ABSTRACT

SAN MARTIN DIAZ, VIVIANA EDITA. Effects of 1-α-hydroxycholecalciferol and Other Vitamin D Analogs on Live Performance, Bone Development, Meat Yield and Quality, and Mineral Digestibility on Broilers. (Under the direction of Dr. Edgar O. Oviedo-Rondón).

Total Ca levels in broiler diets can be under or overestimated. The reasons for this can go from simple failures on the mixing to the current tendency to considering lower dietary Ca. Vitamin D plays an important role in intestinal Ca absorption that leads to adequate blood levels of Ca and P for an adequate bone development and health. An analog molecule of $VitD_3$, 1-alpha–hydroxycholecalciferol ($1\alpha(OH)D_3$), has been demonstrated to fulfill these functions. However, $1\alpha(OH)D_3$ its effect under different Ca levels has not been evaluated. To address this question, two experiments were conducted. Male broiler chicks were reared until 14d of age using 5 different Ca levels between 0.80 and 1.40% (Chapter 2) or until 35 d using 4 Ca levels between 0.54 and 1.20% (Chapter 3). In starter diets, an improvement in FCR was detected in diets with 0.80% Ca when $1\alpha(OH)D_3$ is supplemented. Also, this supplementation affected bone development inducing lower bones relative asymmetries, increased mineral digestibility when no deficient Ca diets were used. In the grower phase study, parameters related to bone development and meat quality were assessed. No effects of $1\alpha(OH)D_3$ were detected on these parameters. However, an effect of dietary Ca level was detected on valgus, meat color and spaghetti muscle myopathy incidence. Similarly, regression analyses indicated a quadratic effect of Ca on carcass and breast meat yield, with the higher yields detected at 0.76% Ca inclusion. Bone development, tibia strength and thickness and ash tibia ash content. $1\alpha(OH)D_3$ increased bone ash content as well. A third study was conducted to compare three $VitD_3$ metabolites: $1\alpha(OH)D_3$, $25(OH)D_3$ and $1,25(OH)_2D_3$ (Chapter 4). These metabolites were added on top of a basal diet containing 4,000 IU/kg of $VitD_3$ as part of the mineral premix. Chickens fed with $1\alpha(OH)D_3$ obtained similar BW and BWG than chickens fed with feed

with no metabolite addition. This treatment also obtained higher values when compared to the chickens fed with $25(OH)D_3$ and $1,25(OH)_2D_3$ diets. It was also detected that chickens fed the $1,25(OH)_2D_3$ had the worst FCR compared to the other treatments. Chickens fed $1\alpha(OH)D_3$ also obtained lower bone length relative asymmetry in comparison to other treatments. Furthermore, in meat quality, less cook loss was obtained by the non-supplemented and $1\alpha(OH)D_3$ treatments. In relation with myopathies, the highest and lower presentation of wooden breast were obtained with $25(OH)D_3$ and $1\alpha(OH)D_3$, respectively. Therefore, it can be concluded that adequate dietary Ca levels have considerable effects on live performance, bone health and meat quality. Based on these results, addition of $1\alpha(OH)D_3$ has positive effects on improving mineral digestibility and bone development. Likewise, the potency of each $VitD_3$ metabolite varies differently according to the desired variable to be improved.

Effects of 1-α-hydroxycholecalciferol and Other Vitamin D Analogs on Live Performance, Bone Development, Meat Yield and Quality, and Mineral Digestibility on Broilers.

by
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DEDICATION

To my beloved parents, Iris y Pedro, for their support, love and for being the best example to follow in my life.

To my brothers, Susana y Pedro, for trusting and always being there for me.

To my whole family, for simply being as they are.

BIOGRAPHY

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CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Vitamin D, a fat-soluble vitamin also known as calciferol, is a nutrient, necessary for an adequate intestinal calcium (Ca) absorption and transportation, which guarantee bone health and strength. It is also important for various physiological processes. Phosphorous absorption and homeostasis with Ca, as well as micro mineral regulation (P, Mn, I, Cu, Zn), and even immunity of the bird can be affected by blood VitD levels (Biehl et al., 1995; Vazquez et al., 2018).

VitD can be synthesized in the skin by sun exposure and ultraviolet B radiation. Therefore, for many decades, since broiler rearing conditions made sunlight access limited, it is impossible or limited that the skin of the animal receive the necessary radiation to synthetize adequate VitD levels. For this reason, the consideration of VitD in poultry feed formulation is necessary, especially because of the rapid bone and body growth rate present in broilers.

Different VitD precursors have been study in the past decades (Haussler et al., 1973; Mitchell and Edwards, 1995; Han et al., 2009; Bachmann et al., 2013). The interest in searching for alternative molecules to provide cholecalciferol in the diets is due to the different biopotency and faster metabolic activity that can allow to obtain better broiler live performance. How they affect broiler performance and mineral metabolism under different conditions is a topic of truly importance (Biehl et al., 1995; Rao et al., 2009; Han et al., 2016).

There are three metabolites of VitD currently used in poultry and animal nutrition. The 25-hydroxycholecalciferol ($25(OH)D_3$), 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and 1-alpha-hydrocxicholecalciferol ($1\alpha(OH)D_3$) are metabolites that can be found as commercial products. To determine the effect of these molecules is necessary and valuable for the poultry industry, where stressors, anti-nutritional factors and toxic compounds like mycotoxins and heavy metals, can affect liver and kidney function (Devegowda, 2009; Bryden, 2012; Murugesan et al., 2015) or the Ca dietary content can be compromised (Van Kempen et al., 2001; Adedokun and Adeola 2013; Li et al., 2017).

In the present study, the focus was on the effect produced by $1\alpha(OH)D_3$ in broiler performance, bone development and bone mineralization, as well as mineral retention and digestibility under different dietary Ca levels. Although this analog molecule of the active $VitD_3$ has been previously studied under various conditions and demonstrated its positive effects increasing bone mineralization and obtaining better performance parameters with higher BW and low FCR (Edwards et al., 1992; Biehl et al., 1995; Edwards Jr. et al., 2002; González et al., 2015), a detailed study of its effects under different dietary Ca levels in broiler chickens reared under the same conditions has not been carried out. Furthermore, mineral digestibility and retention of trace minerals under these conditions have not been reported. In the present study P, Ca, Mn, Cu, Zn were the minerals selected for the determination of its digestibility and/or retention, due to their participation in many digestive and physiological processes, including bone development.

The present review will deal with the topics of VitD metabolism and its relationship with the Ca and P homeostasis. Likewise, aspects related to leg bone development and mineral digestibility will be developed. The intention of this revision is to be able to have a clear vision of the findings obtained in the experiments that constitute this thesis.

VITAMIN D AND ITS METABOLISM

Vitamin D is a fat-soluble vitamin that synthesized in the outer skin layers when they are exposed to sufficient sunlight (McDowell, 2000). In the nature, ergocalciferol ($VitD_2$) and cholecalciferol ($VitD_3$) are two natural forms of VitD. The first one, $VitD_2$, occurs predominantly in plant sources and it is generated by the ultraviolet irradiation of the ergosterol (steroid found in plants), while $VitD_3$ is produced mainly in animal tissues upon the irradiation of the 7-dehydrocholesterol (provitamin D) in skin (McDonald et al., 2011).

It is known that $VitD_2$ has little to no-activity (nutritional value) for avian species (NRC, 1994). However, $VitD_3$ is about 10-fold more effective in chicks than $VitD_2$ (Hurwitz et al., 1967; Adedokun and Adeola, 2013). Throughout this document the attention will be centered on $VitD_3$ molecule.

$VitD_3$ is the responsible to ameliorate the effects of a low Ca level, as rickets or dyschondroplasia. Also, it has been detected effects of VitD on immunological responses, where immune cells containing VitD receptors (VDR) can react to $VitD_3$ modulating innate and adaptive immune responses. (Baeke et al., 2010; Aranow, 2011). One international unit (IU) of VitD is defined as the activity of 0.025 μg of $VitD_3$ (NRC, 1994). As mentioned previously, VitD may be supplied through the diet or by UV irradiation of the skin. Natural cholecalciferol is produced exclusively in animal products by UV irradiation of 7-dehydrocholesterol from the sunlight. 7-dehydrocholesterol is derived from cholesterol or squalene, which is synthesized in the body and present in large amounts in skin, intestinal wall, and other tissues (McDowell, 2000). Nevertheless, the VitD obtained by chickens from many years ago is too little or null. Consequently, the source of VitD in chickens comes from its dietary ingestion as form of cholecalciferol.

To obtain the active $VitD_3$, the dietary cholecalciferol molecule needs to undergo two consecutive hydroxylations. The first reaction will be done in the liver, where the 25-hydroxylase catalyzes the transformation of cholecalciferol into 25-hydroxycholecalciferol by a hydroxylation at position 25. Later, at the proximal tubules of the kidney, 1-alpha-hydroxylase will catalyze the hydroxylation of 25-hydroxycholecalciferol into 1,25 dihydroxycholecalciferol (calcitriol). This last molecule is the active $VitD_3$ that will act in the specific tissues. Other VitD metabolites can be produced in the kidney, especially in situations of sufficient Ca levels in plasma. In this situation 24,25-dihydroxycholecalciferol is produced. Nevertheless, its effectiveness and physiological importance of this molecule is under discussion and investigation (Henry, 2011). Figure 1.1 shows the metabolic pathway of $VitD_3$ activation.

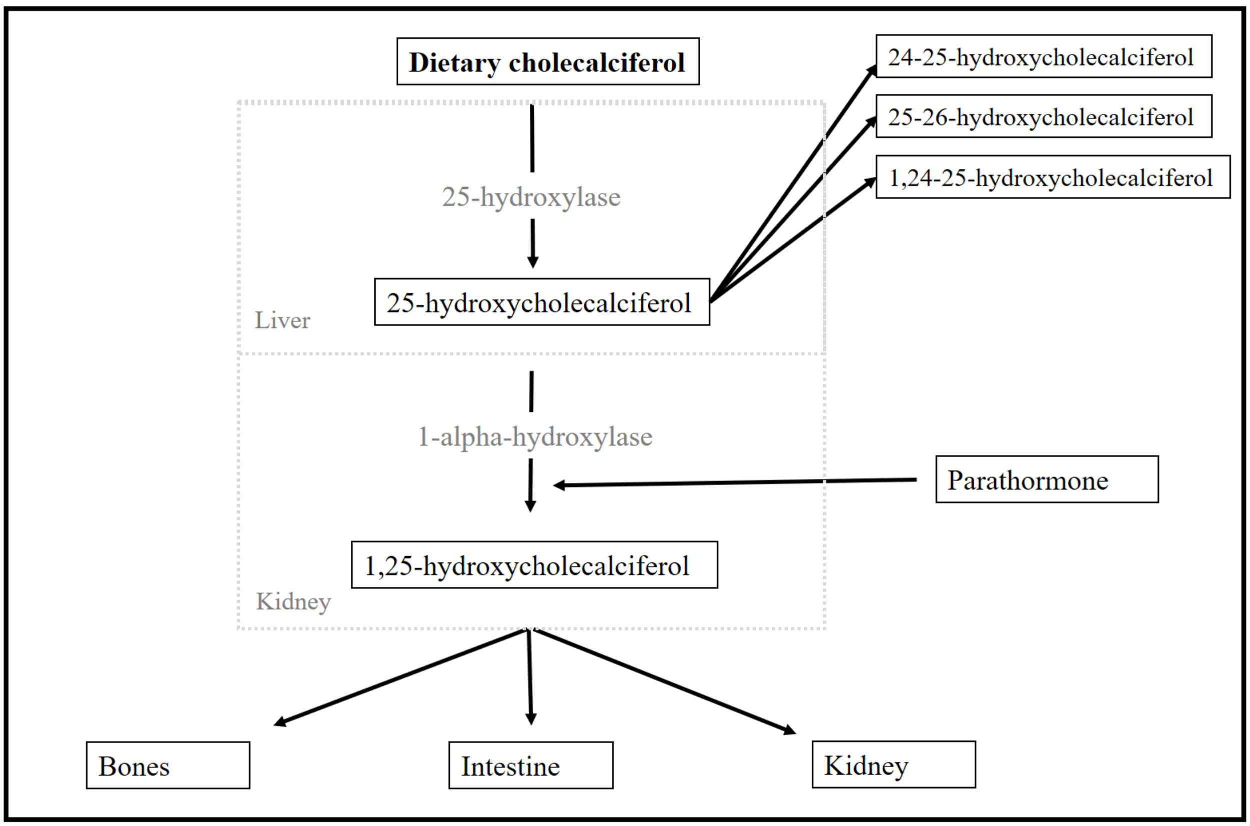

Figure 1.1. Metabolic pathway in chickens of dietary Vitamin D (cholecalciferol) and hormonal activation.

Mucosal epithelial cells, in the intestine, kidney, cartilage, osteocytes and osteoblasts, have receptors for $1,25(OH)_2D_3$. (Veum, 2015). $1,25(OH)_2D_3$ plays role in the regulation of the Ca absorption by the regulation of the specialized protein calbindin. This protein will be in charge of binding Ca^{2+} molecules to allow its absorption and further transport of Ca^{2+} through the intestinal cell (Christakos et al., 2014).

VITAMIN D3 ANALOGS

Vitamin D is generally provided to poultry by supplementation of the diet with crystalline forms of cholecalciferol (Vitamin D_3) (Fritts and Waldroup, 2003). It is known that the $VitD_3$ requirement is dependent on the dietary Ca and available phosphorus (avP) contents in diets (Rath et al., 2000).

The National Research Council (NRC, 1994) indicated a minimum requirement of 200 IU/kg of $VitD_3$ for poultry diets. However, under current commercial practices, broiler and turkey diets contain from 2,000 to over 5,000 IU/kg (Applegate and Angel, 2014). In addition to these high concentrations of $VitD_3$, the supplementation of animal feed with metabolites products from the hydroxylation pathway is becoming frequently. There are three different metabolites of $VitD_3$ commercially available: 25(OH) D_3 (HyD®, DSM Nutritional Products Inc, Parspany NJ), the $1,25(OH)_2D_3$ (Panbonis®, Herbonis Animal Health GmbH, Switzerland) and the $1\alpha(OH)D_3$ (AlphaD3®, Premex, Medellin, Colombia).

The first one, $25(OH)D_3$, is a synthetic analog that have been studied widely and demonstrated its bioactivity in bone development, bone mineralization and performance (Parkinson and Cransberg, 2004; Chou, et al., 2009; Han et al., 2016;Vazquez et al., 2018). It only requires a hydroxylation at the kidney for becoming active $VitD_3$. This decreases the time of activation and the expense of energy in the hydroxylation. However, it is known that the effectiveness of the hydroxylation at the liver is about 95%, decreasing the percentage of the activated product. Nowadays, commercial products with more stable molecules have been study under commercial conditions. Han et al. (2016) showed that $25(OH)D_3$ was 2.03 times more active than $VitD_3$ in growth performance and bone mineralization in chickens. Similar results were obtained by Fritts and Waldroup (2003), who also detected a reduction in tibia dyschondroplasia with $25(OH)D_3$ supplementation. Moreover, its effects has been demonstrated on broiler immunity with an increment of cellular immune response (Vazquez et al., 2018).

The $1,25(OH)_2D_3$ is the active hormone metabolite of Vitamin D that does not require hydroxylation in the liver or kidney as other analogs. This metabolite, $1,25(OH)_2D_3$, is commercially available as Panbonis®. It comes from natural extract of *Solanum glaucophyllum* leaves, which contains $1,25(OH)_2D_3$-glycosides (Bachmann et al., 2013). Because this product comes from a natural source, it can be make less consistent in its activity and biopotency than other analogs (European Food Safety Authority, 2015). Previous studies demonstrated the ability of $1,25(OH)_2D_3$ to improve body weight as well as its additive effect with phytase for Ca, P and Zn retention (Roberson and Edwards, 1994; Mitchell and Edwards, 1996; Edwards Jr, 2002). Its effectiveness in leg health on prevention of tibia dyschondroplasia have been demonstrated as well (Rennie et al., 1995; Rennie and Whitehead, 1996).

Finally, $1\alpha(OH)D_3$ was first described by Haussler et al. (1973), who defined this vitamin D analog as to its benefits in rachitic chicks. $1\alpha(OH)D_3$ lacks the hydroxyl group at the 25-carbon position, requiring a hydroxylation process in the kidney to get to the active $1,25(OH)_2D_3$ (González et al., 2015). Dietary inclusion of $1\alpha(OH)D_3$ has the advantage of being quickly hydroxylated in the kidney to the active $1,25(OH)_2D_3$ (Edelstein et al., 1978). An important fact is the lower cost in the production of this synthetic $1\alpha(OH)D_3$ in comparison to other $VitD_3$ pre-metabolites (Biehl et al., 1995; Biehl and Baker, 1997; González et al., 2015).

Several studies have been conducted demonstrating effectiveness of utilization of $1\alpha(OH)D_3$, together or as a replacement of $VitD_3$, including improvements in growth performance (Snow et al., 2004; Han et al., 2012), decreased incidence of tibia dyschondroplasia (Rennie and Whitehead, 1996), or changes in meat quality parameters like breast and tights color and lightness changes (Han et al., 2009, 2012). Moreover, research on dietary mineral bioavailiability has been conducted determining the increment of phytate utilization with $1\alpha(OH)D_3$ supplementation (Biehl et al.,1995; Snow et al., 2004).

CALCIUM AND PHOSPHORUS ABSORPTION

Ca and P, two of the most abundant minerals in the body, must maintain homeostasis for important vital functions in the organism. In broilers, dietary Ca:P ratio should be maintained around 2:1 for an adequate balance (NRC, 1994). Changes in this ratio could affect the percentage of Ca retention and P utilization, as well as the intestinal abortion of both minerals (Plumbstead et al, 2008; Quian et al., 1997; Proszkowiec-Weglarz and Angel, 2013). This homeostasis is driven primarily by dietary levels and the feedback regulation of plasma levels of these minerals. Plasma levels of Ca and P will influence the release of hormones that regulate intestinal absorption, bone resorption and kidney excretion of Ca and P (Veum, 2015).

Among the metabolic effects of VitD, the regulation of the essential genes for the absorption of Ca can be considered the most important (DeLuca, 2014; 2016; Pike et al., 2018). Active $VitD_3$ acts through a nuclear receptor to express functions such as intestinal absorption of Ca and P, bone homeostasis, Ca mobilization in the bone, and Ca reabsorption in the kidney (Bouillon et al., 2003). These mechanisms can be considered the most important role of VitD, as it consequently maintains Ca and P homeostasis (Pike et al, 2018).

The active transport of Ca and P from the intestine is increased by a metabolite of VitD (Barrett et al., 2010). $VitD_3$ synthesis is regulated by a feedback mechanism associated with plasma concentration of Ca and P. Organs that participate in this homeostatic regulation include the parathyroid gland, intestine, kidney and bones, where are regulated by calcitriol ($VitD_3$) or calcitonin for maintaining the adequate level of Ca and P in the body (Figure ***1.2***). This regulation will depend of the Ca status level in plasma.

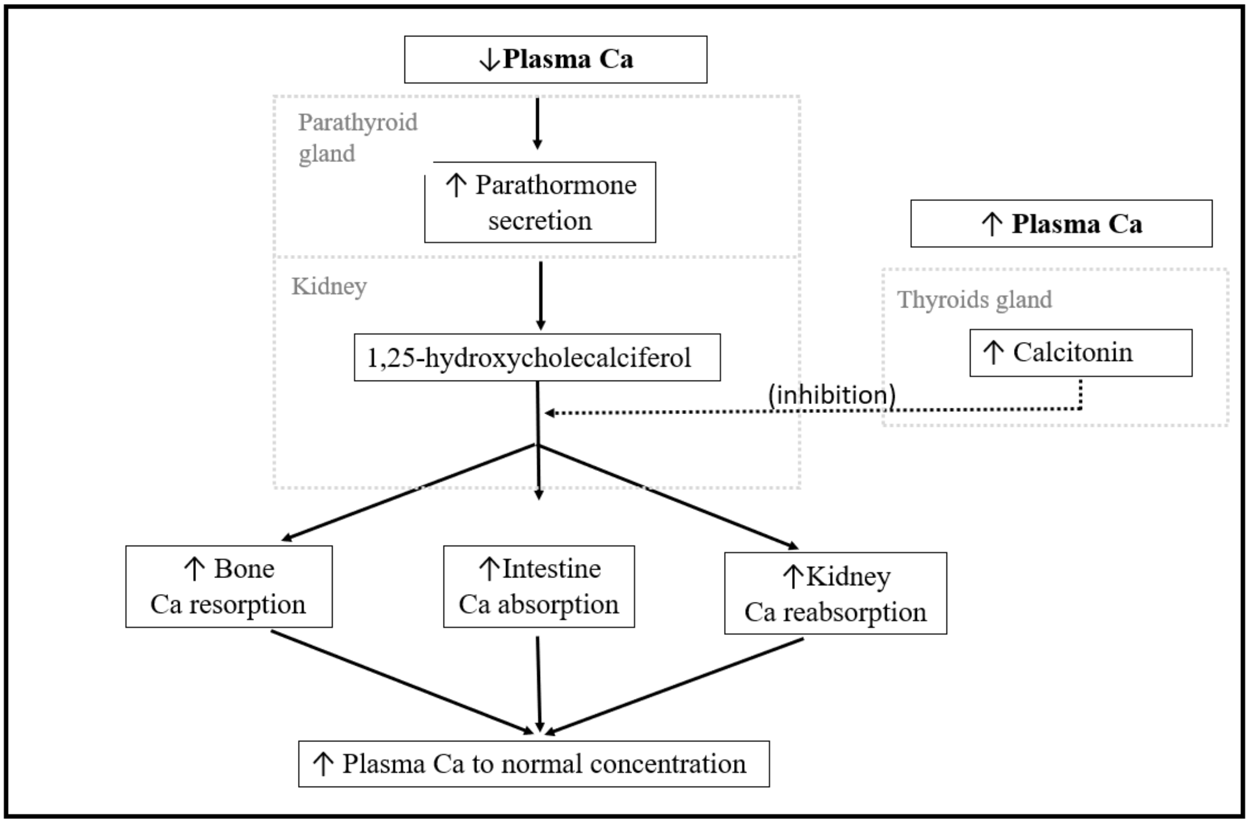

Figure 1.2. Hormonal regulation of Ca plasma levels to homeostasis.

When plasma Ca concentration level is low, the parathyroid will release parathyroid hormone (PTH), which induces the bioconversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ in kidneys by stimulating the expression of 1-hydroxylase. The hydroxylation to $1,25(OH)_2D_3$ is inhibited when plasma Ca concentration levels are adequate, thereby reducing $1,25(OH)_2D_3$ production. Therefore, $1,25(OH)_2D_3$ and plasma Ca homeostasis is regulated by controlling 1-hydroxylase and the inhibition of PTH expression (Bouillon et al., 2003). Parathyroid hormone will also activate Ca reabsorption from the kidneys and mobilize Ca from bones to plasma. Furthermore, when the plasma Ca^{2+} concentration exceeds the physiological threshold, C cells in the thyroid gland release calcitonin (CT), a peptide hormone that lowers plasma Ca^{2+}, by decreasing or preventing bone resorption. (Veum, 2015). Plasma phosphate levels also affect the PTH to regulate physiological plasma concentrations of Ca^{2+} (DeLuca, 2004).

Intestinal Ca is absorbed across the intestinal wall via two pathways: The transcellular and paracellular routes. The paracellular absorption is a passive transport through the cellular tight junctions between the cells, where the quantity of Ca transported will depend in the digesta Ca concentration in the intestinal lumen (Adedokun and Adeola, 2013; Veum, 2015). In contrast, transcellular absorption occurs by active transport through the intestinal enterocytes via epithelial Ca channels, intracellular calbindins, and the ATP-activated basolateral membrane Ca pump (Adedokun and Adeola, 2013; Veum, 2015). In the transcellular absorption, calmodulin, a Ca-binding protein, function as a Ca channel protein to transport the Ca^{2+} away from the brush border of intestinal cells (Brooner, 2003). Intracellular calbindins are Ca-binding proteins located in the intestine and kidney. They transport Ca across the intestinal cell from the apical (lumen) side to the basolateral side (Veum, 2015). This is a VitD dependent transport protein, which is upregulated as plasma Ca and P concentration levels decrease, while it is down-regulated when dietary Ca intake increases (Bar et al., 2003; Bouillon et al., 2003).

