning. Valgus/varus deformities, TD incidence, and TD severity were all affected by the Ca:nPP ratio rather than the individual levels of these minerals, and widening the ratio (above or below 2:1) resulted in higher probability of incidence of those problems. Furthermore, low levels of Ca and nPP (< 0.45% and < 0.20%, respectively) reduced the probability of incidence of total leg problems and crooked toes.

In summary, performance, bone mineralization, and bone strength were improved by using diets with high levels of Ca and nPP during the starter and grower phases. No significant effects of the treatments fed during the starter phase were observed on leg abnormalities and walking ability at market age. However, broilers fed high levels of Ca and nPP during the grower phase had higher probability of incidence of bone and leg problems. In order to obtain maximum bone mineralization and strength at 49 days, the levels of Ca and nPP fed during the finisher phase were higher than those that resulted in optimum performance, and these high levels did not reduce the probability of incidence of bone and leg abnormalities. P retention responded to dietary Ca and nPP differently in each dietary

phase, and also showed effects of the levels fed in starter and grower diets in the subsequent feeding periods.

APPENDICES

Appendix A

House temperature experiment 1 (Chapter II):

Age	Average	Maximum	Minimum
days	----------°C----------		
0 - 3	37.9	41.4	31.4
4 - 7	32.4	36.5	29.4
8 - 14	27.6	29.9	25.5
15 - 21	25.1	27.6	22.5
22 - 28	23.0	25.5	20.0
29 - 35	22.8	25.9	18.3
36 - 42	22.4	25.1	19.6
43-49	20.4	23.5	17.3

House temperature experiment 2 (Chapter III):

Age	Average	Maximum	Minimum
days	----------°C----------		
0 - 3	35.9	38.9	31.4
4 - 7	30.3	32.7	28.4
8 - 14	28.1	30.7	25.3
15 - 21	26.2	28.7	24.0
22 - 28	25.0	28.2	20.7
29 - 35	25.0	29.0	21.4
36 - 42	24.3	32.2	19.1
43-49	24.9	30.7	21.1

House temperature experiment 3 (Chapter IV):

Age	Average	Maximum	Minimum
days	----------°C----------		
0 - 3	34.1	39.6	32.6
4 - 7	32.4	36.3	28.8
8 - 14	29.5	33.7	25.2
15 - 21	28.7	33.4	24.2
22 - 28	27.5	34.1	20.7
29 - 35	26.5	32.3	21.7
36 - 42	26.7	33.0	20.9
43-49	26.8	33.9	21.0

Appendix B

ANOVA results of variables evaluated in Experiment 1 (Chapter II):

Term	BWG 0-18 d		FI 0-18 d	FCR 0-18 d	BWG 0-49 d		Mortality 0-49 d	
	Females	Males			Females	Males	Females	Males
Intercept	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.6161	0.0161
Ca	0.0031	<.0001	0.0627	0.0003	0.5416	0.2045	0.6465	0.5320
nPP	0.5651	0.4204	0.0971	0.6841	0.1794	0.0764	0.9595	0.4017
Ca^2	0.0097	0.0029	0.0177	0.0194	0.0559	0.9914	0.1607	0.0426
Ca*nPP	0.5566	0.5788	0.9511	0.4719	0.1605	0.7055	0.3230	0.7545
nPP^2	0.4001	0.1085	0.0236	0.4414	0.4233	0.5518	0.1607	0.3793

Term	Ca retention		P retention		Excretion 0-18 d	
	10 d	45 d	10 d	45 d	Ca	P
Intercept	<.0001	<.0001	<.0001	<.0001	0.0156	0.4744
Ca	0.0016	0.2064	0.7383	0.0161	<.0001	0.6575
nPP	0.8052	0.4287	0.0383	0.0249	0.800	<.0001
Ca^2	0.7267	0.0115	0.9365	0.0135	0.8951	0.4175
Ca*nPP	0.1268	0.7768	0.3714	0.3259	0.0734	0.4668
nPP^2	0.0429	0.1724	0.0503	0.9219	0.0875	0.0962