In summary, metabolic Ca and P levels are regulated by feedback mechanisms and hormonal signals, where parathormone and VitD participate in the activation of VDR that lead Ca absorption. Three are the principal organs participating in this regulation: intestine, kidney and bone. These three are responsible of Ca homeostasis and maintain adequate levels of Ca through renal resorption or bone resorption.

CA:P - BONE DEVELOPMENT/MINERALIZATION AND STRENGTH

Calcium is the most abundant mineral in the body located principally in bones. Primary mineral salt in bone occurs as hydroxyapatite crystals, $Ca_{10}(PO_4)_6(OH)_2$. Around 98% of body Ca and 80% of the P are contained in the skeleton (Adedokun and Adeola, 2013), which makes it a key mineral for bone development. Adequate Ca absorption depends on different factors, especially on Ca and P concentrations and ratio, as well as the PTH activation by active vitD_3. Three organs are actively involved in the regulation of dietary Ca absorption: the small intestine, the kidneys, and bones, while VitD plays and important role in the mobilization of Ca from these organs (DeLuca, 2004).

Bone is a metabolically active tissue, and the Ca turnover rate in bone varies widely depending on the type of bone (Williams et al., 2000). The trabecular bone is important for Ca and P resorption and maintaining the mineral plasma levels to assure Ca:P homeostasis (Eklou-Kalonji et al., 1999). Calcium in bone is in a constant turnover depending on the needs and PTH signals received. Bone resorption is accomplished by the osteoclast, which is stimulated by the PTH that is stimulated by $1,25(OH)_2D_3$. Apparently, this resorption is mediated through other cells that have the PTH receptors, such as osteoblasts or chondrocytes (Veum, 2015).

Two types of Ca reserves can be found in a bone: one is readily exchangeable, and the other is a much larger pool of stable Ca that is only slowly exchangeable (Barrett et al., 2010). Moreover, there are three cell types in the bone responsible for bone formation, mineralization and maintenance: osteoblasts, osteocytes, and osteoclasts (Rath et al., 2000). The osteoblasts are responsible for bone formation, while the osteoclasts are involved with bone resorption. Osteoblasts, which are modified fibroblast, have receptors for PTH and $1,25(OH)_2D_3$, and these hormones modulate the activity of alkaline phosphatase and the transport characteristics of Ca2+

in bone growth and remodeling (Murrills, 2006). In contrast, osteoclasts, multinucleated giant cells that develop from of the monocyte/macrophage lineage, have the ability to recognize and degrade bone. (Yan et al., 2004; Barrett et al., 2010).

This turnover of minerals in the bone is associated with adequate bone mineralization and thereby development and strength (Williams et al., 2000). The organic matrix of bone must be mineralized, primarily with Ca and P, to produce a mature, strong bone (Veum, 2010). It has been demonstrated that dietary Ca restriction diminishes bone ash content, and this reduction is more notable with dietary P restriction (Bar et al., 2003). Phosphorus also fulfills important functions in bone mineralization and development of the bird, among other functions such as nerve function, eggshell component, phospholipids and nucleic acid (Li et al., 2017).

Maintainance of dietary Ca:P ratio plays an important role in the metabolic functions of these minerals. Ca and P imbalances could generate skeletal abnormalities, impair in growth performance and affect the digestibility and absorption of mineral by the formation of complexes that could impede adequate nutrient utilization (Qian et al., 1997; Gautier et al., 2017). Ca:P ratio recommended for poultry by the US National Research Council (NRC, 1994) is of 2:1, however more recent studies showed that different factors must be considered when using an optimum Ca:P ratio (Proszkowiec and Angel, 2013). The source and the availability of Ca and P molecules will affect its bioavailablility. Also, the effect of dietary phytases on P bioavailablility from phytates must be taken in to account (Proszkowiec and Angel, 2013; Li et al., 2017). The last edition of the Brazilian tables makes a recommendation of a Total Ca:available P ratio of 2.13 (Rostagno et al., 2017).

It is important to consider the relationship between Ca, P and $VitD_3$ (Whitehead et al., 2004). As mentioned previously in this document, metabolic Ca homeostasis is performed by feedback mechanisms regulated by proteins, hormones, intestinal receptors and vitamins. This includes different molecules such as the PTH, calcitonin, calbindin, as well as $VitD_3$ activity. An adequate level of these molecules and regulation of their action leads to the adequately activation of receptors in the small intestine, bone, and kidney getting to appropriate Ca and P homeostasis in the organism (Veum, 2010; Proszkowiec-Weglarz and Angel, 2013).

MINERAL RETENTION AND DIGESTIBILITY

In poultry, Ca digestibility must be determined at the ileal level to avoid the possible urine Ca contamination at the excreta level (Anwar and Ravindran, 2016). Calculations based on poultry excreta reflect retainable Ca, rather that digestible values (Anwar et al., 2017). The analysis of mineral digestibility gives an important knowledge of the real bioavailability for the animal to use the mineral for its metabolic process.

The bioavailability analysis includes the determination of the amount of a specific mineral with feed intake content and the quantity excreted from the feces (mineral retention) or from the ileal digesta (ileal digestibility). Both mineral retention and ileal digestibility measurements have some limitations, but they give a valid approach to recognize the real utilization of the dietary mineral. However, the measurement of total feed intake - excreta output is not 100% definable in birds, principally because of the urine content in feces, as well as the contamination caused from the excreta collection method and its contamination with scurf or feathers (Sales and Janssens, 2003).

Currently, the utilization of mineral markers to determine the mineral digestibility of feeds is widely used. The most used dietary markers for digestibility studies are the celite, chromium oxide and titanium oxide. Although there is no ideal marker for digestibility studies (Sales and Janssens, 2003), acid insoluble ash (AIA) is a widely used technique that is based in quantifying the indigestible mineral components in the sample. This technique was first described by Vogtmann et al. (1975), who utilized Celite® as an indigestible dietary marker and a solution of 4N HCl to calculate the insoluble ash content in the samples. The content of ashes obtained is correlated with the mineral content in the sample.

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CHAPTER II

Effect of 1-alpha-hydroxy-vitamin-D_3 on Performance, Bone Development and Mineral retention in starter Diets containing different Calcium levels for Ross-708 broilers

ABSTRACT

The effect of 1-alpha-hydroxy-VitD_3 (1α(OH)D_3) supplementation under different dietary calcium (Ca) content was evaluated in performance, bone development and mineral digestibility during the starter period (1 – 14 d). A factorial arrangement, with 5 dietary levels of Ca (0.8; 0.95; 1.10; 1.25; and 1.40%), and 2 levels of 1α(OH)D_3 supplementation (0 and 0.00125%) were evaluated. Four-hundred males Ross-708 day-old chicks weredistributed among 80 cages in four Petersime batteries. Interaction effects ($P < 0.05$) on BW, BW gain, feed intake, and FCR were observed at 14d. BW and BW gain were reduced ($P < 0.05$) in chicks fed 1α(OH)D_3-supplemented diets containing 1.4% dietary Ca. Moreover, 1α(OH)D_3 supplementation improved FCR up to 7 points, when dietary Ca content was 0.8% as compared to chicks fed non-supplemented diets. A quadratic effect ($P < 0.05$) due to dietary Ca level in the diet was observed on FCR at 14d. An interaction effect ($P < 0.05$) was detected on flock uniformity at 14d. Chicks fed dietary Ca levels of 0.95% and 1.10% and supplemented with 1α(OH)D_3 had the best flock uniformity (5.88% and 6.56%, respectively). Dietary supplementation of 1α(OH)D_3 reduced the RA of tibia length by 0.38 points ($P < 0.05$). Chickens fed non-supplemented diets with 1.25 % dietary Ca had greater tibia ash than chicks fed non-supplemented diets containing 0.80 and 1.40% dietary Ca (36.15 *vs*. 32.65 and 32.72 %, respectively). Interaction effects ($P < 0.05$) were detected on Ca, P, Mn, and Zn digestibilities. Ca and P digestibility was improved by 1α(OH)D_3 supplementation in diets containing 0.95% Ca, whereas in diets containing 0.80% dietary Ca enhanced Mn digestibility up to 2 percent. In conclusion, at high dietary Ca level (1.40% Ca), 1α(OH)D_3 supplementation

decreased BW and BWG. However, $1\alpha(OH)D_3$ supplementation at low dietary Ca content (0.8% Ca) ameliorated the negative effect of on FCR up to 7 point at 14d. Also, $1\alpha(OH)D_3$ supplementation improved Ca, P, and Mn digestibilities in diets containing low dietary Ca and reduced tibia RA length.

INTRODUCTION

Ca and Vitamin D (VitD) are two important nutrients involved in overall body functionality and physiology (Delezie et al., 2012). These two nutrients work together; VitD regulates intestinal Ca absorption, and dietary Ca levels modulates the required hydroxylation to form the active $VitD_3$ metabolite (Wasserman and Fullmer, 1995). Therefore, VitD is required by broilers for a proper Ca and P metabolism (Henry, 2011). It is known that bone mineralization and mineral digestibility could be also affected by dietary Ca level, VitD metabolites, and its bioavailability (Roberson et al., 1994; Mutucumarana et al., 2014a; Paiva et al., 2013, 2014).

The dietary levels of VitD3, Ca, and P used in commercial poultry diets is not consistant with the NRC (1994) recommendations. The NRC (1994) recommends 200 IU/kg of $VitD_3$, although different authors reported higher levels of inclusion (1,000-2,000 IU/kg) are necessary for adequate bone development. Different research groups (Fritts and Waldroup., 2003; Whitehead et al., 2004; Świątkiewicz et al., 2016) dietary inclusion levels used commercial industry poulry feeds for VitD3 has increased over the years to be as high as 20 times the recommendation of the NRC (1994) (Applegate and Angel, 2014). In contrast, there is a trend in the commercial feed industry is to provide lower dietary levels of Ca due to the common use of phytases to guarantee and improve the availability of P in phytate (Snow et al., 2004; Delezie et al., 2012). Nevertheless, there is considerable discussion about whether the ubiquitous use of supplemental phytase provides enough Ca to meet the metabolic demands of today's broiler genetics (Angel and Ashwell, 2014) and at what levels the availability of Ca could be affected. Under these current tendencies of feed formulation, Ca levels could be underestimated compared to what is needed for the chick.

Regardless of the dietary requirements of today's broilers for Ca, variability in dietary Ca concentration is a very common problem in feed ingredients and commercial least-cost feed formulation (Li et al., 2017). There are many situations presented in the industry can affect the final dietary Ca concentration in the feed that chickens received. Failures in ingredient handling, mixing, and feed manufacturing often results in suboptimal dietary Ca levels, or insufficient or inconsistant doseing or application of phytase (Van Kempen et al., 2001; Adedokun and Adeola, 2013; Li et al., 2017), which all increases risk of effect Ca formulation errors.

Ca particle size and solubility, are characteristics that can also affect broiler responses. Better BWG, FI and FCR, as well an improve tibia ossification have been observed with the utilization of Ca carbonate particles over 150 microns (Guinotte et al., 1991). Also, coarse particle size have been demonstrated to obtain a better nutrient digestibility. In an study performed by Anwar et al., (2016), a higher digestibility, and lower Ca *in vitro* solubility (0.33), was obtained with the utilization of coarse limestone particle size (1-2 mm). Lower solubility of Ca particle seems increase Ca absorption due to a slow mineral release, while the opposite occurs with the high solubility Ca with a faster Ca absorption (Zhang and Coon, 1997). There are reports indicating that the utilization of high Ca levels in broiler diets leads to bad results of P duodenal digestibility (Mutucumarana et al., 2014a).

An effective dietary VitD source could help to minimize any possible marginal deficiency of Ca and P (Światkiewicz et al., 2017). VitD sources with more biopotency may ameliorate the variability of dietary Ca content in poultry feeds that affect live performance and productivity. For example, increasing the level of cholecalciferol up to 1,250 mg/kg in Ca and P deficient diets or the addition of its metabolites at concentration of 10μg/kg of $1,25(OH)_2D_3$ in 0%P dietary diet P, increases the ability of phytate phosphorus utilization in chickens (Mohammed et al., 1991; Edwards, 1993).

The $1\alpha(OH)D_3$ is a synthetic VitD analogue that requires one hydroxylation at C25 to form biologically active $VitD_3$, and it has been demonstrated to have positive effects on the growth performance and bone development of broiler chickens (Edwards Jr. et al., 2002; Snow et al., 2004). Its effect on preventing tibia dyschondroplasia and rickets were demonstrated as well (Rennie and Whitehead, 1996; Edwards Jr. et al,. 2002; Driver et al., 2005). Dietary supplementation of $1\alpha(OH)D_3$ help to improve P utilization in broilers (Han et al., 2009) as well as other minerals like Mg and Zn (Biehl et al., 1995). Bone mineralization and ash percentage is also positively affected by $1\alpha(OH)D_3$ (Edwards Jr, 2002).

It was hypothesized that $1\alpha(OH)D_3$ could be affected by Ca levels and it can affect chicken live performance, bone mineral and mineral utilization.

MATERIALS AND METHODS

Treatments and Chicken Husbandry

One experiment was conducted using a randomized complete block design of 2 x 5 factorial arrangement of two dietary levels of $1\alpha(OH)D_3$ (0 and 5 µg/kg feed (1,600 IU/kg $VitD_3$/kg feed)) and 5 levels of Ca (0.80, 0.95, 1.10, 1.25, and 1.40 %) to obtain 10 treatment combinations. Chicks were placed in 80 Petersime battery cages with 8 replicate cages per treatment, and all were located in a room with controlled temperature. A total of 400 Ross 708 male-chicks were placed with 5 male chicks per cage.Room temperature was recorded and adjusted daily to guarantee thermoneutral temperatures. Feed and water were offered *ad libitum* to the chicks during the whole experimental period. Galvanized metal linear feeder and drinker were used; one of each were placed in a side of each cage.

Diets

Chicks received corn-soybean meal crumble diets formulated to meet Ross 708 requirements (Aviagen, 2014). The basal diet (Table 2.1) was supplemented with $VitD_3$ as cholecalciferol (4,000 IU/kg). A commercial phytase (Natuphos®) was used in all diets up to 500 FTU/kg, expecting to release 0.12% Ca and 0.12% P. The levels of Ca and P were obtained adding proportions of limestone, dicalcium phosphate and sand. The limestone particle sized used as Ca source had a granulometry of 1129µ and a solubility of 81.72% in 60 minutes of dissolution in EDTA.

Live Performance

Group BW and feed intake were obtained at 7 and 14 days, and BW gain and FCR (adjusted for mortality) were calculated. Flock uniformity expressed as CV% of individual BW were evaluated at 14 days of age.

Bone development and mineralization

At 16 days of age, three chicks per pen were selected, euthanized and leg bones removed. After defleshing, the weights and lengths of femurs, tibia and tarsus-metatarsus (shanks) from both legs were recorded to calculate bone Relative Asymmetry (RA). The RA was calculated ($|L-R|/[(L+R)/2]x100$) as described by Møller et al. (1995, 1999). Subsequently, femur bones were placed in porcelain crucible and incinerated in a muffle furnace at 600°C for 8 hours. The resulting bone ashes were weighted, and ash percentage was calculated as a measure of bone mineralization.

Mineral digestibility and retention

Between 11 and 13 d of age, excreta samples were collected from all cages. Ileal content from three birds per cage was collected at 16 days of age. Jejunum content was washed with deionized water and collected in Falcon tubes. Ileal samples were freeze-dried and further analyzed by inductively coupled plasma optical emission spectroscopy (Optima 8000, Perkin Elmer Corporation, Waltham, MA) and acid insoluble ash methodology, with Celite® as marker, for obtaining Ca and P digestibility. Ileal digestibility was calculated for dry matter, Ca and P. From excreta samples mineral retention of Ca, P, Mn, and Zn were assessed on dry matter basis.

All the procedures involving the birds used in the present experiment were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Statistical Analysis

Data were analyzed in a complete randomized block design using a two-way ANOVA and regression analysis for Ca levels. A 2 x 5 factorial arrangement of treatments was used with 8 replicates cages per treatment in a mixed model design. The main factors were the dietary Ca level and the inclusion levels of $1\alpha(OH)D_3$. Each of the four battery units were considered as a block (random effect). Mean separation was done using Tukey's or Student's t tests. All percentage data were transformed using the best Box-Cox transformation prior to analyses. All data were evaluated using JMP 13 software (SAS Inst. Inc., Cary, NC)

RESULTS

Performance

Significant interaction effects ($P < 0.05$) were detected on BW, BW gain, feed intake, and FCR at 14d. Feed intake, BW and BW gain were reduced ($P < 0.05$) in chicks fed $1\alpha(OH)D_3$-supplemented diets when dietary Ca content was 1.4%. (Table 2.2). Moreover, dietary supplementation $1\alpha(OH)D_3$ improved FCR up to 7 points, when dietary Ca content was 0.80% as compared to chicks fed non-supplemented diets (Table 2.3). In addition, a quadratic effect ($P < 0.05$) of dietary Ca levels was observed on FCR at 14d (Table 2.4), with the best FCR achieved at 1.01% and 1.19% of Ca for $1\alpha(OH)D_3$ supplemented and non-supplemented diets, respectively. A significant interaction effect ($P < 0.05$) was detected on flock uniformity (CV%) at 14d. Groups of chicks fed dietary Ca levels of 0.95% and 1.10% and supplemented with $1\alpha(OH)D_3$ had the lowest coeficient of variation (5.88% and 6.56%, respectively).

Bone development and mineralization

A treatment interaction effect ($P < 0.05$) was observed for tibia ash at 16 d (Table 2.5). A positive quadratic effect of Ca was observed for chicks fed diets without $1\alpha(OH)D_3$. The regression equation indicated an optimum level of 1.11% Ca for tibia ash (Table 2.7). Also chickens fed non-supplemented diets with 1.25 % dietary Ca had more ($P < 0.05$) tibia ash content compared to chicks fed non-supplemented diets containing 0.80 and 1.40 % dietary Ca (36.15 *vs*. 32.65 and

32.72 %, respectively). The supplementation with 1α(OH)D_3 resulted in intermediate responses, therefore supporting bone mineralization regardless of the dietary content of Ca.

No interaction effects were detected for bone RA. However, an effect of Ca was detected for tibia and shank weight RA (P < 0.05) at 16 d (Table 2.5). Regression analysis indicated a negative quadratic effect of Ca on tibia weight RA with a calculated optimum RA at 1.06% of Ca (Table 2.7). Furthermore, the lowest shank RA was detected at 1.10% Ca inclusion.

Mineral digestibility and retention

Results obtained in ileal digestibility analysis suggested that the supplementation with 1α(OH)D_3 increased Ca (51.22 *vs*. 38.75%) and P (58.63 *vs.* 46.11%) ileal digestibility when dietary Ca level was 0.95% when compared to diets without 1α(OH)D_3 inclusion (Table 2.6). Moreover, a linear effect was detected on Ca ileal digestibility due to dietary Ca level, only in chicks fed 1α(OH)D_3 supplemented diets (Figure 2.2).

For mineral retention analysis, interaction effects (P<0.05) were observed on the apparent digestibility coefficient of dry matter (CDADM), Ca, P, Mn, and Zn at 14 d (Table 2.8). The supplementation of 1α(OH)D_3 in diets containing 0.95% dietary Ca increased Ca and P retention, whereas diets containing 0.80% dietary Ca showed greater (P < 0.001) Mn retention (89.83 vs 91.62 %). In addition, a positive quadratic effect (P < 0.05) was detected for chicks fed diets regardless of 1α(OH)D_3 for CDADM with optimum estimated at 1.19 and 1.29% of Ca for 1α(OH)D_3 supplemented and no supplemented diets, respectively (Table 2.10). Positive quadratic effects were also observed in diets without 1α(OH)D_3 on Mn and Zn retention (Figure 2.6 ; 2.7). Regression analysis indicated a positive quadratic effects of Ca on P retention with a calculated optimum value of 1.14% Ca (Table 2.10)

A comparison of ileal digestibility and retention percentage was calculated for each one of the treatments. This comparison was defined as the difference of ileal digestibility *vs* retention (ileal digestibility % - retention %). The differences obtained in each treatment were statistically analyzed. (Mutucumarana et al., 2014b; Perryman et al., 2016).

An interaction effect ($P < 0.05$) was detected for Ca and P difference between Ca ileal digestibility and retention (Table 2.9). The difference between Ca ileal digestibility and retention increased significantly with the addition of $1\alpha(OH)D_3$ at 1.40% Ca Figure 2.10). Furthermore, an effect of Ca was detected ($P < 0.01$) for Ca and P. It was observed that a negative difference between the digestion and retention tends to become positive as the dietary Ca level increase. Ca and P digestibility *vs*. retention regressions had a quadratic effect of Ca levels. No effect ($P > 0.05$) of $1\alpha(OH)D_3$ in the difference between digestibility and retention was observed (Table 2.9).

DISCUSSION

Previous studies indicated that the utilization of $1\alpha(OH)D_3$ in poultry diets, improved chick BW gain and FCR during the first three weeks of chick age (Biehl and Baker 1997; Snow et al., 2004; Driver et al., 2005). Furthermore, Han et al. (2012) proposed that the addition of $1\alpha(OH)D_3$ can correct Ca deficiencies in diets, increasing BW gain in birds during the period of 21 to 42 d of age. Same effect was detected in the present study for the first two weeks of chicken life, where FCR and BWG improved at low Ca levels with the supplementation of $1\alpha(OH)D_3$.

The main objective of the present study was to determine the effect of $1\alpha(OH)D_3$ under different dietary Ca levels, due to the actual variability that exist in the industry on Ca dietary concentrations, when failures in the mixing, or the tendency in feed formulation can affect the adequate or expected Ca content getting to either deficiencies or overdosification, that can affect the performance and ossification of broilers.

Regression analysis showed a quadratic effect of Ca levels on the performance of chickens and growth. It is proposed that this quadratic effect could be due to effects on feed intake caused by the hormonal regulation of Ca absorption and it homeostasis at the bone level (Proszkowiec-Weglarz and Angel, 2013).

At low Ca inclusion, the organism would not have a source of Ca to supply for bone mineralization and development. On the other side, in an excess of Ca level, the body cannot regulate Ca as it normally would, generating an inefficient bone development in chicks at this early age. Also, as proposed by Shafey (1993), an excess of Ca can reduce soluble fraction of other minerals used for growth. In this way their availability for its absorption is reduced, generating growth depression in chickens.

Rath et al. (2000) mentioned that in addition to the importance of the presence of collagen crosslinks in the bone for mechanical strenght, Ca homeostasis is an essential driving force in the maintenance of bone strength. This statement can be reinforced by the present bone mineralization results that indicated that bone ash content increased by dietary Ca levels fitting to a quadratic effect. This means that at the lowest or highest Ca level, where Ca homeostasis with P is harder to obtain, the mineralization of the bone seems to be more affected. Likewise, the results of the present experiment suggested a positive influence of $1\alpha(OH)D_3$ on bone mineralization, increasing the percentage of bone ash. This result agrees with many previous studies (Biehl and Baker, 1997; Edwards et al., 2002; Snow et al., 2004; Han et al., 2009, 2012) that have shown that the inclusion of $1\alpha(OH)D_3$ used at different doses (from 5 to 20 μg/kg) increased tibia ash percentage.

Interestingly, tibia ash levels at 16 days showed an interaction effect, in which it was observed a consistent tibia ash increment with $1\alpha(OH)D_3$. This could suggest the ability of

$1\alpha(OH)D_3$ to regulate the deficit or excess of dietary Ca, guaranteeing in this way bone mineralization (Edwards Jr, 2002; Han et al., 2012).

The RA is a parameter that indicates the fitness of bilateral development, and in bones it has been studied to evaluate bird responses to their environment. The RA has been suggested as a tool to indicate animal wellbeing (Møller et al., 1999; Van Poucke et al., 2007). RA have been positively related with chicken growth rate too. (Møller, et al., 1995, 1999). There was not found previous studies that analyzed the effect of $1\alpha(OH)D_3$ in bone RA data, so this research and the data presented in it becomes the first study relating these data. The $1\alpha(OH)D_3$ inclusion showed to have a positive effect in the symmetric development of bone size, being reflected in the decrease of tibia RA length. This confirm that $1\alpha(OH)D_3$ had a positive influence in bone quality development. Also, in the present study, dietary Ca levels showed to have an influence on the RA of tibia and shank weight at 16 days. Tibia weight RA fitted to a quadratic regression indicating an optimum level of Ca at 1.06%, for the best tibia symmetry, while shank RA of weight indicated lower relative asymmetry at 1.10 and 1.25% of dietary Ca.

Mineral retention analyses were obtained for Ca, P, Cu, Mn and Zn in excreta. For all the minerals, the dietary Ca level exert a significant effect. For Ca retention, the dietary Ca produced a negative linear effect in diets without $1\alpha(OH)D_3$, decreasing the retention as the dietary Ca concentration increased. With the inclusion of $1\alpha(OH)D_3$, this effect became quadratic. The estimated optimum level for Ca retention with $1\alpha(OH)D_3$ was 0.84% Ca. Suggesting that at this point, levels of Ca absorbed and retained in the organism are increased by $1\alpha(OH)D_3$ supplementation. Similar results were obtained with P retention. The inclusion of $1\alpha(OH)D_3$ generated higher P retention than in groups without $1\alpha(OH)D_3$. This increment of retention of Ca and P was previously detected by Quian et al (1997), when the addition of 600µg/kg of cholecalciferol produced and increment on Ca and P retention compared to a diet with 66 µg/kg of cholecalciferol. The interaction effect indicated quadratic effects with and without 1α(OH)D_3 supplementation and the optimum levels of Ca were 1.11% and 1.16%, respectively.

Ca and P digestibility showed similar patterns, increasing mineral ileal digestibility with 1α(OH)D_3 inclusion, and improving the digestibility in the groups with 0.95%. It can be said that 1α(OH)D_3 promotes a favorable effect increasing the Ca and P intestinal absorption and utilization. Bielh et al, (1995), detected a trend ($P < 0.09$) of increasing plasma cholecalciferol with the utilization of 1α(OH)D_3 regardless the utilization or not of 120 FTU phytase.

Ca levels had a quadratic effect on Mn and Zn retention when diets had no 1α(OH)D_3. For Mn, the addition of 1α(OH)D_3 caused a negative linear effect, that decreased the retention as the Ca levels increased. This effect allows to increase Mn retention when diets are deficient of Ca. (Figure 2.5)

The difference between the ileal digestibility and excreta retention for Ca and P was calculated. In Ca analysis, it was observed that at high Ca levels. Plumstead et al. (2008), quantify the apparent digestibility and the retention of P and Ca in corn and soybean meal diets with the objective to get to the optimal ratio of Ca to nonphytate P (Ca:NPP) in diets with different phytate levels. Different Ca levels were used (0.47 to 1.16%). When working with the results presented in their publication, it was observed that patterns similar to the obtained in the present study were obtained. The difference between the ileal digestibility and excreta retention at the lowest Ca levels were a negative difference (-4.04 to -6.91), that becomes positive (4.77) in the treatment with highest Ca inclusion (1.16%). For P, the opposite occurred, observing positive differences in the diets with lower Ca inclusion and becoming negative in the highest inclusion (1.16%).

One interesting detail detected in these analyses is that the differences calculated goes from negative to positive values in both groups. This means that at lower Ca levels the mineral digested is lower than the retained. This suggested that in a situation of mineral deficiency, chickens started to reabsorb and retained higher levels of minerals to equilibrate the homeostasis generated by this low Ca levels. Focusing on how the chick could retain additional Ca after ileum stage, it is suggested that in chicks there also exist a kind of reabsorption happening at cecum and kidney levels. Svihus et al., (2013) mentioned that the nutritional significance of the caeca in chickens remains unclear. However, previous studies suggested based on the proximal ceca surface structure, with similarities to the jejunum, that nutrient absorption may take place there (Ferrer et al., 1991). Ceca Ca absorption is not well documented in chicks, nevertheless, a research done in rats by Nellans and Goldsmith (1981), concluded that cecum possess the highest density of Ca transport sites and receptors in the intestine. Further research in ceca mineral absorption in chicks would be necessary. On the other side, it also must be considered renal Ca and P metabolism that can be affecting these results. In addition to intestinal absorption and bone resorption, it is known that Ca homeostasis is obtained by renal excretion or reabsorption of Ca (Wideman, 1987). In situations of high demand of Ca, this excretion can be reduced, increasing the reabsorption from renal tubes to try to reach the required Ca levels by the organism (Proszkowiec-Weglarz and Angel 2013).

CONCLUSION

$1\alpha(OH)D_3$ supplementation in addition to the premix cholecalciferol during the starter phase is effective for better chick performance at low dietary Ca levels. Supplementation with $1\alpha(OH)D_3$ in starter phase resulted in higher Ca, P, Mn and Zn retention when low levels of Ca were used, and increased Ca and P digestibility in diets with levels higher than 0.90%Ca.

Furthermore, $1\alpha(OH)D_3$ allows better ossification of tibia especially at low Ca levels with better tibia development.

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Table 2.1. Ingredient composition and calculated nutrient content of starter basal diet (1-16 days of age) for Ross-708 broilers

Ingredient Name	(%)	Nutrient	%
Corn	53.25	Dry matter	88.55
Soybean meal, 46%	30.94	Moisture	11.45
Corn gluten meal	5.00	Crude protein	22.71
Poultry fat	0.00	Calcium	0.30
Soybean oil	4.20	Total phosphorous	0.38
Celite	1.00	Nonphytate phosphorous	0.18
Filler (Limestone+DiCaP+Sand)	3.15	Phytate phosphorous	0.24
Salt (NaCl)	0.29	Total methionine	0.67
DL-Methionine, 99%	0.30	Total cysteine	0.36
Sodium bicarbonate	0.31	Total lysine	1.39
Dicalcium phosphate[1]	0.27	Total tryptophan	0.25
Mineral premix[2]	0.20	Total threonine	0.98
Limestone Cerne Pure Cal 12-40[3]	0.17	Total isoleucine	0.94
L-Lysine-HCl, 78.8%	0.38	Total valine	1.05
Choline chloride, 60%	0.18	Total leucine	2.13
L-Threonine, 98%	0.15	Total arginine	1.40
Selenium premix	0.05	Total Sulphur amino acids	1.04
Vitamin premix[4]	0.10	Total glycine	0.88
Coccidiostat[5]	0.05	Sodium	0.22
Natuphos E®[6]	0.01	Potassium	0.85
Total	100.00	Chloride	0.29
		Digestible lysine	1.28
		Digestible methionine	0.63
		Digestible cysteine	0.31
		Digestible total Sulphur amino acids	0.94
		Digestible threonine	0.85
		Digestible tryptophan	0.22
		Digestible isoleucine	0.84
		Digestible leucine	2.04
		Digestible valine	0.95
		Digestible arginine	1.30
		Metabolizable Energy, kcal/kg	3,000
		Dietary electrolyte balance, mEq/100 g	254

[1] Dicalcium phosphate contains 19.79% Calcium, 17.9091% phosphorus and 17.73% available phosphorus (99%).
[2] Trace minerals provided per kg of premix: manganese ($MnSO_4$), 60 g; zinc ($ZnSO_4$), 60 g; iron (FeSO4), 40 g; copper ($CuSO_4$), 5 g; iodine ($Ca(IO_3)_2$),1.25 g.
[3] Limestone (Cerne Pure Cal 12-4) contains 39.467% Calcium;
[4] Vitamins provided per kg of premix: vitamin A, 13,227,513 IU; vitamin D_3, 3,968,253 IU; vitamin E, 66,137 IU; vitamin B12, 39.6 mg; riboflavin, 13,227 mg; niacin, 110,229 mg; d-pantothenic acid, 22,045 mg; menadione, 3,968 mg; folic acid, 2,204 mg; vitamin B6, 7,936 mg; thiamine, 3,968 mg; biotin, 253.5 mg.
[5] Coban® 90 (Monensin), Elanco Animal Health, Greenfield, IN, at 500 g/ton in the starter and grower diets.
[6] Natuphos E® (500 FTU/kg, 50 g/ton FTU)

Table 2.2. Effect of supplementation of $1\alpha(OH)D_3$ on top of $VitD_3$ with varying levels of calcium in starter diets for Ross 708 male broilers from 0-7d[1]

Ca	VitD	BW		BWG	Feed intake	FCR adj**
		Hatch	7d	0 – 7d	0 – 7d	0 – 7d
		------------------------- (g)		----------------------		--- (g:g) ---
0.80		46.0	167[ab]	121[ab]	152[a]	1.289[a]
0.95		46.2	171[a]	125[a]	153[a]	1.222[b]
1.10		46.1	170[ab]	124[ab]	151[ab]	1.219[b]
1.25		46.1	163[bc]	117[bc]	143[bc]	1.223[b]
1.40		46.2	157[c]	111[c]	141[c]	1.277[a]
SEM		0.15	1.71	1.71	2.26	0.01
	VitD	46.2	166	119	150[a]	1.269[a]
	VitD+$1\alpha(OH)D_3$	46.1	166	120	146[b]	1.224[b]
	SEM	0.13	1.09	1.09	1.54	0.01
0.80	VitD	46.0	164[ab]	118[ab]	154	1.364[a]
	VitD+$1\alpha(OH)D_3$	45.9	170[ab]	124[ab]	150	1.215[b]
0.95	VitD	46.2	168[ab]	122[ab]	152	1.248[ab]
	VitD+$1\alpha(OH)D_3$	46.2	174[a]	128[a]	153	1.196[b]
1.10	VitD	46.2	167[ab]	120[ab]	151	1.260[ab]
	VitD+$1\alpha(OH)D_3$	46.0	174[a]	128[a]	150	1.179[b]
1.25	VitD	46.3	168[ab]	121[ab]	146	1.206[b]
	VitD+$1\alpha(OH)D_3$	46.0	159[bc]	113[bc]	141	1.241[b]
1.40	VitD	46.1	162[bc]	116[bc]	147	1.267[ab]
	VitD+$1\alpha(OH)D_3$	46.3	152[c]	106[c]	136	1.287[ab]
	SEM	0.19	4.00	4.00	3.60	0.01
CV%		0.78	4.13	5.75	5.78	5.75
Source of variation		---------------------------------P – values ------------------------------				
Ca		0.3280	<.0001	<.0001	0.0002	0.0098
VitD source		0.2225	0.9503	0.8703	0.0441	0.0063
Ca*VitD source		0.4114	0.0003	0.0002	0.3289	0.0032
		-----------------------------Regression P – values ----------------------				
Ca linear		0.4286	0.0169	0.0181	<.0001	0.1324
Ca*Ca quadratic		0.1052	0.0213	0.0167	0.5320	0.0007
VitD source		0.5998	0.0012	0.0012	0.0040	0.0159
Ca*VitD source		0.8429	0.2820	0.2728	0.8991	0.0446

[a-d] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's t or Tukey's test.

[1] Values are means of 10 cages per treatment combination with 5 male chicks.

*FCR was adjusted by mortality weights.

Table 2.3. Effect of supplementation of 1α(OH)D_3 on basal diets containing VitD_3 and different Ca levels during the starter phase for Ross 708 male broilers on performance from 0 to 14 d[1]

Ca	VitD	BW	BWG	FI	FCR	FCR adj*
		14d	0–14d	0 – 14d	0 – 14d	0 – 14d
		---------------- (g)	----------------	----------------	--------- (g:g)	-----------
0.80		470^{ab}	424^{ab}	539^{ab}	1.273^{ab}	1.273^{ab}
0.95		487^{a}	441^{a}	553^{a}	1.254^{ab}	1.246^{ab}
1.10		486^{a}	440^{a}	553^{a}	1.258^{b}	1.247^{b}
1.25		465^{b}	419^{b}	521^{b}	1.244^{ab}	1.244^{ab}
1.40		434^{c}	388^{c}	495^{c}	1.2771^{a}	1.277^{a}
SEM		5.1	5.3	6.1	0.013	0.011
	VitD	470	424	538	1.270^{a}	1.270^{a}
	VitD+1α(OH)D_3	467	421	527	1.252^{b}	1.245^{b}
	SEM	3.2	3.3	3.6	0.009	0.008
0.80	VitD	460^{bc}	414^{ab}	539^{ab}	1.305^{a}	1.305^{a}
	VitD+1α(OH)D_3	481^{abc}	435^{ab}	540^{ab}	1.242^{bc}	1.242^{bc}
0.95	VitD	482^{abc}	435^{ab}	552^{a}	1.268^{abc}	1.268^{abc}
	VitD+1α(OH)D_3	494^{ab}	447^{a}	555^{a}	1.241^{bc}	1.225^{bc}
1.10	VitD	478^{abc}	432^{ab}	545^{ab}	1.261^{abc}	1.261^{abc}
	VitD+1α(OH)D_3	494^{a}	448^{a}	561^{a}	1.255^{c}	1.232^{c}
1.25	VitD	475^{abc}	429^{ab}	533^{ab}	1.244^{bc}	1.244^{bc}
	VitD+1α(OH)D_3	455^{c}	409^{b}	509^{b}	1.243^{bc}	1.243^{bc}
1.40	VitD	456^{c}	410^{b}	521^{ab}	1.273^{abc}	1.273^{abc}
	VitD+1α(OH)D_3	413^{d}	367^{c}	469^{c}	1.282^{ab}	1.282^{ab}
	SEM	7.3	7.4	8.8	0.014	0.015
	CV%	4.43	4.94	4.78	2.84	0.03
Source of variation		----------------------	----------------------	P – values	----------------------	----------------------
Ca		<.0001	<.0001	<.0001	0.0106	0.0060
VitD source		0.5327	0.5327	0.0541	0.0025	0.0007
Ca*VitD source		<.0001	<.0001	0.0028	0.0353	0.0246
		----------------------	----------------------	Regression P – values	----------------------	----------------------
Ca linear		0.0001	0.0001	0.9176	0.9176	0.8538
Ca*Ca quadratic		0.0001	0.0001	0.0004	0.0004	0.0001
VitD source		0.5474	0.5648	0.0023	0.0023	0.0005
Ca*VitD source		0.0001	<.0001	0.0032	0.0032	0.0011

[a-d] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's *t* or Tukey's test

[1] Values are means of 10 cages per treatment combination with 5 male chicks

*FCR was adjusted by mortality weights.

Table 2.4. Equations for effects of supplementation of 1α(OH)D_3 on a basal diet containing VitD_3 and different Ca levels during the starter phase on live performance for Ross 708 male broilers from 0 to 7d and 0-14d.

	Equation	*P*-values[1]			Optimum Calcium level (%)	R^{2*}
		Intercept	Ca	Ca^2		
BW VitD 7d	$70.0524 + 177.2262*Ca - 79.7619*Ca^2$	0.1223	0.0388	0.0401	1.112	0.06
BW VitD + 1α(OH)D_3 7d	$63.4016 + 229.8175*Ca - 119.8413*Ca^2$	0.0496	0.0003	<.0001	0.959	0.64
BWG VitD 7d	$28.1337 + 168.8294*Ca - 75.7937*Ca^2$	0.5277	0.0476	0.0496	1.114	0.05
BWG VitD + 1α(OH)D_3 7d	$17.1603 + 230.8968*Ca - 120.6349*Ca^2$	0.583	0.0003	<.0001	0.957	0.66
Feed Intake 7d	$171.2708 - 21.1667*Ca$	<.0001	<.0001	-	-	0.20
FCR adj VitD 7d	$2.5110 - 2.1779*Ca + 0.9190*Ca^2$	<.0001	0.0054	0.0091	1.185	0.24
FCR adj VitD + 1α(OH)D_3 7d	$1.8590 - 1.3356*Ca + 0.6643*Ca^2$	<.0001	0.0286	0.0169	1.005	0.23
BW VitD 14d	$176.2781 + 565.0691*Ca - 261.1905*Ca^2$	0.1369	0.0130	0.0114	1.082	0.12
BW VitD + 1α(OH)D_3 14d	$35.8345 + 939.4905*Ca - 479.6150*Ca^2$	0.0496	0.0003	<.0001	0.979	0.76
BWG VitD 14d	$132.6313 + 560.5206*Ca - 259.2064*Ca^2$	0.2636	0.0144	0.0126	1.082	0.12
BWG VitD + 1α(OH)D_3 14d	$-11.5097 + 942.3275*Ca - 481.0731*Ca^2$	0.583	0.0003	<.0001	0.979	0.76
Feed intake VitD 14d	$577.2783 - 35.9167*Ca$	<.0001	0.0617	-	-	0.06
Feed intake VitD + 1α(OH)D_3 14d	$139.9860 + 858.4412*Ca - 446.5774*Ca^2$	0.1513	<.0001	<.0001	0.997	0.74
FCR VitD 14d	$1.7802 - 0.9000\ 4*Ca + 0.3825*Ca^2$	<.0001	0.0253	0.0352	1.185	0.14
FCR VitD + 1α(OH)D_3 14d	$1.6447 - 0.8133*Ca + 0.3945*Ca^2$	<.0001	0.0097	0.0059	1.005	0.24

[1]Regression analyses with $P < 0.05$ indicated non-significant effect of Ca on specific interaction effects.

* Regression analyses correspond to a mixed model and very low R^2 may not indicate lower adjustment.

Table 2.5. Effect of supplementation of $1\alpha(OH)D_3$ on basal diets containing $VitD_3$ and different Ca levels during the starter phase for Ross 708 male broilers on tibia ash and bone relative length and weight at 16d[1]

Ca	VitD	Tibia Ash	Tibia		Femur		Shank	
			RA weight	RA length	RA weight	RA length	RA weight	RA length
		(%)						
0.80		33.46^{b}	6.54^{ab}	1.86	5.88	0.65	2.15^{b}	0.97
0.95		34.86^{ab}	5.99^{b}	1.31	5.11	0.55	2.97^{a}	1.21
1.10		34.18^{ab}	5.63^{b}	1.53	4.31	0.71	2.04^{b}	1.09
1.25		35.35^{a}	5.70^{b}	1.27	5.14	0.56	2.07^{b}	0.91
1.40		33.54^{b}	9.03^{a}	1.67	4.73	0.79	2.86^{a}	0.97
SEM		0.64	1.02	0.27	0.61	0.08	0.24	0.11
	VitD	34.14	6.82	1.72^{a}	4.90	0.67	2.47	1.02
	VitD+$1\alpha(OH)D_3$	34.42	6.34	1.34^{b}	5.17	0.64	2.36	1.04
	SEM	0.55	0.77	0.23	0.39	0.05	0.16	0.07
	VitD+$1\alpha(OH)D_3$	34.28^{ab}	6.52	1.68	5.88	0.72	2.58	1.02
0.95	VitD	35.20^{ab}	6.50	1.48	5.83	0.66	2.99	1.10
	VitD+$1\alpha(OH)D_3$	34.51^{ab}	5.48	1.13	4.39	0.45	2.95	1.32
1.10	VitD	33.96^{ab}	6.63	1.94	3.67	0.66	2.35	1.14
	VitD+$1\alpha(OH)D_3$	34.41^{ab}	4.64	1.12	4.94	0.76	1.73	1.04
1.25	VitD	36.15^{a}	4.38	1.55	4.93	0.52	2.37	0.88
	VitD+$1\alpha(OH)D_3$	34.55^{ab}	7.01	1.00	5.36	0.61	1.77	0.94
1.40	VitD	32.72^{b}	10.04	1.58	4.18	0.91	2.95	1.06
	VitD+$1\alpha(OH)D_3$	34.36^{ab}	8.03	1.75	5.27	0.66	2.78	0.89
	SEM	0.76	1.34	0.33	0.87	0.12	0.34	0.16
Source of variation		*P* – values						
Ca		0.005	0.0398	0.1719	0.4759	0.2520	0.0081	0.3064
VitD		0.4493	0.5325	0.0265	0.6258	0.7179	0.6029	0.7969
Ca*VitD		0.0232	0.2867	0.4486	0.5332	0.3145	0.1593	0.7087
		Regression *P* – values						
Ca linear		0.6167	0.0929	0.5941	0.3106	0.3241	0.4584	0.4227
Ca*Ca quadratic		0.0046	0.0101	0.0555	0.3557	0.2944	0.3853	0.3284
VitD		0.4603	0.5827	0.0255	0.6333	0.6631	0.7327	0.7369
Ca*VitD		0.7429	0.8964	0.4477	0.3966	0.3927	0.1129	0.3284

[a-b] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's *t* or Tukey's test

[1] Values are means of 10 cages per treatment combination with 5 male chicks

Table 2.6. Effect of dietary Ca levels and 1α(OH)D_3 supplementation on ileal digestibility of Ross 708 male broilers at 16 d of age

Ca	VitD	Dry matter	Ca	P
		-----------------%-------------------		
0.80		66.89^{b}	45.56	51.21
0.95		68.13^{b}	44.98	52.37
1.10		72.39^{a}	47.56	54.68
1.25		72.46^{a}	47.39	50.77
1.40		72.59^{a}	49.97	52.93
SEM		0.63	47.15	52.33
	VitD	69.58^{b}	44.63^{b}	50.35^{b}
	VitD + 1α(OH)D_3	71.41^{a}	49.56^{a}	54.43^{a}
	SEM	0.36	47.13	52.29
0.80	VitD	69.34^{bc}	47.62^{abc}	54.70^{ab}
	VitD + 1α(OH)D_3	64.45^{d}	43.49^{abc}	47.72^{bc}
0.95	VitD	65.25^{cd}	38.75^{c}	46.11^{c}
	VitD + 1α(OH)D_3	71.01^{ab}	51.22^{ab}	58.63^{a}
1.10	VitD	71.57^{ab}	47.83^{abc}	55.19^{ab}
	VitD + 1α(OH)D_3	73.22^{ab}	47.30^{abc}	54.16^{abc}
1.25	VitD	70.48^{ab}	42.27^{bc}	47.99^{bc}
	VitD + 1α(OH)D_3	74.43^{a}	52.52^{a}	53.54^{abc}
1.40	VitD	71.25^{ab}	46.66^{abc}	47.78^{bc}
	VitD + 1α(OH)D_3	73.93^{ab}	53.27^{a}	58.07^{a}
	SEM	0.93	2.16	1.84
CV%		3.59	12.50	9.61
Source of variation		-------------*P*-values---------------		
Ca		<.0001	0.1853	0.2433
VitD source		0.0044	0.0006	0.0009
Ca*VitD source		<.0001	0.0007	<.0001
		-------------Regression *P*-values--------------		
Ca linear		<.0001	0.0391	0.7743
VitD		0.0143	0.0022	0.0061
Ca*VitD source		0.0086	0.0586	0.0056
Ca*Ca quadratic		0.0709	0.6246	0.4363

[a-d] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's *t* or Tukey's test

[1] Values are means of 10 cages per treatment combination with 5 male chicks

Table 2.7. Equations for effects of supplementation of 1α(OH)D_3 on a basal diet containing VitD_3 and different Ca levels during the starter phase on ileal digestibility of Ross 708 male broilers at 16 d of age

	Equation	*P* – values[1]			Optimum Ca level (%)	R^{2*}
		Intercept	Ca	Ca^2		
Dry matter digestibility VitD	63.5109 + 5.6250*Ca	<.0001	0.0260	---	---	0.11
Dry matter digestibility VitD + 1α(OH)D_3	2.8210 + 113.6025*Ca - 44.9056*Ca^2	0.8231	<.0001	0.0002	1.26	0.72
Ca Digestibility VitD + 1α(OH)D_3	34.1160 + 14.1209*Ca	<.0001	0.0054	---	---	0.19
P Digestibility VitD	59.6421 - 8.4272*Ca	<.0001	0.0692	---	---	0.06
P Digestibility VitD + 1α(OH)D_3	43.1682 + 10.2114*Ca	<.0001	0.031 7	---	---	0.10
Tibia ash VitD	1.1389 + 61.5595*Ca - 27.6479*Ca^2	0.8936	0.0002	0.0002	1.11	0.10
Tibia RA weight VitD	37.8595 - 61.5077*Ca + 29.1017*Ca^2	0.0361	0.0667	0.0555	1.06	0.02

[1]Regression analyses with $P > 0.05$ indicated non-significant effect of Ca on specific interaction effects.
* Regression analyses correspond to a mixed model and very low R^2 may not indicate lower adjustment.