Term	Tibia ash 17 d		BMC 49 d		BMD 49 d	
	Females	Males	Females	Males	Females	Males
Intercept	<.0001	<.0001	0.0117	0.6674	<.0001	0.0011
Ca	0.0005	0.7152	0.8058	0.2357	0.3688	0.0050
nPP	0.0039	0.0018	0.3786	0.9227	0.0231	0.9355
Ca^2	0.9495	0.8754	0.4998	0.5776	0.6498	0.5496
Ca*nPP	0.9517	0.7079	0.1403	0.7077	0.8424	0.0990
nPP^2	0.9062	0.7667	0.0347	0.0141	0.8225	0.0033
BW			0.0005	0.0001	0.9852	0.0006

Term	Mechanical Deboning 49 d	Tibia Breaking strength - Force 17 d	
	Diaphyseal breakage	Females	Males
Intercept	0.4340	<.0001	0.0067
Ca	0.0313	0.3686	0.0033
nPP	0.4551	0.7479	0.1697
Ca^2	0.0224	0.6158	0.3114
Ca*nPP	0.8499	0.4080	0.7589
nPP^2	0.4563	0.3607	0.6116
Sex (female)	0.1297		

ANOVA results of variables evaluated in Experiment 2 (Chapter III):

Term	BWG 18-35 d		FI 18-35 d	FCR 18-35 d	BWG 0-49 d	
	Females	Males			Females	Males
Intercept	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Ca	0.0324	0.0709	0.1982	0.1296	0.0487	0.0012
nPP	0.2253	0.0515	0.8681	0.0068	0.8274	0.1451
Ca^2	0.0177	0.0397	0.4208	0.0035	0.5972	0.7191
Ca*nPP	0.1313	0.0546	0.0869	0.9724	0.5366	0.0178
nPP^2	0.3033	0.4723	0.4988	0.2062	0.5209	0.8034

Term	Mortality 18-35 d	Mortality 0-49 d
	Males	Females
Intercept	0.6578	0.4513
Ca	0.6384	0.8474
nPP	0.6381	0.0313
Ca^2	0.6365	0.1815
Ca*nPP	0.0169	0.1564
nPP^2	0.3280	0.3616

Term	Ca retention		P retention		Excretion 18-35 d	
	31 d	44 d	31 d	44 d	Ca	P
Intercept	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Ca	<.0001	0.1852	<.0001	0.8780	<.0001	<.0001
nPP	0.3683	0.6145	0.6015	0.2277	0.2714	<.0001
Ca^2	0.0291	0.0046	0.7272	0.0444	0.0023	0.7453
Ca*nPP	0.0829	0.9767	0.3132	0.6540	0.0310	0.0024
nPP^2	0.0064	0.8756	0.0002	0.5325	0.0234	0.0348

Term	Tibia ash 35 d		BMC 49 d		BMD 49 d	
	Females	Males	Females	Males	Females	Males
Intercept	<.0001	<.0001	<.0001	0.5820	<.0001	<.0001
Ca	0.0008	0.0117	0.1745	0.4054	0.3328	0.5637
nPP	0.0043	0.0125	0.5250	0.0065	0.2564	0.0087
Ca^2	0.3425	0.6303	0.5235	0.2519	0.0639	0.4369
Ca*nPP	0.2035	0.2463	0.4968	0.3773	0.5495	0.1482
nPP^2	0.0993	0.6289	0.2256	0.8198	0.1642	0.6848
BW			<.0001	<.0001	0.0084	<.0001

Term	Tibia strength - Force 35 d		Tibia strength - Shear stress 35 d	
	Females	Males	Females	Males
Intercept	<.0001	<.0001	<.0001	<.0001
Ca	<.0001	<.0001	<.0001	0.0145
nPP	0.3881	0.9078	0.4798	0.2597
Ca^2	0.0392	0.8834	0.3808	0.7414
Ca*nPP	0.1270	0.5953	0.0058	0.8185
nPP^2	0.1967	0.0807	0.0125	0.3929