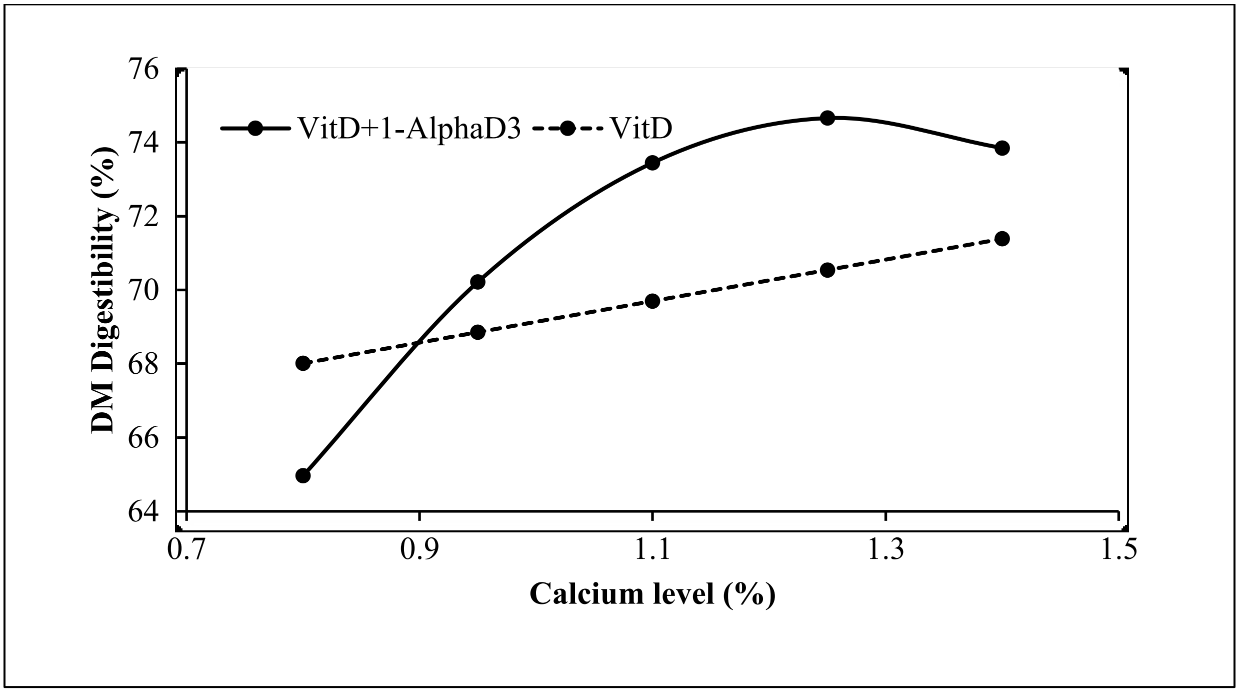

Figure 2.1. Quadratic effect of calcium level with 1α(OH)D_3 and linear effect without 1α(OH)D_3 on dry matter digestibility from broiler males at 16 d of age.

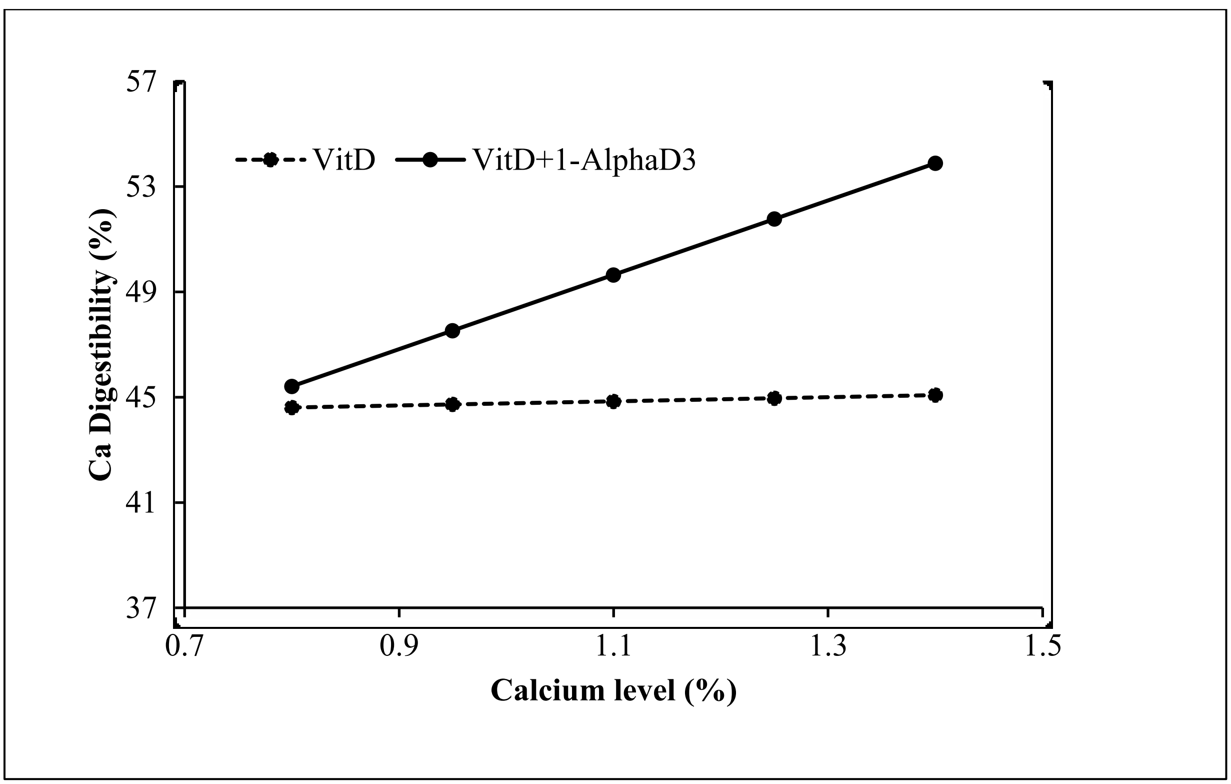

Figure 2.2. Linear effect of calcium level with and without 1α(OH)D_3 on calcium digestibility from broiler males at 16 d of age.

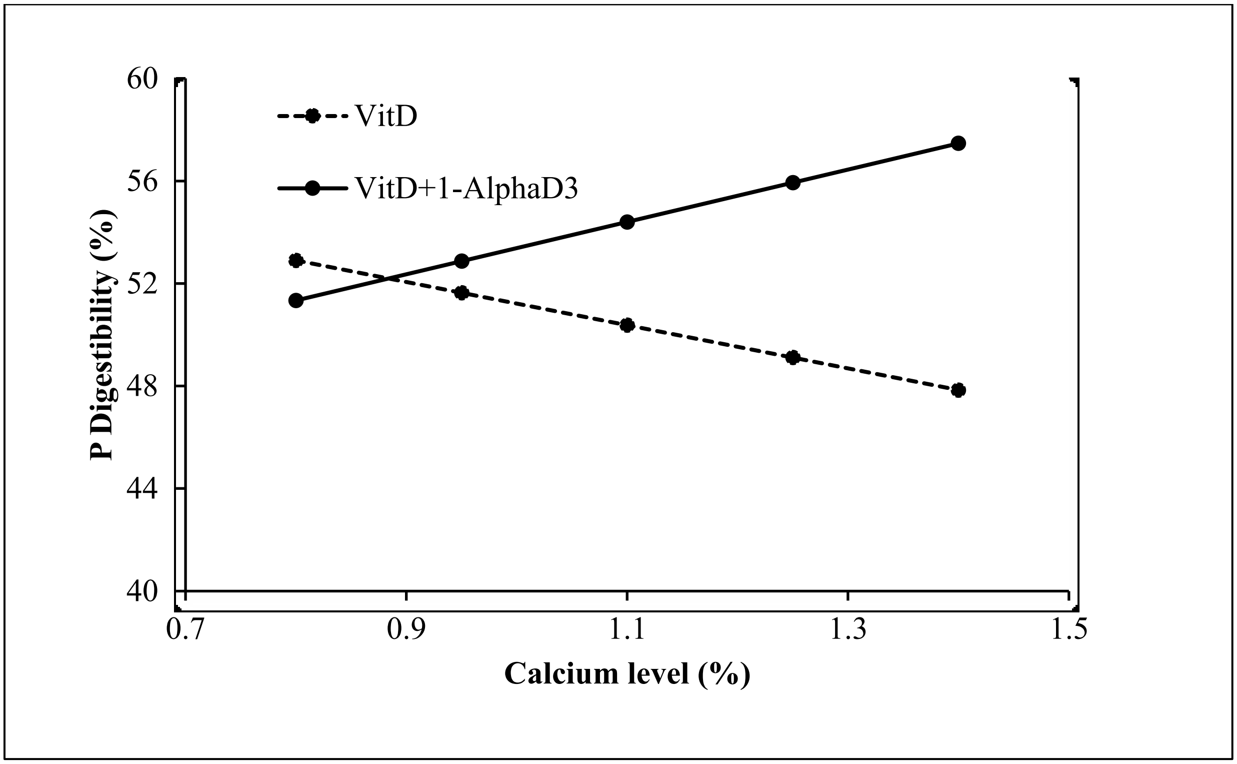

Figure 2.3. Effect of calcium level with and without 1α(OH)D_3 on phosphorous digestibility from broiler males at 16 d of age.

Table 2.8. Effect of supplementation of $1\alpha(OH)D_3$ on basal diets containing $VitD_3$ and different Ca levels during the starter phase for Ross 708 male broilers on mineral retention at 14 days

Ca	VitD	Dry matter	Ca	P	Cu	Mn	Zn
		---------------------------- (%) ---------------------------------					
0.80		71.91^{c}	58.51^{a}	52.78^{d}	76.81^{ab}	90.72^{b}	92.16^{b}
0.95		72.89^{bc}	54.38^{a}	58.85^{bc}	81.25^{a}	91.39^{ab}	92.69^{ab}
1.10		76.15^{a}	54.81^{a}	62.80^{a}	75.20^{ab}	91.59^{a}	92.99^{a}
1.25		76.00^{a}	43.22^{b}	60.63^{ab}	78.13^{ab}	91.44^{ab}	92.96^{ab}
1.40		75.06^{ab}	33.46^{c}	56.68^{c}	73.25^{b}	90.73^{b}	92.54^{ab}
SEM		0.57	2.32	0.92	1.98	0.18	0.19
	VitD	73.25^{b}	46.97^{b}	56.36^{b}	76.97	91.22	92.60
	VitD+$1\alpha(OH)D_3$	75.55^{a}	50.79^{a}	60.33^{a}	76.89	91.14	92.73
	SEM	0.32	1.64	0.55	1.35	0.11	0.10
0.80	VitD	70.92^{cd}	55.68^{ab}	50.91^{e}	75.87	89.83^{c}	91.76^{b}
	VitD+$1\alpha(OH)D_3$	72.89^{bcd}	61.35^{a}	54.65^{de}	77.75	91.62^{a}	92.57^{ab}
0.95	VitD	69.51^{d}	47.19^{bc}	52.40^{de}	82.18	91.24^{ab}	92.15^{ab}
	VitD+$1\alpha(OH)D_3$	76.27^{ab}	61.56^{a}	65.31^{a}	80.32	91.54^{a}	93.23^{a}
1.10	VitD	76.62^{ab}	57.70^{ab}	64.67^{a}	76.05	92.14^{a}	93.19^{a}
	VitD+$1\alpha(OH)D_3$	75.67^{ab}	51.92^{ab}	60.94^{abc}	74.35	91.05^{ab}	92.78^{ab}
1.25	VitD	74.75^{abc}	37.83^{cd}	57.90^{bcd}	78.72	91.46^{a}	93.02^{ab}
	VitD+$1\alpha(OH)D_3$	77.26^{a}	48.61^{abc}	63.35^{ab}	77.54	91.43^{a}	92.91^{ab}
1.40	VitD	74.47^{abc}	36.43^{cd}	55.94^{cde}	72.01	91.42^{a}	92.90^{ab}
	VitD+$1\alpha(OH)D_3$	75.66^{ab}	30.49^{d}	57.42^{bcd}	74.49	90.05^{bc}	92.18^{ab}
	SEM	0.84	3.15	1.33	2.72	0.26	0.28
	CV%	3.30	17.38	6.57	9.69	1.00	0.90
Source of variation		----------------------------*P* – values --------------------------					
Ca		<.0001	<.0001	<.0001	0.0413	0.001	0.0393
VitD		<.0001	0.049	<.0001	0.9634	0.6221	0.4854
Ca*VitD		0.0009	0.0017	<.0001	0.8679	<.0001	0.0115
		-------------------------Regression *P* – values ---------------------					
Ca linear		<.0001	<.0001	0.0097	0.0938	0.8979	0.1084
Ca*Ca quadratic		0.0064	0.0726	<.0001	0.1803	<.0001	0.0046
VitD		0.0004	0.0747	0.0002	0.9643	0.6409	0.4741
Ca*VitD		0.1872	0.0724	0.1012	0.8761	<.0001	0.0014

[a-e] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's *t* or Tukey's test

[1] Values are means of 10 cages per treatment combination with 5 male chicks

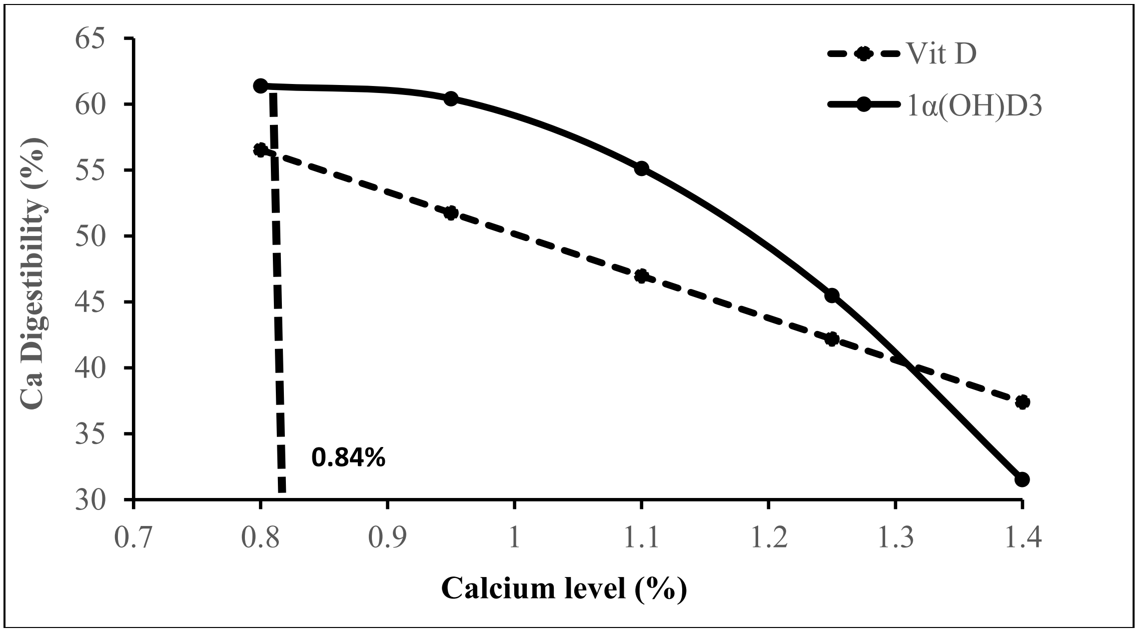

Figure 2.4. Quadratic effect of calcium levels with and without 1α(OH)D_3 on calcium retention from 0-14 days in male broilers

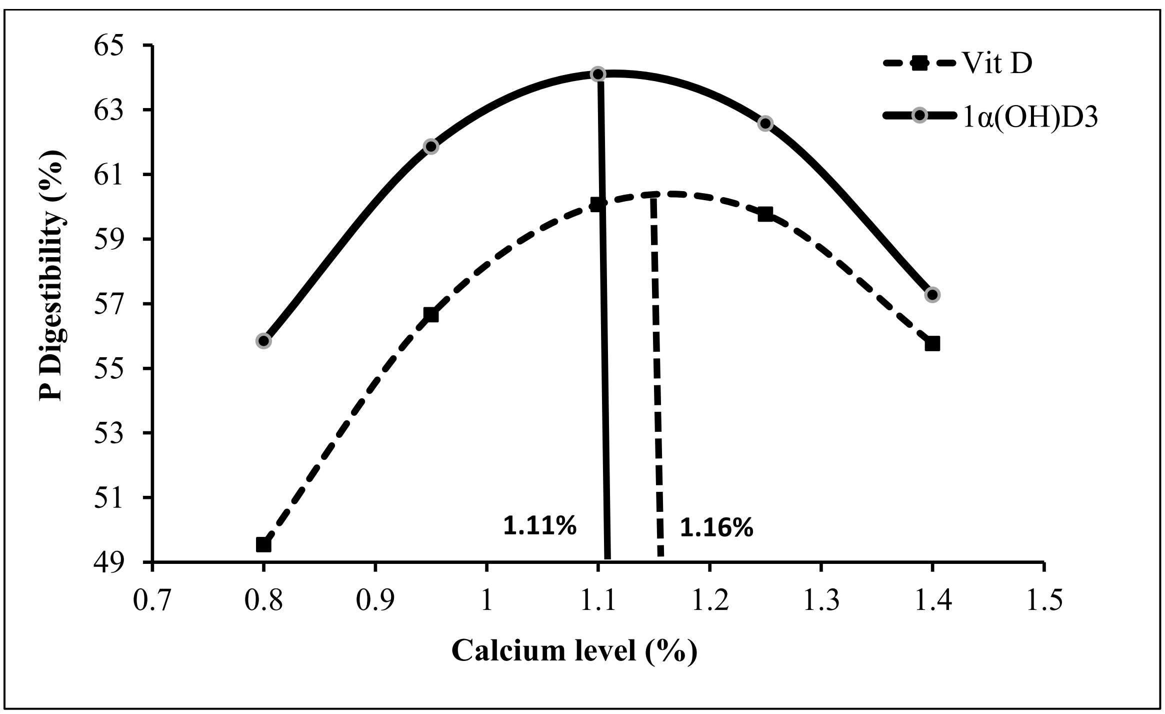

Figure 2.5. Quadratic effect of calcium levels with and without 1α(OH)D_3 on phosphorous retention from 0-14 days in male broilers.

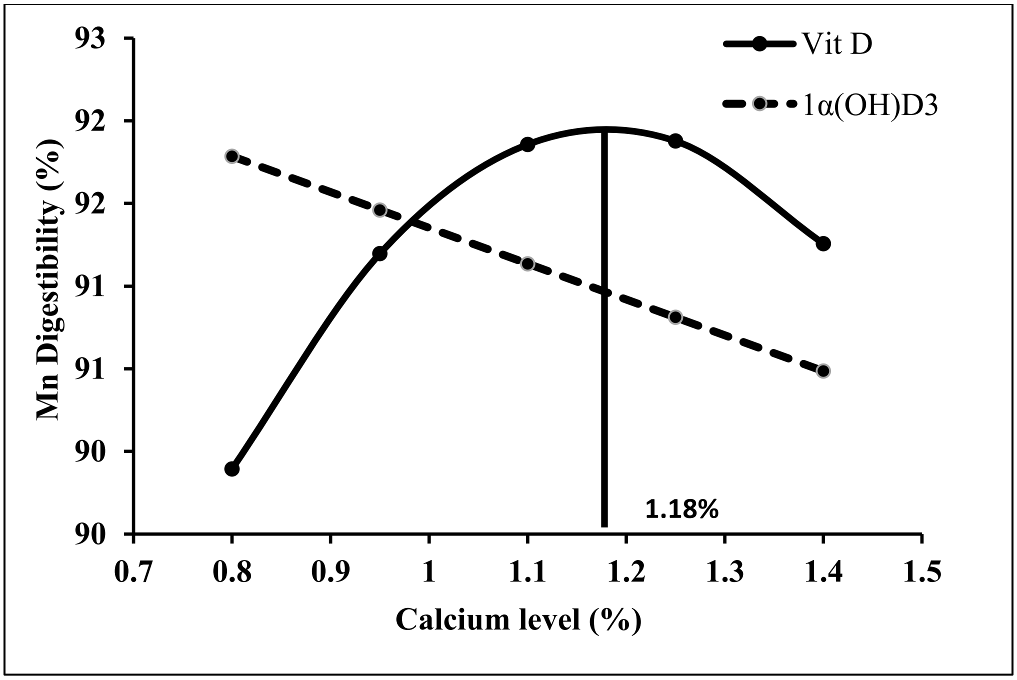

Figure 2.6. Quadratic effect of calcium levels with and without 1α(OH)D_3 on manganese retention from 0-14 days in broiler males

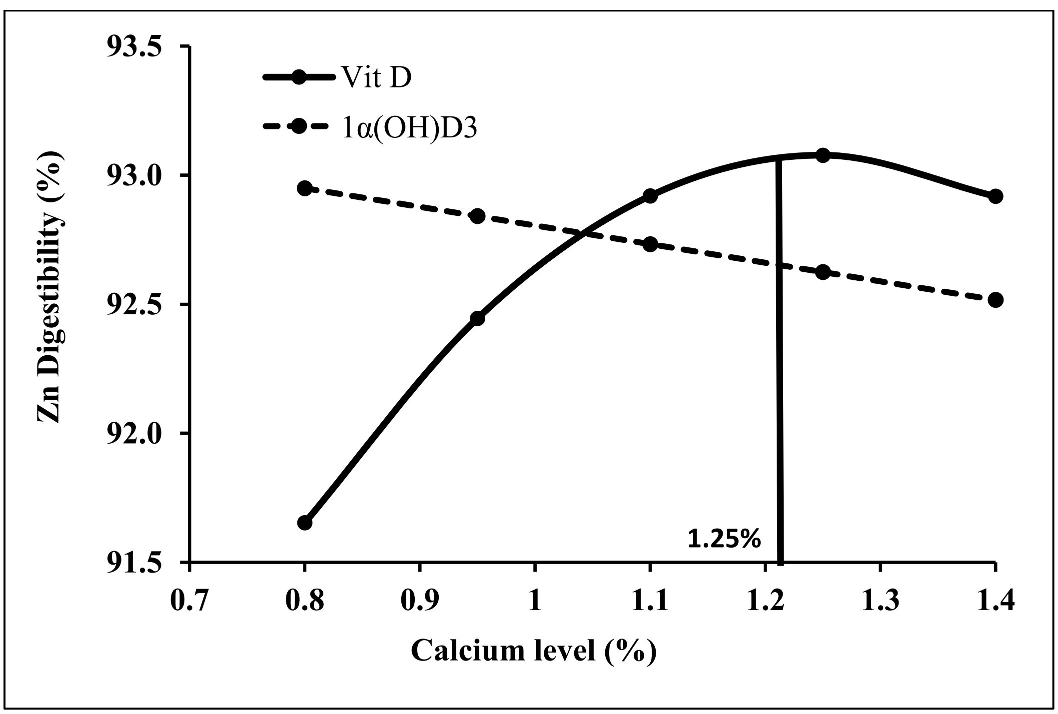

Figure 2.7. Quadratic effect of calcium level with and without 1α(OH)D3 on zinc retention from 0-14 days in broiler males.

Table 2.9. Effect of dietary Ca levels and 1α(OH)D_3 supplementation on Ca and P ileal digestibility vs. Retention of Ross 708 male broilers at 16 d of age.

Ca	VitD	Ca	P
		---------------- % -----------------	
0.80		-13.12^{c}	-2.68^{a}
0.95		-9.99^{c}	-7.57^{ab}
1.10		-8.02^{c}	-9.26^{ab}
1.25		3.65^{b}	-10.50^{b}
1.40		17.25^{a}	-4.82^{ab}
SEM		2.82	1.63
	VitD source	-2.82	-7.03
	VitD + 1α(OH)D_3	-1.27	-6.90
	SEM	2.15	0.95
0.80	VitD	-8.23^{cde}	3.26^{a}
	VitD + 1α(OH)D_3	-18.02^{e}	-8.62^{b}
0.95	VitD	-9.33^{cde}	-7.68^{ab}
	VitD + 1α(OH)D_3	-10.64^{de}	-7.46^{b}
1.10	VitD	-9.38^{cde}	-9.93^{b}
	VitD + 1α(OH)D_3	-6.65^{cde}	-8.58^{b}
1.25	VitD	2.46^{bcd}	-11.29^{b}
	VitD + 1α(OH)D_3	4.85^{bc}	-9.71^{b}
1.40	VitD	10.40^{ab}	-9.50^{b}
	VitD + 1α(OH)D_3	24.10^{a}	-0.14^{ab}
	SEM	3.68	2.35
CV%		-351.17	-92.75
Source of variation		------------------*P*-value------------------	
Ca		<.0001	0.0097
VitD source		0.4684	0.9327
Ca*VitD source		0.0208	0.0012
		-----------Regression *P*-value-----------	
Ca linear		<.0001	0.0972
VitD source		0.5273	0.9166
Ca*VitD source		0.0012	<.0001
Ca*Ca quadratic		<.0001	0.0013

[a-e] Means in a column not sharing a common superscript are significantly different ($P \leq 0.05$) by Student's t or Tukey's test

[1] Values are means of 10 cages per treatment combination with 5 male chicks

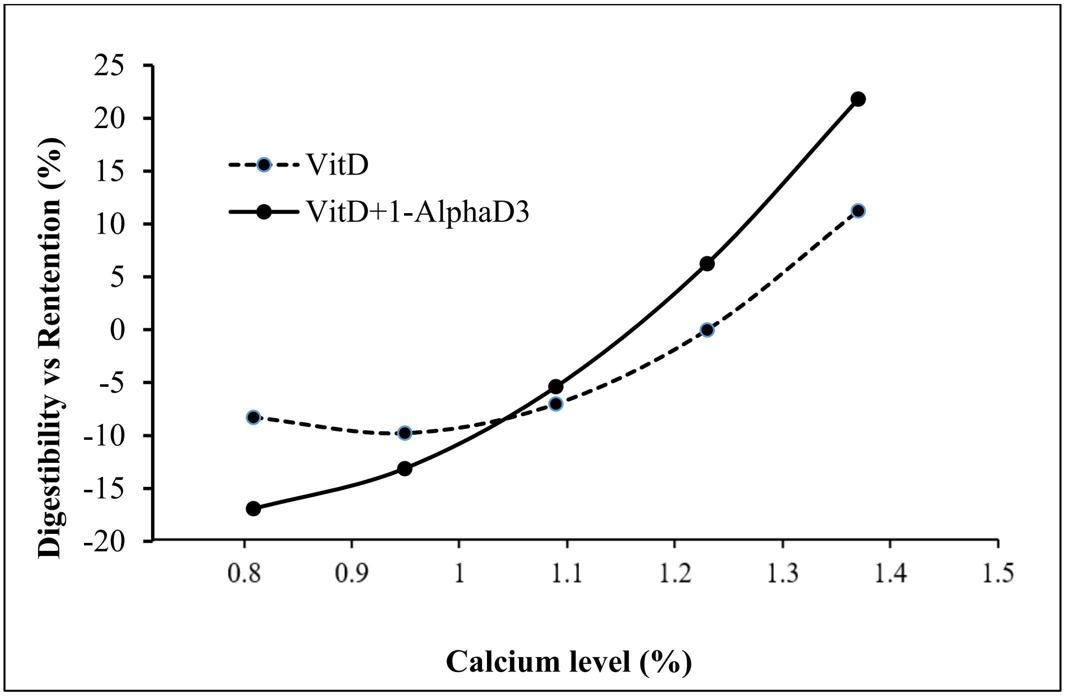

Figure 2.8. Quadratic effect of calcium level with and without 1α(OH)D_3 on Ca digestibility vs. retention percentage in diets with and without 1α(OH)D_3 from broiler males at 16d of age.

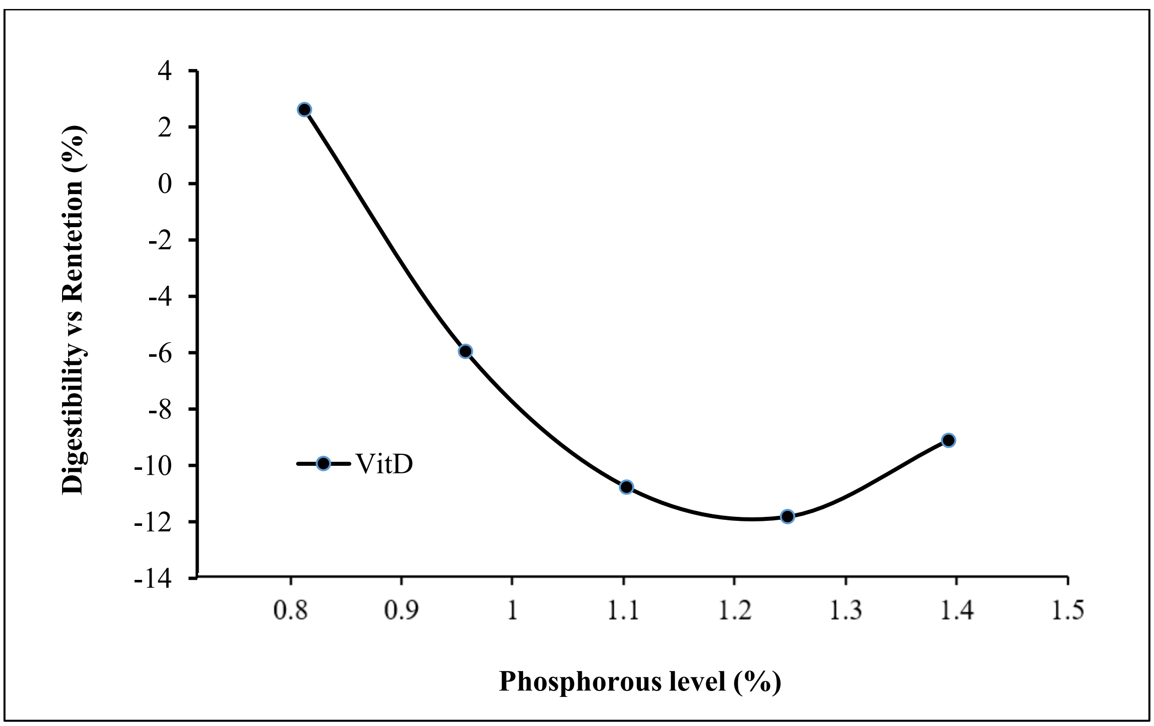

Figure 2.9. Quadratic effect of calcium level on P digestibility vs retention percentage in diets with similar level of VitD from broiler males at 16 d of age.

Table 2.10. Equations for effects of supplementation of 1α(OH)D_3 on a basal diet containing VitD_3 and different Ca levels during the starter phase on mineral retention and mineral digestibility vs. retention for Ross 708 male broilers from 0 to 14 d.

	Equation	*P* – values[1]			Optimum Calcium level (%)	R^{2*}
		Intercept	Ca	Ca^2		
Dry matter Dig. 14d VitD	$39.3862 + 55.0964*Ca - 21.3056*Ca^2$	0.0103	0.0503	0.0925	1.29	0.28
Dry matter Dig. 14d VitD + 1α(OH)D_3	$41.9703 + 58.7231*Ca - 24.7143*Ca^2$	0.0018	0.0164	0.0251	1.19	0.18
Ca Dig. 14d VitD	$82.0599 - 31.9033*Ca$	<.0001	0.0006	-	-	0.25
Ca Dig. 14d VitD + 1α(OH)D_3	-6.6894 + 162.1675*Ca - 96.3413*Ca2	0.8460	0.0155	0.002	0.84	0.73
P Dig 14d, *n*= 80	$-45.4573 + 189.1492*Ca - 83.0754*Ca^2$	0.0117	<.0001	<.0001	1.14	0.31
P Dig. 14d VitD	$-50.9910 + 191.5512*Ca - 82.3532*Ca^2$	0.0294	<.0001	0.0001	1.16	0.40
P Dig. 14d VitD + 1α(OH)D_3	$-39.9235 + 186.7473*Ca - 83.7976*Ca^2$	0.0922	0.0001	0.0001	1.11	0.30
Mn Dig. 14d, *n*= 80	$79.5977 + 21.8081*Ca - 9.8889*Ca^2$	<.0001	0.0004	0.0004	1.10	0.13
Mn Dig. 14d VitD	$72.1358 + 33.5859*Ca - 14.2341*Ca^2$	<.0001	<.0001	<.0001	1.18	0.56
Mn Dig. 14d VitD + 1α(OH)D_3	$93.5179 - 2.1658*Ca$	<.0001	0.0022	-	-	0.10
Zn Dig. 14d, *n*=80	$83.7222 + 16.1457*Ca - 7.0238*Ca^2$	<.0001	0.0053	0.0073	1.15	0.09
Zn Dig. 14d VitD	$82.0729 + 17.6131*Ca - 7.0476*Ca^2$	<.0001	0.0155	0.0311	1.25	0.30
Zn Dig. 14d VitD + 1α(OH)D_3	$93.5266 - 0.7217*Ca$	<.0001	0.2920	-	-	0.00
Ca digest. vs. retent., VitD	$71.5180 + 175.3464*Ca + 94.4971*Ca^2$	0.2086	0.1018	0.0536	0.9278	0.31
Ca digest. vs. retent, VitD + 1α(OH)D_3	$28.9424 + 126.9714*Ca + 87.0510*Ca^2$	0.4379	0.0760	0.0095	0.7293	0.80
P digest. vs. retent, VitD	$111.8344 - 203.3680*Ca + 83.5576*Ca^2$	0.4323	0.0018	0.0041	1.2169	0.43

[1]Regression analyses with $P > 0.05$ indicated non-significant effect of Ca on specific interaction effects.

* *Regression analyses correspond to a mixed model and very low R^2 may not indicate lower adjustment.*

CHAPTER III

Effect of 1-alpha-hydroxy-vitamin-D_3 on Performance, Bone Development, Meat yield and Quality and Mineral Digestibility in Grower Diets Containing Different Calcium levels for Ross-708 broilers

ABSTRACT

1,152 Ross 708 male chicks were used to evaluate the effect of $1\alpha(OH)D_3$ supplementation during the grower phase (16-34 d) in diets containing variable Ca levels. Chickens were fed with one starter diet containing $1\alpha(OH)D_3$ at 5 µg/kg and eight different grower dietary treatments from a factorial arrangement of four Ca levels (0.54; 0.76; 0.98, and 1.20%), and two doses of $1\alpha(OH)D_3$ (0 *vs*. 5µg/kg feed). FCR regression analysis indicated a quadratic effect of Ca levels (P<0.05), in which the best FCR was estimated at 0.91% Ca. Quadratic effects (P<0.01) of Ca levels were detected on carcass yields with calculated higher yields at 0.90% Ca level. Effects of Ca were observed (P<0.05) in cook loss, breast meat color lightness and spaghetti muscle incidence. Ca levels decreased (P < 0.05) valgus incidence in diets containing 1.20% Ca. Interaction effect of main factors (P<0.05) were detected on FPD and hock burns. $1\alpha(OH)D_3$ supplementation caused less (P<0.05) tibia weight RA compared with chickens non-supplemented (0.16 *vs*. 0.19). High dietary Ca (1.20%) resulted in greater tibia thickness (P<0.05), and ash content (P<0.05) as compared with chickens fed diets with low dietary Ca (0.54%). Also, tibia from those broilers were able (P<0.01) to support higher force stress to be broken. Ca level affected (P<0.05) dry matter and P digestibility. Ca levels had a negative quadratic effect on Ca dig. (P<0.01) in diets without $1\alpha(OH)D_3$ inclusion. Ca levels had a positive linear effect on P dig. $1\alpha(OH)D_3$ supplementation increased P dig. as Ca levels increased compared to diets without $1\alpha(OH)D_3$. Highest P digestibility was observed at 0.54% Ca. In conclusion, Ca levels performed quadratic effects in carcass yield, breast meat cut-up and lightness. $1\alpha(OH)D_3$ supplementation reduced FPD

incidence and provided more symmetric bone development at a low Ca level. At high Ca levels bones with less angulation deformities, greater bone strength and ash content are obtained. Finally, $1\alpha(OH)D_3$ supplementation improved digestibility of P as Ca levels increased from 0.98% to 1.20%.

KEY WORDS:

1-alpha-vitamin D_3, Calcium, performance, carcass traits, meat quality, leg health, relative assymetry, bone development, mineral digestibility.

INTRODUCTION

Currently, the poultry industry needs to face different challenges in an environment where stressors, anti-nutritional factors and toxic compounds, as mycotoxins and heavy metals, can affect liver and kidney function (Zhang et al., 2012; Imran et al., 2014; Murugesan et al., 2015). In addition, changes in broiler nutrition, where Ca levels are reduced and phytase use is widespread, give unknown panorama of how this last one can liberate Ca or make it more digestible under some conditions.

Vitamin D (VitD), a liposoluble vitamin with hormonal activities that affect primarily intestinal Ca absorption and its homeostasis in the body (Brooner and Pansu, 1999; DeLuca, 2016). Consequently, an effective VitD source could help to minimize any possible marginal Ca deficiency. This is done by the increment of Ca intestinal absorption, mobilization of intracellular Ca from bone to the extracellular fluid, as well as Ca reabsorption distal renal tubules (McDowell et al., 2000; McDonald et al., 2011).

1-α-hydroxycholecalciferol (1α(OH)D_3) is a VitD analog that have been demonstrated to have a better biopotency and faster metabolic activity (Haussler et al., 1973; Boris et al., 1977; Edwards Jr. et al., 2002). However, since 1α(OH)D_3 is a very potent metabolite, it is important to evaluate the impact on the effects produced in broilers at different dietary Ca levels, and the possible interaction between them.

A previous study was conducted in our laboratory to study the effect of 1α(OH)D_3 and different dietary Ca levels for broilers during the first 14 days of age. From this experiment, it was demonstrated that Ca and 1α(OH)D_3 affected chick live performance, as well as affect mineral digestibility and bone development and ossification during the starter phase. Therefore, it was of interest to conduct a study to determine these effects of 1α(OH)D_3 when used during the next phase of the broiler development. Consequently, the general objective of this project was to evaluate the effects of $1\alpha(OH)D_3$ on grower diets with different dietary Ca levels on performance, meat quality parameters, leg development and health, as well as Ca and P digestibility.

MATERIALS AND METHODS

Dietary treatments and chicken husbandry

All the procedures involving the birds used in the present experiment were approved by the North Carolina State University Institutional Animal Care and Use Committee. One experiment was conducted using a randomized complete block design of 2 x 5 factorial arrangement of treatments. Chickens received a common corn soybean meal starter diet from 0 to 17 days, that contained 0.99% of Ca, 0.58 %P, 4,000 IU/kg of $VitD_3$ as cholecalciferol, plus 5 µg/kg of feed of $1\alpha(OH)D_3$ (1,600 IU/kg $VitD_3$/kg feed). This starter diet also contained phytase Natuphos® at 500FTU/kg (Table 3.1; 3.2). Water was provided *ad libitum* during the whole experiment. Starter phase diet was offered at 0.90 kg/chick, and grower was fed *ad libitum* until the end of the study.

Experimental diets were formulated to meet or exceed nutrient recommendations for Ross 708 broiler chickens (Aviagen, 2014). Pelleted grower diets were offered from 18 to 35 days of age. The eight grower dietary treatments resulted from a factorial combination where the main factors were the inclusion or not of $1\alpha(OH)D_3$ in a dose of 5 µg/kg feed (1,600 IU/kg $VitD_3$/kg feed) and four levels of Ca (0.54, 0.76, 0.98, 1.20 %). The limestone particle sized used as Ca source had a geometric mean diameter of 1,129µ and a geometric standard deviation of 1.42. The solubility was of 81.72% in 60 minutes of dissolution in EDTA. Celite® was utilized as a marker. Water and diets were provided *ad libitum* during the whole experiment.

Broiler live performance

Group BW and feed intake were obtained per each pen. BW gain and FCR adjusted for mortality weights were calculated at the end of each phase: 17 and 34 days of age. Flock uniformity was obtained by weighing individually all broilers at 34 days of age and calculating CV% for each pen.

Leg health evaluation

At 31d of age, all chickens were individually evaluated for incidence of leg problems such as valgus, varus, hock burns, and twisted legs. Also, footpad dermatitis (FPD) was evaluated and scored using the system suggested by Allain et al., (2009) with scores from 0-9.

Processing, cook loss and bone collection

At 35 days, 4 chickens per pen were selected for processing obtaining a total of 24 chickens per treatment. Broilers were subjected to a 12-h of feed withdrawal the day before the processing and were slaughtered at the NCSU pilot processing plant. At processing chickens were weighed, electrically stunned for 11s, killed by exsanguination, and allowed to bleed for 90s. Broilers were then scalded at 55°C for 90 s, picked for 30 s, and manually eviscerated. Carcasses were dressed by removing liver, gizzard, heart, oil gland, crop, proventriculus, lungs, and viscera. After evisceration, carcasses were air-chilled for 6 h, to proceed a manually deboning on stationary cones. Parts of the leg quarters, breast fillets (*Pectoralis major*), breast tenders (*Pectoralis minor*), wings, and rack with skin were weighed. The carcass yield was calculated as the chilled carcass weight compared to live weight. Carcass part yields were expressed as a percentage of the chilled absolute carcass weight.

For meat quality evaluation drip loss, cook loss, shear force, color, pH, and pectoral myopathies such as wooden breast (WB), white striping (WS), and *spaghetti muscle* (SM) were evaluated. Only one experienced person performed the myopathy scoring to avoid subjective variations due to the evaluator.

Drip loss was evaluated to obtain the water holding capacity. Breast fillets were weighted and hanged from a hook in a refrigerator at 4-6°C for 24h. After this period each fillet were gently cleaned with an absorbent paper, weighted, and the difference of weight was calculated. For cook loss evaluation, the breast fillets were weighed, placed on aluminum pans, and cooked in a forced air oven (SilverStar Southbend, Model SLES/10sc, gas type, NC, USA). After reaching the internal temperature of 75°C, which took approximated 35mins, and was measured with a Therma Plus thermocouple with a 10-cm needle temperature probe (ThermoWorks Model 221-071, UT, USA), the oven was turned off and the fillets cooled to room temperature to be weighed one more time and calculate the cooked weight relative to the raw weight.

For pH determination, a portable pH meter (Oakton-Eutech Instruments® waterproof pH Tester 30) was used on breast fillets at 6 and 24 h after processing. Breast meat color values were determined (L* lightness, a* redness, and b* yellowness) using a colorimeter Minolta Chroma Meter CR-400. (Konica Minolta Sensing, Inc., Japan).

For pectoral myopathies sensorial evaluation and scoring system was conducted by experienced people to identify the presence of WS (1-3), WB: (1-4) or SM (presence or absence) myopathies. (Kuttaappan et al., 2016; Baldi et al, 2018)

Leg asymmetry, bone strength and tibia ash

From the processed chickens, three legs per pen were selected with a total of 36 legs per treatment. Legs were taken out and meat, connective tissue and fibula were removed. The weight of each bone and length were measured and recorded. A digital caliper was used to obtain the longitudinal measurements and a scale with a precision of 0.001g was used for the weights. These measures were used to calculate the relative asymmetry (RA) of the bones. The RA of the bones was calculated using the formula described by Møller et al. (2001, 2003).

$$(|L-R|/[(L+R)/2]x100)$$

After obtaining these measurements, left tibias were used for determining the bone breaking-strength. Each one of the left tibias were placed individually in a texture analyzer (TA-HD plus. Stable Micro Systems, UK) to record the bone strength. Furthermore, bone thickness was recorded. Four measurements were taken from each one of the two parts of the broken bone (frontal, caudal, medial and lateral from proximal and distal brake bone). These measurements, with the values obtained with the texture analyzer were used to calculate each bone bending moment and its maximum stress as described by Crenshaw et al. (1981).

Lipid content was extracted from the broken bones using diethyl ether as a lipid solvent. After 7 days the bones in diethyl ether solution, bones were dried overnight at room temperature, and 24 hours in an 80°C oven. The dried bones were heated in a muffle furnace at 600°C for 7 hours. The ash sample was re-weighed for calculation of the percentage of total mineral content.

Ca and P Digestibility

At 35 days, two chickens per pen were selected for ileal content collection. For the collection is was utilized the technique described by Huang et al. (2007). Jejunum content was washed with deionized water and the content collected in Falcon tubes. Samples were stored at -20°C until taken them to the laboratory for freeze-dried and further analyzed by inductively coupled plasma optical emission spectroscopy (Optima 8000, Perkin Elmer Corporation, Waltham, MA) and acid insoluble ash methodology, with Celite® as marker, for obtaining Ca and P digestibility.

Statistical analyses

Data was analyzed in a randomized complete block design with a 2 x 4 factorial arrangement of treatments and 6 replicate pens per treatment in a mixed model design. Regression analyses for inclusion levels of Ca under each VitD source and ANOVA to determine effects of Ca levels and 1-α-hydroxycholecalciferol in a factorial arrangement of treatments were used. Blocks were each one of the three rows where 16 pens, two replicates per treatment were located within the house, and were considered as a random effect in the mixed model. All percentage data were transformed using the best Box-Cox transformation prior to analyses. JMP® 13 (SAS Institute, 2017) software was the statistical tool.

RESULTS

Broiler live performance

On live performance parameters, no interactions were detected ($P > 0.05$) on BW, BW gain or flock uniformity (Table 3.3). For FCR, the regression analyses indicated a quadratic effect due to Ca levels ($P<0.05$), in which the best FCR was estimated at 0.91% Ca. (Table 3.5).

Leg health evaluation

In the leg health parameters, it was observed that diets containing 1.20% Ca level resulted in the lowest ($P<0.05$) valgus incidence (Table 3.9). An interaction effect ($P<0.05$) was detected on FPD. Broilers fed diets supplemented with 1α(OH)D_3 containing 0.54% dietary Ca had the lowest FPD score and obtained 1 point less average FPD score when compared to diet with the same Ca level (0.54%) without 1α(OH)D_3 supplementation. However, at 0.98%Ca the supplementation with $1\alpha(OH)D_3$ increased FPD when compared with the no supplemented group at the same Ca level. Regression analyses indicated an effect of Ca on FPD severity where the optimum Ca level was calculated at 1.4%. An interaction effect ($P < 0.05$) was detected for hock burn presentation. Chickens no supplemented with $1\alpha(OH)D_3$ presented the highest HB when compared to chickens with no supplemented diets with 0.98 a 1.20%Ca.

Processing, carcass and cut up yield, pH, and meat quality

No interaction ($P > 0.05$) on hot and cold carcass yields were detected. Nevertheless, quadratic effects ($P < 0.001$) of Ca levels on hot and cold carcass yield were detected (Table 3.5). The regression analyses for both variables indicated the highest carcass yields at 0.90% Ca level. On breast meat quality parameters, no interaction effects ($P > 0.05$) was observed (Table 3.7).

Interaction effect was detected ($P < 0.05$) for breast meat pH at 6 and 24 hours after processing. The diet supplemented with $1\alpha(OH)D_3$ with 1.20%Ca decreased the breast meat pH at both sampled hours (6 and 24hr), compared to the same dietary Ca level diet without $1\alpha(OH)D_3$ supplementation (Table 3.6). Also, with the utilization of $1\alpha(OH)D_3$ in a 1.20%Ca diet, the pH decreased as compare to a supplemented diet at 0.76%Ca.

Effects of Ca were detected ($P < 0.05$) in cook loss and color lightness value (Table 3.7). Dietary Ca levels of 1.20% obtained the lowest cook loss% with 2.63% less cook loss% than the diet with 0.98%Ca. No effects of treatments ($P > 0.05$) were observed in WB or WS scores; however, spaghetti muscle increased ($P < 0.05$) with 0.54% Ca level (Table 3.8).