Term	Type of leg abnormality 49 d		TD incidence 35 d	Mechanical Deboning 49 d
	Severe valgus	Crooked toes		Epiphyseal breakage
Intercept	0.0937	0.0573	0.2644	0.7995
Ca	0.3117	0.0254	0.5409	0.9707
nPP	0.1166	0.1145	0.0203	0.7884
Ca^2	0.0881	0.1830	0.8852	0.4638
Ca*nPP	0.1392	0.0217	0.2979	0.0586
nPP^2	0.0201	0.5141	0.0061	0.8001
Sex (female)	0.0005	<.0001	0.5738	0.2047

ANOVA results of variables evaluated in Experiment 3 (Chapter IV):

Term	FI 36-49 d	FCR 36-49 d	FCR 0-49 d	BW CV% 49 d	
				Females	Males
Intercept	<.0001	<.0001	<.0001	<.0001	<.0001
Ca	0.8671	0.6523	0.8086	0.0983	0.1426
nPP	0.0100	0.7568	0.9053	0.7737	0.3818
Ca^2	0.9551	0.7081	0.5142	0.4213	0.6257
Ca*nPP	0.8632	0.0476	0.9496	0.0556	0.5307
nPP^2	0.9054	0.0632	0.0889	0.3958	0.9200

Term	Ca retention	P retention	Excretion 36-49 d	
	44 d	44 d	Ca	P
Intercept	0.0001	<.0001	0.0002	<.0001
Ca	0.1375	0.0041	<.0001	0.0595
nPP	<.0001	0.0001	<.0001	0.0022
Ca^2	0.2528	0.4761	0.3198	0.2111
Ca*nPP	0.0020	0.0104	0.7639	0.0055
nPP^2	0.9590	0.0529	0.8114	0.5663

Term	Tibia ash 49 d		BMC 49 d		BMD 49 d	
	Females	Males	Females	Males	Females	Males
Intercept	<.0001	<.0001	0.0076	0.0002	<.0001	<.0001
Ca	0.1678	0.0218	0.0019	0.044	0.0081	0.0042
nPP	0.0126	0.4124	0.3023	0.7445	0.3405	0.2138
Ca^2	0.6896	0.7668	0.0144	0.5315	0.4754	0.7374
Ca*nPP	0.7672	0.0776	0.6123	0.4386	0.0496	0.4518
nPP^2	0.9612	0.7245	0.7872	0.7216	0.3336	0.9488
BW			<.0001	0.2108	0.0306	0.2605

Term	Tibia strength - Force 49 d		Tibia strength - Shear stress 49 d	
	Females	Males	Females	Males
Intercept	<.0001	<.0001	<.0001	<.0001
Ca	0.1584	0.0005	0.6087	0.0015
nPP	0.2328	0.7784	0.341	0.7872
Ca^2	0.2355	0.7404	0.7634	0.7451
Ca*nPP	0.4086	0.6508	0.6877	0.9489
nPP^2	0.4474	0.5601	0.8896	0.7003

Term	Type of leg abnormality 49 d			Tibial dyschondroplasia	
	Total	Crooked toes	Valgus/Varus	Incidence	Severity
Intercept	0.1072	0.0067	0.2767	0.1124	0.0932
Ca	0.0013	<.0001	0.016	0.323	0.4549
nPP	0.7515	0.1334	0.9382	0.0421	0.0393
Ca^2	0.0077	<.0001	0.0538	0.9003	0.8813
Ca*nPP	0.1116	0.0554	0.1721	0.0651	0.1309
nPP^2	0.8146	0.5894	0.6115	0.1162	0.0709
Sex (female)	<.0001	0.0203	0.0034	<.0001	0.0006