Leg asymmetry, bone strength and tibia ash

Chickens fed high dietary Ca (1.20%) resulted in a greater tibia thickness ($P<0.05$), and ash content ($P < 0.05$) than chickens fed diets with low dietary Ca (0.54%) (Table 3.10).

Additionally, an interaction effect was detected for tibia ash% (*P* < 0.05). The supplementation of 1α(OH)D_3 in diets with 1.20% Ca increased tibia ash percentage as compared with diets of 0.54%Ca levels, regardless the supplementation of 1α(OH)D_3. A quadratic positive effect was obtained for Ca on tibia strength (Table 3.11). The regression analysis indicated an optimum Ca levels at 1.1% Ca for the higher tibia strength.

Ca and P digestibility

An interaction effect was detected (*P* < 0.05) for Ca dig. The regression analyses for Ca dig. revealed a negative quadratic effect of Ca levels (*P* < 0.01) in diets without 1α(OH)D_3 inclusion and no changes (*P* < 0.05) in Ca dig. as Ca increased in diets with 1α(OH)D_3. On the mineral, Ca and P, digestibility analysis it was detected a main effect caused by Ca level (*P* < 0.05) on dry matter and P digestibility. The worst dry matter digestibility was observed at 0.54% Ca level. Dietary Ca had a positive quadratic effect on dry matter dig. (*P* < 0.05) with a calculated optimum at 0.99% Ca (Table 3.13). For P digestibility, no interaction effect (*P* > 0.05) was detected; however, Ca levels had a negative linear effect (*P* < 0.01) and 1α(OH)D_3 supplementation increased P dig. as Ca levels increased compared to diets free of 1α(OH)D_3. The highest P dig. was observed at 0.54% Ca.

DISCUSSION

Supplementation of 1α(OH)D_3 showed no effect in growth performance parameters between 17 and 34 d of age, which differed with previous studies where the use of 1α(OH)D_3 increased performance parameters like BW, BWG or FCR (Snow et al., 2004; Han et al., 2009; 2012). When comparing with these studies, it can be observed that similar conditions and doses of 1α(OH)D_3 (5μg/kg) were used, however the responses were different in between these studies.

Snow et al., (2004), using 1α$(OH)D_3$, with or without the supplementation of 300 units of phytase, obtained an increment in BW, FI and G:F. In the same way, Han et al., (2009) obtained an increment in these parameters with the utilization of 5μg/kg of 1α$(OH)D_3$, when using 1,000 IU VitD_3/kg, unlike the present study, where 4,000 IU VitD_3/kg were used.

An important factor to mention and probably the cause of the different responses is related to the fact that in the present study 1α$(OH)D_3$ was offered to all the chickens from 1 to 35 days of age. Han et al. (2009) analyzed the effects caused during the grower phase but supplemented feed with only from 21 to 42d. In the present study, the 1α$(OH)D_3$ metabolite was fed to all chickens since the first day with the intention to mimic the current utilization in the poultry industry. Consequently, 1α$(OH)D_3$ supplementation in the starter phase may have an effect even in the grower phase reducing chances to detect differences among treatments in the present study.

The same author in other study, Han et al. (2012), improved tibia weight and strength by the utilization of 1α$(OH)D_3$ at a dose of 10 μg/kg of feed. However, in the group with addition of 5 μg/kg, that is the dose used in the present study and currently in the industry, no improvement on weight or strength was detected as it was observed in the present study. This suggested that probably a higher dose of 1α$(OH)D_3$ is required to obtain an improvement in bone strength. As mentioned by Han et al. (2012) this increment in tibia strength can be obtained by the increment of Ca and P in the tibia.

Nonetheless, at this dose level (5 μg/kg) of 1α$(OH)D_3$, it was obtained lower tibia weight RA, as well as, an increment in tibia ash percentage. It has been demonstrated that tibia ash levels recovered to normal levels in P deficient diets when phytase was used (Biehl et al., 1995). Also, that the use of phytases has an additive effect when 1α$(OH)D_3$ is used, what indicates a positive effect of 1α$(OH)D_3$ in P utilization (Biehl and Baker 1997; Edwards, 2002).

Water holding capacity can be evaluated by three different methods; drip loss, squeezable water and cooking loss (Huff-Lonergan and Lonergan, 2005; Bozkurt et al., 2017). In the present study, it was detected an effect in the cook loss percentage at the higher values of Ca% inclusion. The post mortem expected increment of lactic acid, will decrease meat pH, allowing the enzymatic degradation of myofibril structures and allowing the release of water hold in the muscle cell (Roque-Specht et al., 2009; Garcia et al., 2013). Lower water holding capacity means a higher cook loss%.

An interaction effect was detected on breast meat pH at 6 and at 24 hours, maintaining the same effect in both sampling moments. Here, it was noticed again that the meat from the treatments with the highest dietary level of Ca (1.20%) obtained the lowest value of pH when it was supplemented with $1\alpha(OH)D_3$. Previous studies (Han et al., 2009; Garcia et al., 2013) did not detect an effect of $1\alpha(OH)D_3$ supplementation in breast pH when diets were formulated with regular Ca inclusion, what suggested that the effect caused $1\alpha(OH)D_3$ is obtained only at higher dietary Ca.

Also, different studies have indicated that meat pH (Allen et al., 1998; Roque-Specht et al., 2009; Han et al., 2012), and $1\alpha(OH)D_3$ supplementation (Wiegand et al., 2002; Han et al., 2009; Garcia et al., 2012) can affect meat color presentation. In the current study only a slightly effect of dietary Ca was observed on L*. The lower L* values that were obtained in the treatments with the highest Ca concentrations indicating a darker meat in comparison to the other treatments with less dietary Ca. Lightness have been related with denaturalization of meat protein (Qiao et al., 2002; Garcia et al., 2012). It can be suggested that Ca level is limiting the denaturalization at high levels, getting less lightness in the meat.

Similarly, an effect of Ca on spaghetti muscle presentation was detected with higher presentation at 0.54%Ca. No effect was detected for the other studied myopathies (WS, WB) from

Ca, nor from 1α$(OH)D_3$. Spaghetti myopathy is a relatively new myopathy that is obtaining interest and recently started to be studied because of the aspect of the meat where bundles of muscles are presented and can be detected by a naked eye (Kuttappan et al., 2016; Baldi et al., 2018). Few information is known about the factors that are generating this spaghetti myopathy. Recently, Baldi et al., (2018) found a decrease in the protein content and increase moisture in affected broiler fillet samples, but more studies must be conducted.

Furthermore, breast meat showed an effect on Ca, where the higher yield was detected at 0.90%Ca. This same treatment indicated the best hot and cold carcass yields. The regression analysis of these variables indicated a quadratic effect where is observed that at highest and lowest Ca% the carcass yields decrease. Moreover, an effect of Ca was also detected for feed intake, where chickens fed with dietary 0.76%Ca, have the lower feed consumption during the experiment, what indicated that at 0.76%Ca chickens were able to get these high yields despite the low food intake. The data suggest that dietary excess or deficient level of Ca can impair the development of muscle cells that can be reflected in the increase of yield.

Previous studies have demonstrated that 1α$(OH)D_3$ increased Ca (Snow et al., 2004) and P (Edwards et al, 2002) utilization . In our study we also detected an increment in P digestibility when 1α$(OH)D_3$ was offered. For Ca, this positive effect of 1α$(OH)D_3$ was also observed as Ca levels increased from 0.98% to 1.20%. It has been demonstrated that the addition of $VitD_3$ improve P utilization P (Mohamed et al., 1991; Qian et al., 1997), and it can be expected that this same reaction is happening with the supplementation of 1α$(OH)D_3$ in P digestibility.

Also interestingly, Mutucumarana et al. (2014) performed digestibility essays where compared the dietary Ca content vs mineral digestibility. In their results it was obtained that the increment of dietary Ca reduced the P digestibility in the intestinal segments that were evaluated (jejunum, upper and lower ileum). This agree with the findings of the experiment presented herein, where the digestibility of P falls notoriously as the Ca% increased regardless the supplementation or not of $1\alpha(OH)D_3$. Probably the excess of Ca can be forming complexes with the phytic phosphate (Angel et al., 2003). These complexes made the P molecules not available for enzymatic hydrolysis by phytases producing the reduction in digestibility (Plumstead et al., 2008). P digestibility was also affected by the supplementation of $1\alpha(OH)D_3$ increasing the digestibility % in 2.5%. In a study conducted by Bielh et al. (1995) it was concluded that the utilization of $1\alpha(OH)D_3$ supplemented at 20µg/kg improve the utilization of P and it have additive effects when a phytase is supplemented at 1200units/kg.

CONCLUSION

In conclusion, no consistent effects on live performance of $1\alpha(OH)D_3$ inclusion were observed during the grower phase, when starter diets contained $1\alpha(OH)D_3$. However, $1\alpha(OH)D_3$ improved tibia ash content and decreased tibia weight RA. A diet supplemented with $1\alpha(OH)D_3$ combined with a low Ca level reduced FPD incidence in broilers and provided *per se* a more symmetric bone development. Inclusion of about 1.20% Ca level on diets could develop bones with less angulation deformities, greater bone strength and ash content.

On the other side, it was confirmed that Ca levels during the grower phase play an important role on live performance, carcass yield, and meat quality parameters. Also, dietary Ca level in grower diets affected Ca and P digestibility. The supplementation of $1\alpha(OH)D_3$ improved digestibility of P as Ca levels increased from 0.98% to 1.20%.

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Table 3.1. Ingredient composition of starter diet and grower basal diets for Ross-708 male broilers.

Ingredient Name	Starter (1-15d)	Grower (16-34d)
Corn	55.84	57.59
Soybean Meal, 46%	31.70	26.89
Corn Gluten Meal, 60%	4.90	5.00
Soybean Oil	3.12	4.85
Dicalcium phosphate[1]	1.37	0.31
Limestone Cerne Pure Cal 12-40[2]	1.06	0.01
Sodium bicarbonate	0.36	0.23
L-Lysine-HCl, 78.8%	0.36	0.31
DL-Methionine, 99%	0.29	0.25
Salt (NaCl)	0.28	0.28
Mineral premix[3]	0.20	0.20
Choline chloride, 60%	0.18	0.18
L-threonine	0.13	0.10
Vitamin premix[4]	0.10	0.10
Anticoccidial[5]	0.05	0.05
Selenium premix	0.05	0.05
Sand	0.01	3.60
Celite	-	1.00
Limestone dicalcium base	-	2.60
$1\alpha(OH)D_3$	0.00125	-
Natuphos E® [6]	0.005	0.005
Total	100.00	100.00

[1]Dicalcium phosphate contains 19.79% Calcium, 17.9091% phosphorus and 17.73% available phosphorus (99%).
[2]Limestone (Cerne Pure Cal 12-4) contains 39.01% Calcium;
[3]Trace minerals provided per kg of premix: manganese (Mn SO4), 60 g; zinc (ZnSO4), 60 g; iron (FeSO4), 40 g; copper (CuSO4), 5 g; iodine (Ca(IO3)2),1.25 g.
[4]Vitamins provided per kg of premix: vitamin A, 13,227,513 IU; vitamin D_3, 3,968,253 IU; vitamin E, 66,137 IU; vitamin B12, 39.6 mg; riboflavin, 13,227 mg; niacin, 110,229 mg; d-pantothenic acid, 22,045 mg; menadione, 3,968 mg; folic acid, 2,204 mg; vitamin B6, 7,936 mg; thiamine, 3,968 mg; biotin, 253.5 mg.
[5]Coban® 90 (Monensin), Elanco Animal Health, Greenfield, IN, at 500 g/ton in the starter and grower diets.
[6]Natuphos E® (500 FTU/kg, 50 g/ton FTU)

Table 3.2 Nutritional content of basal starter and grower diets for Ross-708 male broilers.

Nutrient	Starter (1-15d)	Grower (16-34d)
Crude protein, %	23.14	20.94
Calcium, %	0.87	0.24
Total phosphorous, %	0.58	0.36
Non phytate phosphorous, %	0.38	0.18
Phytate phosphorous, %	0.24	0.22
Total methionine, %	0.65	0.59
Total cysteine, %	0.38	0.35
Total lysine, %	1.41	1.23
Total tryptophan, %	0.27	0.24
Total threonine, %	0.97	0.86
Total isoleucine, %	0.97	0.87
Total valine, %	1.09	0.98
Total leucine, %	2.15	2.00
Total arginine, %	1.43	1.27
Total sulphur amino acids, %	1.03	0.93
Total glycine	0.90	0.81
Digestible lysine, %	1.28	1.12
Digestible methionine, %	0.63	0.56
Digestible cysteine, %	0.32	0.29
Digestible total sulphur amino acids, %	0.94	0.85
Digestible threonine, %	0.85	0.75
Digestible tryptophan, %	0.23	0.21
Digestible, isoleucine, %	0.88	0.79
Digestible leucine, %	1.99	1.85
Digestible valine, %	0.96	0.87
Digestible arginine, %	1.33	1.18
Sodium, %	0.23	0.19
Potassium, %	0.88	0.781
Chloride, %	0.28	0.275
Metabolizable energy, kcal/kg	3,000.00	3,100.00
DietaryElectrolyteBa mEq/kg	267.53	227.10

Table 3.3. Effect of $1\alpha(OH)D_3$ on grower broiler diets (17-35d) with different dietary calcium levels for male broilers Ross-708[1].

Ca	VitD	BW			Flock	BWG	
		0d	16d	34d	uniformity	0-16d	16-34d
		-----------------g----------------			-----%----	--------g-------	
0.54		44.5	676	2,569	7.33	631	1,889
0.76		44.6	673	2,607	7.38	628	1,921
0.98		44.5	672	2,611	8.11	627	1,933
1.20		44.5	678	2,604	7.92	633	1,920
SEM		0.06	5.36	14.16	0.51	5.48	14.1
	VitD	45	675	2,607	7.69	630	1,923
	VitD+$1\alpha(OH)D_3$	45	675	2,589	7.68	630	1,908
	SEM	0.05	4.79	9.61	0.42	4.91	10.34
0.54	VitD	45	679	2,586	7.13	634	1,902
	VitD+$1\alpha(OH)D_3$	44	673	2,551	7.54	628	1,877
0.76	VitD	45	667	2,603	7.51	622	1,922
	VitD+$1\alpha(OH)D_3$	45	679	2,612	7.24	634	1,920
0.98	VitD	45	675	2,628	8.53	630	1,944
	VitD+$1\alpha(OH)D_3$	45	670	2,595	7.69	625	1,922
1.20	VitD	45	678	2,611	7.61	633	1,925
	VitD+$1\alpha(OH)D_3$	45	678	2,597	8.24	633	1,914
	SEM	0.1	6.4	20.4	0.65	6.48	19.63
CV%		0.37	1.76	1.97	16.01	1.90	2.46
Source of variation		--------------------------- *P*-values ---------------------------					
Ca		0.8387	0.5741	0.1683	0.4332	0.5697	0.1541
VitD source		0.9239	0.9229	0.2194	0.9658	0.9046	0.2738
Ca*VitD		0.8894	0.2258	0.7055	0.5701	0.2560	0.9233
		----------Regression *P*-values----------					
Ca linear		-	0.7192	0.0979	0.1713	0.7151	0.0921
Ca*Ca quadratic		-	0.2479	0.1196	0.7745	0.2246	0.9880
Metabolite		-	0.9325	0.2105	0.9657	0.9166	0.2622
Metabolite *Ca		-	0.9698	0.8727	0.9817	0.9925	0.8576

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's and student's t-test.

[1]Values are means of 6 pens per treatment with 24 male broiler chickens per pen

Table 3.4. Effect of 1α(OH)D_3 on grower broiler diets (17-35d) with different dietary calcium levels for male broilers Ross-708[1].

Ca	VitD	Feed Intake		FCR adj*		
		0-16d	16-34d	16d	34d	16-34d
		------------(g)------------		--------------(g:g)------------		
0.54		744	2,810^{ab}	1.178	1.402^{a}	1.560^{a}
0.76		744	2,800^{b}	1.183	1.383^{b}	1.529^{b}
0.98		738	2,843^{ab}	1.177	1.388^{b}	1.537^{b}
1.20		750	2,850^{a}	1.172	1.389^{ab}	1.550^{ab}
SEM		6.44	14.63	0.01	0.01	0.01
	VitD	741	2,834	1.178	1.384^{b}	1.533^{b}
	VitD+1α(OH)D_3	746	2,817	1.178	1.397^{a}	1.555^{a}
	SEM	4.64	12.05	0.01	0.01	0.01
0.54	VitD	748	2,815	1.178	1.397	1.550
	VitD+1α(OH)D_3	741	2,805	1.178	1.407	1.570
0.76	VitD	739	2,821	1.188	1.373	1.511
	VitD+1α(OH)D_3	748	2,779	1.177	1.393	1.548
0.98	VitD	736	2,847	1.172	1.378	1.523
	VitD+1α(OH)D_3	739	2,839	1.182	1.398	1.552
1.20	VitD	743	2,855	1.172	1.390	1.548
	VitD+1α(OH)D_3	757	2,846	1.173	1.389	1.552
	SEM	9.02	18.75	0.01	0.01	0.01
CV %		2.94	1.40	1.82	0.85	1.24
Source of variation		----------------------------*P*-values---------------------------				
Ca		0.5950	0.0114	0.7290	0.0030	0.0019
VitD		0.4488	0.1560	0.9857	0.0012	0.0002
Ca*VitD		0.6922	0.7249	0.7086	0.1382	0.1867
		---------------------Regression *P*-values---------------------				
Ca linear		0.7116	0.073	0.8501	0.0447	0.4143
Ca*Ca quadratic		0.3010	0.582	0.9159	0.007	0.0012
Metabolite		0.4406	0.975	0.5272	0.0017	0.0008
Metabolite *Ca		0.3305	0.822	0.2234	0.3529	0.3241

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's and student's *t*-test. [1]Values are means of 6 pens per treatment with 24 male broiler chickens per pen
[*] FCR was adjusted by mortality weights.

Table 3.5. Effect of $1\alpha(OH)D_3$ on grower broiler diets with different dietary calcium levels on cut-up parts yields of Ross-708 males at 34d[1]

Ca	VitD	Cut – up parts relative to carcass weight						
		Hot carcass yield	Cold carcass yield	Wings	Leg Quarters	Skin	Breast meat	Rack
		----------------------(%)----------------------						
0.54		74.21^{c}	73.83^{c}	7.36	22.23	2.36	30.42^{ab}	11.44
0.76		75.22^{a}	74.92^{a}	7.37	22.06	2.54	31.19^{a}	11.54
0.98		74.88^{ab}	74.57^{ab}	7.37	22.41	2.44	30.41^{b}	11.80
1.20		74.64^{bc}	74.24^{bc}	7.31	22.42	2.28	30.42^{b}	11.79
SEM		0.20	0.24	0.05	0.14	0.12	0.20	0.17
	VitD	74.85	74.53^{a}	7.34	22.35	2.47	30.68	11.63
	VitD+$1\alpha(OH)D_3$	74.62	74.24^{b}	7.37	22.21	2.33	30.54	11.65
	SEM	0.17	0.22	0.04	0.10	0.10	0.14	0.26
0.54	VitD	74.30	73.89	7.34	22.47^{ab}	2.37	30.21	11.47
	VitD+$1\alpha(OH)D_3$	74.13	73.77	7.38	21.98^{ab}	2.34	30.64	11.40
0.76	VitD	75.50	75.30	7.38	22.47^{ab}	2.54	31.23	11.49
	VitD+$1\alpha(OH)D_3$	74.94	74.53	7.36	21.65^{b}	2.53	31.15	11.59
0.98	VitD	74.91	74.61	7.34	22.21^{ab}	2.56	30.60	11.77
	VitD+$1\alpha(OH)D_3$	74.84	74.52	7.39	22.62^{a}	2.31	30.23	11.84
1.20	VitD	74.68	74.32	7.29	22.24^{ab}	2.42	30.70	11.80
	VitD+$1\alpha(OH)D_3$	74.60	74.16	7.34	22.61^{a}	2.13	30.14	11.79
	SEM	0.24	0.27	0.07	0.20	0.15	0.29	0.12
CV%		1.21	1.24	4.68	4.16	21.62	4.98	7.96
Source of variation		---------------------------- *P*-values---------------------------						
Ca		0.0001	0.0001	0.7923	0.2423	0.2124	0.0218	0.2537
VitD source		0.1136	0.0418	0.5218	0.3385	0.1183	0.4784	0.8944
Ca*VitD source		0.5620	0.2552	0.9296	0.0071	0.5903	0.3321	0.9805
		----------------------Regression *P*-values---------------------						
Ca linear		0.2289	0.2692	0.5682	0.1368	0.3984	0.2873	0.0410
Ca*Ca quadratic		<0.0001	<.0001	0.3229	0.549	0.0146	0.0133	0.8931
VitD source		0.1029	0.0467	0.3469	0.3202	0.2086	0.3562	0.45404
VitD source *Ca		0.6648	0.8408	0.6735	0.0024	0.1837	0.0918	0.5680

[1]Values are means ± SEM of 6 pens per treatment with 4 male broiler chickens per pen selected for processing

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's and student's *t*-test.

Table 3.6. Effect of 1α(OH)D_3 on grower broiler diets with different dietary calcium levels on meat quality and color parameters[2] of Ross-708 males at 34 d[1].

Ca	VitD	Breast meat pH		Cook	Drip	Color		
		6h	24h	loss	loss	L*	a*	b*
				-------(%)-------				
0.54		6.01	6.06	14.61[ab]	1.39	52.72[ab]	3.25	9.13
0.76		6.01	6.08	14.72[ab]	1.45	54.06[a]	3.57	9.99
0.98		6.03	6.07	16.43[a]	1.51	53.80[a]	3.78	9.75
1.20		5.99	6.05	13.80[b]	1.41	52.09[b]	3.92	9.34
SEM		0.03	0.02	0.81	0.07	0.42	0.20	0.32
	VitD	6.02	6.07	14.98	1.48	53.43	3.65	9.45
	VitD+1α(OH)D_3	6.01	6.06	14.80	1.40	52.90	3.61	9.65
	SEM	0.02	0.01	0.64	0.05	0.31	0.13	0.23
0.54	VitD	6.00[ab]	6.05[ab]	14.20	1.46	52.55	3.23	8.94
	VitD+1α(OH)D_3	6.02[a]	6.07[a]	15.01	1.31	54.24	3.41	9.33
0.76	VitD	5.99[ab]	6.06[ab]	14.83	1.44	54.76	3.79	9.84
	VitD+1α(OH)D_3	6.04[a]	6.11[a]	14.61	1.45	52.18	4.20	10.14
0.98	VitD	6.03[a]	6.07[a]	16.23	1.61	52.89	3.27	9.72
	VitD+1α(OH)D_3	6.04[a]	6.08[ab]	16.63	1.42	53.88	3.74	9.77
1.20	VitD	6.04[a]	6.10[a]	14.66	1.40	52.83	3.78	9.29
	VitD+1α(OH)D_3	5.94[b]	5.99[b]	12.95	1.42	51.99	3.64	9.38
	SEM	0.03	0.03	1.08	0.11	0.58	0.29	0.44
CV%		2.06	1.92	35.67	26.36	6.93	33.34	21.60
Source of variation		---------------------------- *P*-values---------------------------						
Ca		0.4723	0.5616	0.0783	0.6638	0.0035	0.1367	0.1969
VitD source		0.8608	0.7808	0.7955	0.3426	0.1851	0.8228	0.4965
Ca*VitD source		0.0315	0.0335	0.6135	0.6852	0.2242	0.5109	0.9732
		----------------------Regression *P*-values---------------------						
Ca linear		0.5602	0.8435	0.8935	0.8764	0.2439	0.0195	0.9128
Ca*Ca quadratic		0.1878	0.0971	0.1228	0.3330	0.0004	0.6534	0.5046
VitD source		0.7451	0.8764	0.7785	0.0184	0.1834	0.8118	0.6584
VitD source *Ca		0.0155	0.0844	0.2947	0.4482	0.3723	0.2476	0.0371

[1] Values are means ± SEM of 6 pens per treatment with 4 male broiler chickens per pen selected for processing
[2]Warner-Bratzler; Color: L∗ = lightness, a∗ = redness, b∗ = yellowness
[a-b] Means in a column not sharing a common superscript are significantly different (P < 0.05) by Tukey's and student's t-test.

Table 3.7. Regression equation of effect of dietary Ca levels and $1\alpha(OH)D_3$ supplementation on performance, yields and meat quality parameters and breast meat color of Ross 708 male broilers at 35 days of age.

	Equation	*P*- values[1]			Optimum Calcium level (%)	R^{2*}
		Intercept	Ca	Ca^2		
FI/bird	2766.0070 + 70.9164*Ca	<.0001	0.0052	-	-	0.15
FCR adj 16-34d	1.7090 - 0.3999*Ca + 0.2239*Ca^2	<.0001	0.0029	0.0035	0.8934	0.15
FCR adj 16-34d VitD	1.7641 - 0.5805*Ca + 0.3349*Ca^2	<.0001	0.0007	0.0006	0.8670	0.38
FCR adj 0-34d	1.4908 - 0.2376*Ca + 0.1317*Ca^2	<.0001	0.0072	0.0089	0.9019	0.12
Cold carcass yield %	68.9827 + 13.0149*Ca level - 7.2392*Ca level^2	<.0001	<.0001	<.0001	0.90	0.11
Cold carcass yield %, vitD	68.1830 + 15.5155*Ca level - 8.7509*Ca level^2	<.0001	<.0001	<.0001	0.89	0.14
Cold carcass yield %, vitD+$1\alpha(OH)D_3$	69.7796 + 10.5223*Ca level - 5.7329*Ca level^2	<.0001	0.37	0.0056	0.92	0.08
Hot carcass yield %	69.9159 + 11.5377*Ca level - 6.3823*Ca level^2	<.0001	<.0001	<.0001	0.90	0.10
Hot carcass yield %, vitD	69.4851 + 13.0998*Ca level - 7.3821*Ca level^2	<.0001	0.0005	0.0006	0.89	0.10
Hot carcass yield %, vitD+$1\alpha(OH)D_3$	70.3463 + 9.9770*Ca level - 5.3834*Ca level^2	<0.0001	0.0042	0.0069	0.93	0.08
Skin %,	1.3228 + 2.8682*Ca level - 1.7350*Ca level^2	0.0282	0.0507	0.0386	0.83	0.02
L*	43.0990 + 26.2803*Ca level - 15.6520*Ca level^2	<.0001	0.0046	0.0032	0.84	0.04
L* , vitD	38.3200 + 38.0659*Ca level - 22.0274*Ca level^2	<.0001	0.0032	0.0029	0.86	0.07
a*	2.7544 + 1.0081*Ca level	<0.0001	0.0076	-	-	-
a* , vitD	2.3556 + 1.4930*Ca level	<0.0001	0.0043	-	-	-
b* , *n*=184	4.9304 + 11.3450*Ca level - 6.4247*Ca level^2	0.0293	0.0388	0.0401	0.88	0.01

[1]Regression analyses with P > 0.05 indicated non-significant effect of Ca on specific interaction effects.
* Regression analyses correspond to a mixed model and very low R^2 may not indicate lower adjustment.

Table 3.8. Effect of $1\alpha(OH)D_3$ on broiler grower diets with different dietary calcium levels on wooden breast sensorial score, white stripping and spaghetti muscle of Ross-708 males at 34d[1]

Ca	VitD	Sensorial scores for breast muscle myopathies		
		White stripping	Wooden breast	Spaghetti muscle
		-------(1-3)------	-------(1-4)------	-------(0-1)------
0.54		1.96	3.06	0.23[a]
0.76		2.10	3.25	0.04[b]
0.98		2.08	3.10	0.13[ab]
1.20		2.04	3.02	0.08[ab]
SEM		0.12	3.11	0.05
	VitD	2.07	3.18	0.12
	VitD+$1\alpha(OH)D_3$	2.02	3.04	0.11
	SEM	0.11	0.07	0.04
0.54	VitD	1.92	3.04	0.25
	VitD+$1\alpha(OH)D_3$	2.00	3.08	0.21
0.76	VitD	2.08	3.20	0.04
	VitD+$1\alpha(OH)D_3$	2.13	3.29	0.04
0.98	VitD	2.21	3.21	0.08
	VitD+$1\alpha(OH)D_3$	1.96	3.00	0.17
1.20	VitD	2.08	3.25	0.13
	VitD+$1\alpha(OH)D_3$	2.00	2.79	0.04
	SEM	0.15	0.16	0.07
CV%		30.98	26.77	271.67
Source of variation		---------------------------*P* - values--------------------------		
Ca		0.6504	0.5272	0.0273
VitD source		0.5582	0.2449	0.8190
Ca*VitD source		0.5151	0.3151	0.5800
		---------------------Regression *P*-values--------------------		
Ca linear		0.5892	0.5498	0.0997
Ca*Ca quadratic		0.2600	0.2155	0.1207
VitD source		0.6554	0.2215	0.8417
VitD source *Ca		0.3016	0.0653	0.9107

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's and student's *t*-test.
[1] Values are means ± SEM of 6 pens per treatment with 4 male broiler chickens per pen selected for processing.

Table 3.9. Effect of 1α(OH)D_3 on broiler grower diets with different dietary calcium levels on the incidence (0-1) of leg health issues or footpad dermatitis average score (0-9) of Ross-708 males at 34 d.

Ca	VitD	Straight	Valgus	Hock burn	Footpad dermatitis
		------------(0 - 1) -----------			---(0 - 9)---
0.54		0.31^{ab}	0.69^{ab}	0.15	1.37^{c}
0.76		0.25^{b}	0.74^{a}	0.12	1.64^{b}
0.98		0.22^{b}	0.77^{a}	0.10	1.95^{a}
1.20		0.39^{a}	0.60^{b}	0.13	2.15^{a}
SEM		0.03	0.03	0.03	0.09
	VitD	0.31	0.68	0.12	1.84
	VitD+1α(OH)D_3	0.27	0.72	0.12	1.72
	SEM	0.02	0.02	0.03	0.07
0.54	VitD	0.37	0.63	0.21^{a}	1.86^{bcd}
	VitD+1α(OH)D_3	0.25	0.74	0.10^{ab}	0.89^{e}
0.76	VitD	0.24	0.76	0.13^{ab}	1.81^{bcd}
	VitD+1α(OH)D_3	0.27	0.71	0.10^{ab}	1.48^{d}
0.98	VitD	0.21	0.78	0.07^{b}	1.75^{cd}
	VitD+1α(OH)D_3	0.23	0.77	0.13^{ab}	2.15^{ab}
1.20	VitD	0.44	0.56	0.08^{b}	1.96^{abc}
	VitD+1α(OH)D_3	0.33	0.65	0.17^{ab}	2.34^{a}
	SEM	0.04	0.04	0.04	0.11
CV%		153.78	64.85	263.59	45.92
Source of variation		-------------------*P*-values-------------------			
Ca		0.0003	0.0003	0.3299	<0.0001
VitD source		0.1360	0.2303	0.9908	0.0540
Ca*VitD source		0.1265	0.1515	0.0029	<0.0001

[a-d] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's and student's *t*-test.

[1] Values are means of 6 pens per treatment with 24 male broiler chickens per pen.

Table 3.10. Effect of dietary calcium level and 1α(OH)D_3 on tibia strength and mineralization of Ross 708 male broiler at 35 d of age[1].

Ca	VitD	Thickness	Inertia	Moment	Max Stress	Max Force	Tibia ash
		---mm---	--m^4--	--Nm--	--MPa--	--Nm--	--%--
0.54		1.47^{b}	2.91E-10	4.71^{b}	72.9^{b}	295.67^{b}	41.44^{b}
0.76		1.57^{ab}	3.01E-10	5.39^{a}	81.1^{a}	300.87^{a}	42.55^{a}
0.98		1.56^{ab}	3.12E-10	5.79^{a}	86.7^{a}	362.85^{a}	42.61^{a}
1.20		1.62^{a}	3.09E-10	5.79^{a}	84.0^{a}	363.53^{a}	42.79^{a}
SEM		0.03	1.16E-11	0.20	2.3	10.59	0.28
	VitD	1.53	3.02E-10	5.30	79.7	333.23	42.09^{b}
	VitD+1α(OH)D_3	1.58	3.05E-10	5.59	82.6	347.84	42.61^{a}
	SEM	0.02	8.20E-12	0.10	1.4	7.16	0.19
0.54	VitD	1.45	2.93E-10	4.71	71.9	292.04	41.11^{c}
	VitD+1α(OH)D_3	1.49	2.89E-10	4.81	73.8	299.20	41.78^{bc}
0.76	VitD	1.55	3.08E-10	5.49	80.5	343.53	42.22^{abc}
	VitD+1α(OH)D_3	1.59	2.94E-10	5.39	81.6	336.76	42.88^{ab}
0.98	VitD	1.55	3.13E-10	5.59	82.7	348.63	42.77^{ab}
	VitD+1α(OH)D_3	1.57	3.11E-10	6.08	90.6	377.07	42.46^{ab}
1.20	VitD	1.57	2.94E-10	5.59	83.8	348.63	42.27^{ab}
	VitD+1α(OH)D_3	1.67	3.25E-10	6.08	84.2	378.44	43.31^{a}
	SEM	0.05	1.64E-11	0.20	3.4	15.20	0.39
	CV%	14.00	24.22	20.25	19.81	20.26	3.40
Source of variation		----------------------------------*P*-values----------------------------------					
Ca		0.024	0.5989	<.0001	0.0015	<.0001	0.0064
VitD source		0.135	0.8269	0.1809	0.2635	0.1831	0.0692
Ca*VitD source		0.827	0.5412	0.5730	0.7150	0.5716	0.3792
		------------------------------- Regression *P*-values-------------------------					
Ca		0.0050	0.2110	<.0001	0.0008	<.0001	0.0002
Ca*Ca		0.5053	0.6183	0.0466	0.0285	0.0449	0.0511
VitD source		0.1239	0.8423	0.1896	0.3092	0.1913	0.0354
VitD source * Ca		0.5087	0.2506	0.2966	0.9363	0.2853	0.8320

[1] Values are means ± SEM of 6 pens per treatment with 4 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's and student's *t*-test.

Table 3.11. Regression equations for effect of supplementation of $1\alpha(OH)D_3$ on top of VitD and dietary Ca levels for Ross 708 male broilers on bone strength, mineralization and on ileal digestibility at 35d.

	Equation	*P* - values[1]			Optimum Ca level (%)	R^{2*}
		Intercept	Ca	Ca^2		
Moment	$0.1617 + 0.7887*Ca - 0.3568*Ca^2$	0.2224	0.0149	0.0523	1.11	0.13
Max Stress	$6660105.1 + 1716759.1*Ca$	<.0001	0.0247	-	-	0.05
Max Stress VitD + $1\alpha(OH)D_3$	$1566084.7 + 14914159*Ca - 7514352.6*Ca^2$	0.5666	0.0274	0.0506	0.99	0.09
Max Force,	$9.9392 + 49.6344*Ca - 22.4809*Ca^2$	0.23	0.0142	0.0505	1.10	0.13
Max Force VitD	$27.2476 + 7.8558*Ca$	<.0001	0.0222	-	-	0.05
Max Force VitD + $1\alpha(OH)D_3$	$24.3110 + 12.7846*Ca$	<.0001	<.0001	-	-	0.17
Tibia ash	$37.4756 + 9.9805*Ca - 4.6406*Ca^2$	<.0001	0.0259	0.0683	1.08	0.09
Tibia ash VitD	$34.6647 + 16.4161*Ca - 8.3844*Ca^2$	<.0001	0.0114	0.0227	0.98	0.11
Tibia ash VitD + $1\alpha(OH)D_3$	$40.8679 + 2.0059*Ca$	<.0001	0.0037	-	-	0.09
CDADM VitD	$28.6982 + 89.9333*Ca - 44.8798*Ca^2$	0.0158	0.0032	0.0092	1.00	0.53
CDADM VitD + $1\alpha(OH)D_3$	$38.0291 + 69.1204*Ca - 35.0169*Ca^2$	0.0019	0.0143	0.0275	0.99	0.38
Ca Dig. VitD	$119.0279 - 160.0598*Ca + 80.3435*Ca^2$	<.0001	0.0052	0.0132	1.00	0.48
P Dig. VitD	$85.3427 - 17.0557*Ca$	<.0001	0.0003	-	-	0.46
P Dig. VitD + $1\alpha(OH)D_3$	$83.3669 - 12.0284*Ca$	<.0001	0.0046	-	-	0.32
CDADM VitD	$28.6982 + 89.9333*Ca - 44.8798*Ca^2$	0.0158	0.0032	0.0092	1.00	0.53

[1]Regression analyses with P > 0.05 indicated non significant effect of Ca on specific interaction effects.
* Regression analyses correspond to a mixed model and very low R^2 may not indicate lower adjustment.

Table 3.12. Effect of dietary calcium level and 1α(OH)D_3 on leg relative weight and relative asymmetry of Ross 708 male broiler at 35d of age[1].

Ca	VitD	Tibia			Femur		
		Relative weight	RA weight	RA length	Relative weight	RA weight	RA length
		----%----			----%----		
0.54		1.17	0.18	0.08	0.91	0.04	0.08
0.76		1.16	0.18	0.09	0.90	0.04	0.08
0.98		1.18	0.18	0.09	0.92	0.04	0.09
1.20		1.19	0.18	0.08	0.92	0.04	0.08
SEM		0.02	0.02	0.01	0.02	0.002	0.01
	VitD	1.16	0.19[a]	0.09	0.91	0.04	0.08
	VitD+1α(OH)D_3	1.19	0.16[b]	0.09	0.92	0.04	0.08
	SEM	0.01	0.01	0.006	0.01	0.001	0.003
0.54	VitD	1.16	0.18	0.08	0.91	0.04	0.08
	VitD+1α(OH)D_3	1.17	0.17	0.09	0.91	0.04	0.09
0.76	VitD	1.15	0.19	0.09	0.90	0.04	0.08
	VitD+1α(OH)D_3	1.16	0.17	0.10	0.90	0.04	0.08
0.98	VitD	1.17	0.21	0.09	0.92	0.05	0.10
	VitD+1α(OH)D_3	1.19	0.15	0.08	0.92	0.04	0.09
1.20	VitD	1.16	0.19	0.09	0.89	0.04	0.08
	VitD+1α(OH)D_3	1.22	0.17	0.08	0.95	0.04	0.08
	SEM	0.03	0.02	0.01	0.02	0.003	0.01
Source of variation		---------------------------------*P*-values---------------------------------					
	Ca	0.485	0.997	0.561	0.769	0.880	0.482
	VitD	0.238	0.027	0.933	0.399	0.175	0.719
	Ca*VitD	0.637	0.522	0.542	0.581	0.409	0.623
		--------------------------------- Regression *P*-values---------------------					
Ca		0.254	0.951	0.790	0.414	0.948	0.750
Ca*Ca		0.513	0.795	0.171	0.816	0.405	0.397
VitD source		0.203	0.050	0.829	0.394	0.176	0.771
VitD source *Ca		0.358	0.433	0.293	0.282	0.160	0.498

[1] Values are means ± SEM of 6 pens per treatment with four male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's and student's *t*-test.

Table 3.13. Effect of dietary Ca levels and $1\alpha(OH)D_3$ supplementation on ileal digestibility of Ross 708 male broilers at 35 d of age[1].

Ca	VitD	Dry matter	Ca	P
		------------------	%	-------------------
0.54		64.53^{b}	51.99^{a}	75.89^{a}
0.76		72.73^{a}	48.47^{a}	76.15^{a}
0.98		71.08^{a}	39.38^{a}	66.77^{b}
1.20		72.09^{a}	47.28^{b}	68.79^{b}
SEM		0.74	1.77	1.06
	VitD	70.70	45.50	70.67^{b}
	VitD + $1\alpha(OH)D_3$	69.52	48.06	73.12^{a}
	SEM	0.44	1.39	0.72
0.54	VitD	65.09	54.42^{a}	75.18
	VitD + $1\alpha(OH)D_3$	63.97	49.56^{ab}	76.60
0.76	VitD	73.65	48.61^{ab}	75.42
	VitD + $1\alpha(OH)D_3$	71.82	48.33^{ab}	76.88
0.98	VitD	71.21	34.51^{c}	65.41
	VitD + $1\alpha(OH)D_3$	70.96	44.25^{b}	68.13
1.20	VitD	72.86	44.47^{abc}	66.69
	VitD + $1\alpha(OH)D_3$	71.32	50.09^{ab}	70.90
	SEM	1.10	2.34	1.54
CV%		3.93	10.99	5.00
Source of variation		-------------	*P*-value	---------------
Ca		<.0001	<.0001	<.0001
VitD		0.1625	0.1056	0.0350
Ca*VitD		0.9066	0.0095	0.8090
		----------	Regression *P*-values	--------
Ca linear		<.0001	0.0124	<.0001
Ca*Ca quadratic		0.0017	0.0041	0.9271
VitD		0.8020	0.1440	0.2959
Ca*VitD		0.5359	0.0107	0.0528

[a-c] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's and student's *t*-test.

[1] Values are means ± SEM of 6 pens per treatment with four male broiler chickens per pen selected for processing.

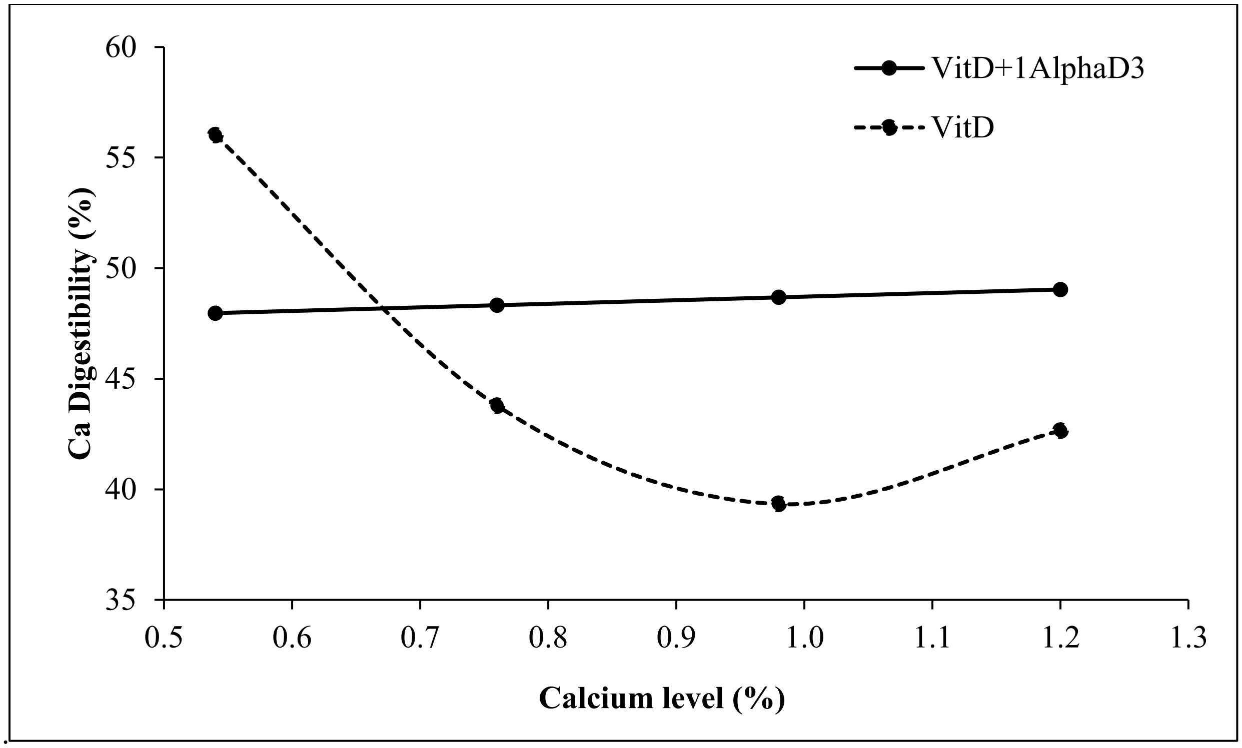

Figure 3.1 Effect of calcium level with and without 1α(OH)D_3 on Calcium digestibility from broiler males at 35 d of age

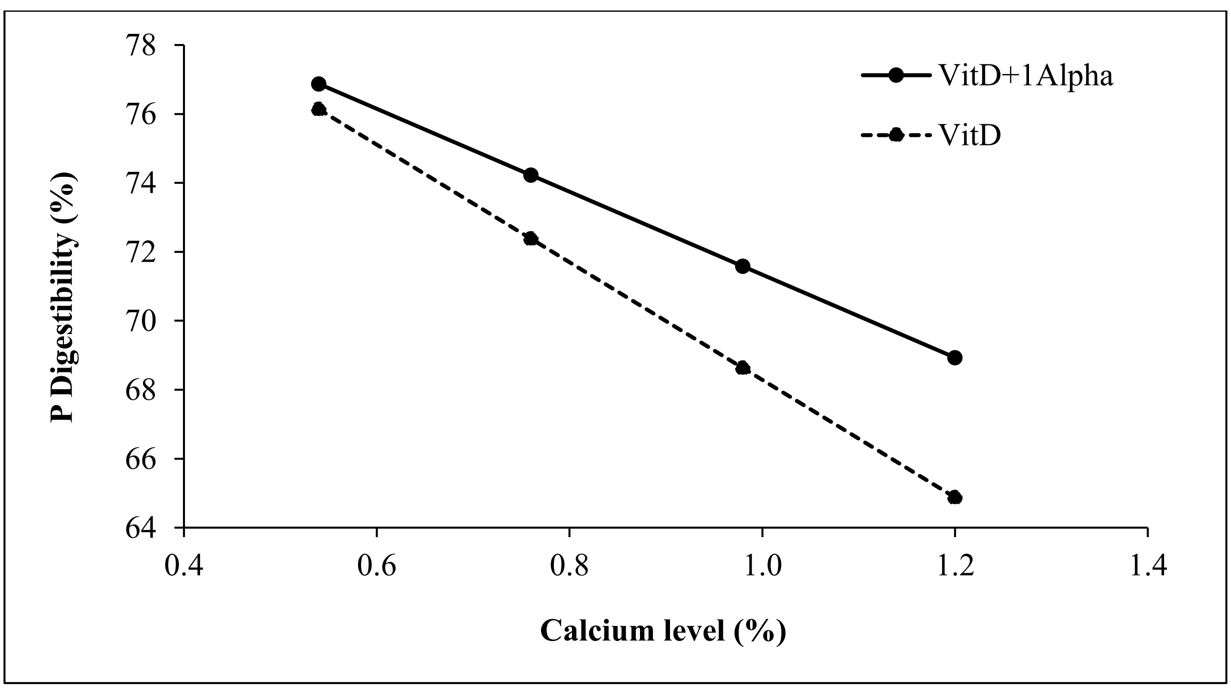

Figure 3.2 Effect of calcium level with and without 1α(OH)D_3 on phosphorous digestibility from broiler males at 35 d of age.

CHAPTER IV

Effect of VitD metabolite sources on broiler performance, carcass yield, meat quality, leg health and bone development

ABSTRACT

The effect of three VitD analogs on live performance, meat yield and quality in Ross 708 male broilers raised up to 42d were evaluated. Four experimental dietary treatments resulted from common basal diets (control diet) that contained 4,000 IU D_3/kg and supplemented on-top with three VitD analogs, $1\alpha(OH)D_3$, $1,25(OH)_2D_3$, and $25(OH)D_3$, at doses recommended by providers. 1,056 one-d-old chicks were randomly placed in 48 floor pens with 22 chicks/pen. At 7, 21, and 41d, growth performance parameters were recorded and FCR calculated. Flock uniformity was assessed at 41d as the CV% of individual BW. At 42d, three broilers/pen were selected for processing. Carcass and cut-up yields, meat quality and incidence of myopathies were evaluated. Data were analyzed using one-way ANOVA in a randomized complete block design in a mixed model. Mean separation was performed using Tukey's test. At 20 d broilers fed $1,25(OH)_2D_3$ gain less weight (P = 0.058) than broilers fed $1\alpha(OH)D_3$ supplemented diets (977 *vs*. 998). At 41d, broilers fed the control diet were heavier ($P < 0.05$) compared to chickens fed diets supplemented with $1,25(OH)_2D_3$ and $25(OH)D_3$, but similar to $1\alpha(OH)D_3$. FCR was worsened ($P < 0.01$) by $1,25(OH)_2D_3$ inclusion. Overall, no effects of treatments ($P > 0.05$) were observed in carcass, and cut up yields, and meat quality parameters except for cook loss. The *P. major* samples from broilers fed diets with $25(OH)D_3$ lose ($P < 0.05$) more weight at cooking compared to samples from chickens fed the control diet and $1\alpha(OH)D_3$-supplemented feed. Interestingly, $1\alpha(OH)D_3$ inclusion reduced overall mean score for wooden breast myopathy (P = 0.055) compared to samples from broilers fed diets with $25(OH)D_3$. In conclusion, $1,25(OH)_2D_3$ supplementation resulted in the

worst live performance whereas $1\alpha(OH)D_3$ and $25(OH)D_3$ inclusion showed intermediate responses up to 41d. In addition, these VitD analogs did not affect carcass and cut up yields, and most of the meat quality parameters for cook loss and wooden breast.

KEY WORDS:

$1\alpha(OH)D_3$, $25(OH)D_3$, $1,25(OH)_2D_3$, live performance, meat quality

INTRODUCTION

There are several metabolites of VitD available in the market that may enhance broiler growth and improve leg health by supporting metabolic functions of VitD (Roberson and Edwards Jr., 1994; Biehl et al., 1995; Mitchell and Edwards Jr., 1996; Edwards Jr., 2002; Rennie and Whitehead, 2007). These sources are 25-hydroxycholecalciferol ($25(OH)D_3$), 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and 1-alpha-hydroxycholecalciferol ($1\alpha(OH)D_3$). (Han et al., 2012; Świątkiewicz et al. 2016; Vazquez et al., 2018). Currently, it can be found in the market these three commercial products named as HyD® (DSM Nutritional Products Inc., Parsippany, NJ), Panbonis® (Herbonis AG, Augst BL, Switzerland) and AlphaD3® (Premex, Antioquia, Colombia), respectively.

These molecules are part of the metabolic activation pathway of the VitD. Consequently, all these molecules need to get to a final $1,25(OH)_2D_3$ to become active and held the activities on the intestinal tract, kidney and bones to maintain Ca and P homeostasis in the body (McDowell, 2000; Pike et al., 2018). The difference between these molecules is primarily in regard of the number of hydroxylations they must complete to be converted into active cholecalciferol ($1,25(OH)_2D_3$).

As is expected, $1,25(OH)_2D_3$ will not require any additional hydroxylation, being ready to carry out the VitD regulation actions (Kumar, 1984). On the other side, $25(OH)D_3$ and $1\alpha(OH)D_3$ require one hydroxylation, in the kidney and liver, respectively. Currently, in the industry we can found a synthetic $25(OH)D_3$ and $1\alpha(OH)D_3$. While the $1,25(OH)_2D_3$, that does not need any hydroxylation, comes from the natural source *Solanum glaucophyllum* (EFSA, 2015).

Since the poultry industry has these options and there is very little data available to choose among them, it is important to compare the effects of these three options under conditions that resemble current commercial poultry conditions, which include the addition of phytases and VitD supplementation. (Rostagno et al., 2017; NRC, 2014). Therefore, the general objective of this project was to compare the effects on broiler live performance, carcass yield, meat quality, bone development and leg health of the three different metabolites of VitD ($25(OH)D_3$, $1,25(OH)_2D_3$, and $1\alpha(OH)D_3$) added on top of broiler diets containing commercial levels of $VitD_3$ up to 42 days of age.

MATERIALS AND METHODS

Treatments and chicken husbandry

All the procedures involving chickens used in the present experiment were approved by the North Carolina State University Institutional Animal Care and Use Committee. Three diets were used: starter (1-7d), grower (8-20) and finisher (21-41d). Basal corn-soybean meal diets, with corn gluten meal and soybean oil were used for each one of the three feeding phases. Diets were formulated to meet or exceed nutrient recommendations (Table 4.1; 4.2) for Ross 708 broiler chickens (Aviagen, 2014). Each diet included a vitamin premix that included 4,000IU/kg of $VitaminD_3$ and a phytate Natuphos® at 500FTU/kg that was expected to release 0.12% Ca and 0.12% P. The four treatments included one control and three treatments with supplementation of one of the three VitD analogs. The three vitamin sources used were: $25(OH)D_3$ at 0.5kg/ton (69 µg/kg - 500.000 IU per gram (1,25%)), $1,25(OH)_2D_3$ at 0.2 kg/Ton (1 µg/kg - 1.000 IU per gram (0,001%)) and $1\alpha(OH)D_3$ at 12.5 g AlphaD3®/ton (5 µg $1\alpha(OH)D_3$/kg feed - 15.380 IU per gram (0.04%). The doses utilized for each metabolite were the ones recommended by the commercial producer of each product. Water was provided *ad libitum* during the whole experiment. Starter and grower phases were offered at 0.19, 1.25 kg/chick, respectively. The finisher was offered *ad libitum* until the end of the study.

Broiler live performance

At placement, 20 and 41 d of age, group BW and feed intake were obtained, and BW gain and FCR adjusted for mortality were calculated at the end of each dietary phase: 20 and 41d of age.

Leg health evaluation

At 38d of age, all chickens were individually evaluated for incidence of leg problems: valgus, varus, hock burns, and twisted legs. Also, footpad dermatitis were evaluated and scored using the grades, from 0 to 9 points, suggested by Allain et al., (2009).

Processing, carcass and cut up yield, and meat quality

At 42 days, broilers were subjected to a 12-h of feed withdrawal before processing. A total of 36 broilers per treatment, 3 per pen, were slaughtered at the NCSU pilot processing plant. Chickens were weighed, electrically stunned for 11s, killed by exsanguination, and allowed to bleed for 90s. Broilers were then scalded at 55°C for 90s, picked for 30s, and manually eviscerated. Carcasses were dressed by removing liver, gizzard, heart, oil gland, crop, proventriculus, lungs, and viscera. After this, carcasses were air-chilled for 6 h, to proceed a manually deboning on stationary cones. Parts of the leg quarters, breast fillets (*Pectoralis major*), breast tenders (*Pectoralis minor*), wings, and rack with skin were weighed. Carcass yield was calculated as the chilled carcass weight compared to live weight. Parts yield were expressed as a percentage of the chilled absolute carcass weight.

For meat quality evaluation drip loss, cook loss, shear force, color, pH, and pectoral myopathies such as wooden breast (WB), white striping (WS), and spaghetti muscle (SM) were evaluated. Only one experienced person performed the myopathy scoring to avoid subjective variations due to the evaluator.

Drip loss was evaluated to obtain the water holding capacity. Breast fillets were weighted and hanged from a hook in a freezer at 4-6°C for 24h. After this period each fillet were gently cleaned with an absorbent paper and weight and the difference of weight were calculated. For cook loss evaluation, the breast fillets were weighed, placed on aluminum pans, and cooked in a forced air oven (SilverStar Southbend, Model SLES/10sc, gas type, NC, USA). After reaching the internal temperature of 75°C, (approximated time: 35mins) as measured by a Therma Plus thermocouple with a 10-cm needle temperature probe (ThermoWorks Model 221-071, UT, USA), the oven was turned off and the fillets cooled to room temperature to be weighed one more time and calculate the cooked weight relative to the raw weight.

For pH determination, a portable spear pH meter (Oakton-Eutech Instruments® waterproof pH Tester 30) was used on breast fillets at 6 and 24 h after processing. Breast meat color values were determined (L* lightness, a* redness, and b* yellowness) using a colorimeter Minolta Chroma Meter CR-400. (Konica Minolta Sensing, Inc., Japan).

For pectoral myopathies sensorial evaluation and scoring system was conducted by experienced people to identify the severity of WS (1-3), WB (1-4) or incidence of SM) myopathies. (Kuttappan et al., 2016; Baldi et al, 2018)

Bone development and mineralization

The RA of femur length was lower ($P < 0.05$) in $1\alpha(OH)D_3$ broilers than the ones fed the with the control diet (Table 4.8). No effect for $1,25(OH)_2D_3$ and $25(OH)D_3$ were manifested when compared to the other supplementations. Moreover, no effects of treatments ($P > 0.05$) were detected on tibia ash at 42d.

Leg asymmetry, bone strength and tibia ash

Three chickens per experimental unit and 36 per each treatment were selected from the processed chickens. Legs were collected, and meat was removed. The weight and length of each bone was measured. These measurements were used to calculate the relative asymmetry (RA) of the bones. The asymmetry of the bones was calculated using the formula described by Møller et al. (1995, 1999), RA = (|L-R|/[(L+R)/2]x100.

After obtaining these measures, left tibias were used for determining the bone breaking-strength. Each one of the left tibias were placed individually in a texture analyzer (TA-HD plus. Stable Micro Systems, UK) to record the bone strength. The values were expressed in kilograms-force (kgf). Furthermore, bone thickness were recorded. Four measurements were taken from each of the two parts of the braked bone (frontal, caudal, medial and lateral from proximal and distal broken bones). These measurements, with the values obtained with the texture analyzer were used to calculate each bone bending moment and its maximum stress as described by Crenshaw et al., (1981).

Lipid content was extracted from the broken bones using diethyl ether as a lipid solvent. After 7 days, the bones in diethyl ether solution, were dried overnight in room temperature, and 24 hours in a 100°C oven. After that, the dried bones were ashed in a muffle furnace at 600°C for 7 hours. The ash sample was re-weighed to calculate the percentage of total mineral content.

Statistical analyses:

Data was analyzed in a randomized complete block design with four treatments and 12 replicate pens per treatment in a mixed model. One-way ANOVA was used to determine effects of treatments. Blocks were each one of the three rows where 16 pens, four replicates per treatment were located within the house and considered as random effect. All percentage data was transformed using the best Box-Cox transformation prior to analyses. JMP® 13 (SAS Institute, 2017) software was the statistical tool.

RESULTS

Broiler live performance

Results obtained at 21 d indicated that broilers fed 1,25$(OH)_2D_3$ gained less weight (P = 0.058) as compared to broilers fed with 1α$(OH)D_3$ supplement diets (977 *vs*. 998 g) (Table 4.4). It was also detected that at 41d (Table 4.3), that broilers fed the control diet were heavier ($P < 0.05$) than chickens fed diets supplemented with 1,25$(OH)_2D_3$ and 25$(OH)D_3$ (3,416 and 3,402 g, respectively). No difference ($P > 0.05$) was detected between the 1α$(OH)D_3$ supplemented chickens and the control without supplementation. In addition, FCR was worsened ($P < 0.01$) by 1,25$(OH)_2D_3$ inclusion at this age (Table 4.5). No difference on FCR were observed between 1α$(OH)D_3$, 25$(OH)D_3$ and no supplemented diets. No effect of treatments ($P > 0.05$) was obtained on feed intake, total mortality, and flock uniformity at 41d (Table 4.4).

Processing, cut up yield and meat quality

Overall, no effect of treatments ($P > 0.05$) was obtained in carcass and cut up yields, and meat quality parameters except for cook loss (Table 4.9). The *P. major* samples from broilers fed diets with 25$(OH)D_3$ lose ($P < 0.05$) more weight at cooking compared to samples from chickens fed the control diet and 1α$(OH)D_3$-supplemented feed. 1,25 $(OH)D_3$ supplementation did not indicate a difference of cook loss % compared with the other three treatments (Table 4.11). Interestingly, in the sensorial score analysis, no effect on WS was detected ($P > 0.05$), while the inclusion of 1α$(OH)D_3$ reduced overall mean score for WB myopathy (P = 0.055) compared to samples from broilers fed diets with 25$(OH)D_3$. (Table 4.12).

DISCUSSION

Metabolites for $VitD_3$ are available in the market and it was found that these molecules can increment the biopotency and guarantee the requirement levels of this VitD (Świątkiewicz et al., 2016). Previous studies have demonstrated the individual effectiveness of these metabolites used in addition or in replacement of a $VitD_3$ (Boris et al., 1977; Soares et al., 1995; Edwards Jr. et al., 2002; Garcia et al., 2013; Fritts et al., 2004; Rath et al., 2007). Different responses have been obtained, and the effectiveness of each metabolite varies depending of the metabolic functions that were studied (Edward Jr., 1993; Roberson and Edwards Jr., 1994; Applegate et al., 2003; Chou et al, 2009; Wideman et al., 2015).

The supplementation with $1\alpha(OH)D_3$ had similar effects to the control group on BW and it was 45 and 31g heavier than $25(OH)D_3$ and $1,25(OH)_2D_3$, respectively. BWG from 21 to 41 days, presented similar pattern. While $1\alpha(OH)D_3$ had no significant difference with the control group, it obtained enumerically 28g more of BWG compare to both metabolites. In FCR the treatment supplemented with $1,25(OH)_2D_3$ were lower than all the other treatments. Probably because of the variations that could exist in this molecule due to its natural origin and extraction from the *Solanum glaucophyllum* (SG) process (Bachmann et al., 2013; EFSA, 2015; Herbonis AG, 2017).

However, no differences were obtained between the control group and the three metabolites in tibia ash content, leg health or bone strength. Which indicate that under these conditions no additional biopotency in leg structure development and mineralization was obtained with these precursors. In comparison to the present study, Han (2012) detected better tibia ash content with the supplementation of $1\alpha(OH)D_3$ at 5 and 10 μg/kg, when working with a Ca and P deficient diet. Similar results were obtained by the same author (Han et al., 2016), when work with $25(OH)D_3$ in Ca-P deficient diets. Probably, the utilization of a deficient Ca-P diet, made more evident bone mineralization the effect of these VitD metabolites, in comparison to the no supplemented group. Also, in contrast to the results in this study, when diets contained adequate levels of Ca and P, Castro et al. (2018) did not detect significant differences on bone mineralization.

Related to bone development, interestingly, similar than in the starter and grower experiments performed by this group and discussed in the previous chapters of this thesis, an effect of $1\alpha(OH)D_3$ in bone RA was detected. One more time, $1\alpha(OH)D_3$ decreased femur length RA as compared by the control group (0.7 and 0,11 respectively). RA have been related with an adequate tonic mobility, presence of tibiochondroplasia and affecting negatively the locomotion. (Moller et al., 1995; Moller, 1999, Moller et al, 1999). This effect on improving RA indicating a better and homogenous bone development have been observed in the three experiments that conform this thesis.

In processing variables, the only difference obtained was the higher cook loss% in the treatment supplemented with $25(OH)D_3$. This increment in cooking loss indicated the reduction of meat water holding capacity when meat is subjected to heat (Bozkurt et al., 2017). This is generally related with lower levels of pH at 6 and 24 hours post mortem, when the levels of lactic acid increase (Roque-Specht, 2009). Under these conditions the degradation of myofibrillar structures occurs due to the action of proteases called calpains. The decrement of meat pH has been correlated with the decline of the calpain activity in vitro postmortem conditions (Kendall et al., 1993). However, in the present study no effect was detected in pH values in between treatments. Bozkurt et al. (2017), working with supplementation of $25(OH)D_3$ at 8.7–15.0 μg/kg in Ross 308, only detected at 38d a slight result in the increment of pH (P = 0.05), while a decrease in cooking loss of breast meat (P < 0.01) was manifested. Garcia et al. (2013), conducted a study comparing these three metabolites effects on meat quality when replacing the total content of $VitD_3$. Like the current results, no difference was detected in breast meat ph, as well as in a* and b*color value, however a difference was obtained in L* value. The treatment using only $VitD_3$ and without any metabolite supplementation obtained the lowest L* value and significantly different with the supplementation of $1,25(OH)D_3$. Similarly, Wilborn et al. (2004), detected no difference in color values (a*, b*), but on lightness (L*). Also obtained the lower L*-values with the utilization of $VitD_3$ at 40,000 and 80,000 IU $VitD_3$/kg compare to the non-inclusion. Wiegand et al., 2002, repeated these results, with pork meat supplemented or not with 500,000IU/kg VitD. No difference in color were obtained at 24hr post mortem, but lower L* values were detected at 7d post mortem in the meat supplemented with VitD.

Finally, $1\alpha(OH)D_3$ obtained the lower presentation score of WB myopathy. A significant difference was detected in this treatment with the chickens supplemented with $25(OH)D_3$, group that presented the higher score of WB myopathy. The presentation of myopathies affects different parameters of meat quality as well as the acceptance of the customers in the market. These will produce economic losses caused by complaints and rejections from customers. WB is recognized by hardness meat with pale areas affecting negatively parameters of meat quality and tenderness of the meat (Shivo et al, 2014, Tijare et al., 2016).

It has been reported the presence of the VitD receptors (VDR) in avian muscle cells, but these findings are uncertain due to the no specificity of many of the VDR antibodies (Girgis et al., 2013). In humans, there exist reports that the deficiency of VitD may result in muscle injuries and the supplementation of VitD improved these conditions (Pfeifer et al, 2000; Bunout et al., 2006; Moreira-Pfrimer et al., 2009), but further studies for support and clarify how beneficial effects of VitD supplementation act in the improvement of muscle strength must be conducted (Rejnmark, 2011).

CONCLUSION

In conclusion, supplementation with $1,25(OH)_2D_3$ resulted in the worst live performance whereas $1\alpha(OH)D_3$ and $25(OH)D_3$ inclusion showed intermediate live performance responses up to 41d. Supplementation with $1\alpha(OH)D_3$ reduced RA in length of the femur indicating a better bone development. In addition, the supplementation with these VitD analogs did not affect carcass and cut up yields, and meat quality parameters except for cook loss and WB presentation.

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Table 4.1. Ingredient composition of starter, grower and finisher basal diets for Ross 708 male broilers.

Ingredient	Starter (1-15d)	Grower (16-34d)	Finisher (35-41d)
	---------------------- (%)----------------------		
Corn	54.73	58.64	64.04
Soybean meal, 46%	33.90	31.06	24.57
Corn gluten meal, 60%	4.45	3.00	3.58
Soybean oil	2.60	3.54	4.41
Dicalcium phosphate[1]	1.35	0.93	0.78
Limestone Cerne Pure Cal 12-40[2]	1.010	0.92	0.78
Sodium bicarbonate	0.34	0.29	0.27
L-Lysine-HCl, 78.8%	0.32	0.31	0.34
DL-Methionine, 99%	0.300	0.30	0.28
Salt (NaCl)	0.27	0.24	0.21
Mineral premix[3]	0.20	0.20	0.20
Choline chloride, 60%	0.18	0.18	0.18
L-threonine	0.11	0.12	0.12
Vitamin premix[4]	0.10	0.10	0.10
Anticoccidial[5]	0.05	0.05	0.05
Selenium premix	0.05	0.05	0.05
Sand[6]	0.05	0.05	0.05
Natuphos E® [7]	0.01	0.01	0.01
Total	100.00	100.00	100.00

[1] Dicalcium phosphate contains 19.79% Calcium, 17.9091% phosphorus and 17.73% available phosphorus (99%).
[2] Limestone (Cerne Pure Cal 12-4) contains 39.01% Calcium.
[3] Trace minerals provided per kg of premix: manganese (Mn SO4), 60 g; zinc (ZnSO4), 60 g; iron (FeSO4), 40 g; copper (CuSO4), 5 g; iodine (Ca(IO3)2),1.25 g.
[4] Vitamins provided per kg of premix: vitamin A, 13,227,513 IU; vitamin D_3, 3,968,253 IU; vitamin E, 66,137 IU; vitamin B12, 39.6 mg; riboflavin, 13,227 mg; niacin, 110,229 mg; d-pantothenic acid, 22,045 mg; menadione, 3,968 mg; folic acid, 2,204 mg; vitamin B6, 7,936 mg; thiamine, 3,968 mg; biotin, 253.5 mg.
[5] Coban® 90 (Monensin), Elanco Animal Health, Greenfield, IN, at 500 g/ton in the starter and grower diets.
[6] According to additive inclusion in the diet (Diets Control: 0.05% Sand; 25(OH)D_3: 0.05% 25(OH)D_3; 1,25$(OH)_2D_3$: 0.01% 1,25$(OH)_2D_3$; 1α(OH)D_3: 0.00125% 1α(OH)D_3).
[7] Natuphos E® (500 FTU/kg, 50 g/ton FTU).

Table 4.2. Nutritional content of basal diets in each feeding phase

Nutrient	Starter (1-15d)	Grower (16-34d)	Finisher (35-41d)
Crude protein, %	23.78	21.82	19.50
Calcium, %	0.97	0.85	0.75
Total phosphorous, %	0.58	0.49	0.44
Nonphytate phosphorous, %	0.50	0.42	0.380
Phytate phosphorous, %	0.25	0.24	0.23
Total methionine, %	0.66	0.64	0.59
Total cysteine, %	0.39	0.36	0.32
Total lysine, %	1.43	1.33	1.19
Total tryptophan, %	0.28	0.26	0.22
Total threonine, %	0.99	0.92	0.82
Total isoleucine, %	1.01	0.92	0.80
Total valine, %	1.12	1.03	0.92
Total leucine, %	2.18	1.97	1.84
Total arginine, %	1.50	1.38	1.19
Total sulphur amino acids, %	1.05	0.99	0.91
Digestible methionine, %	0.64	0.61	0.57
Digestible cysteine, %	0.32	0.30	0.27
Digestible total sulphur amino acids, %	0.96	0.91	0.84
Digestible threonine, %	0.86	0.80	0.72
Digestible tryptophan, %	0.24	0.23	0.19
Digestible isoleucine, %	0.91	0.83	0.73
Digestible lysine, %	1.30	1.21	1.08
Digestible valine, %	0.99	0.91	0.81
Digestible arginine, %	1.39	1.29	1.10
Sodium, %	0.22	0.20	0.18
Potassium, %	0.92	0.87	0.75
Chloride, %	0.27	0.25	0.24
Metabolizable energy, kcal/kg	2,950	3,050	3,175
Dietary electrolyte balance, mEq/100 g	275	256	225

Table 4.3. Effect of VitD sources on body weight of male Ross 708 broilers raised to 41d

VitD source	Body weight (g)				
	0 d	7 d	20 d	41 d	41d adj*
	------------------------------(g)------------------------------				
Control	44.8	192	$1{,}032^{ab}$	$3{,}503^{a}$	$3{,}520^{a}$
$1\alpha(OH)D_3$	44.7	190	$1{,}043^{a}$	$3{,}447^{ab}$	$3{,}492^{ab}$
$25(OH)D_3$	44.6	191	$1{,}034^{ab}$	$3{,}402^{b}$	$3{,}448^{b}$
$1{,}25(OH)_2D_3$	44.7	193	$1{,}021^{b}$	$3{,}416^{b}$	$3{,}450^{b}$
SEM	0.08	1.57	6.29	29.83	20.14
CV%	0.43	2.40	1.84	2.28	1.82
Source of variation	-------------------------------- *P*-values-------------------------------				
VitD Source	0.2752	0.3677	0.0609	0.0152	0.0218

Values are means of 12 pens per treatment with 22 male broiler chickens per pen.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's test.

Table 4.4. Effect of VitD sources on body weight gain and flock uniformity as coefficient of variation of male Ross 708 broilers, and total mortality

VitD source	BWG		Flock uniformity at 41d	Total Mortality
	0-21d	21-41d		
	-------------(g) -------------		------CV %------	-----(%)-----
Control	987^{ab}	$2{,}471^{a}$	7.71	3.79
$1\alpha(OH)D_3$	998^{a}	$2{,}423^{ab}$	7.18	4.55
$25(OH)D_3$	989^{ab}	$2{,}395^{b}$	7.41	2.27
$1{,}25(OH)_2D_3$	977^{b}	$2{,}395^{b}$	7.37	3.41
SEM	6.2	18.2	0.34	1.51
CV%	1.91	2.29	18.38	
Source of variation	----------------------------------- *P*-values -------------------------------------			
VitD Source	0.0576	0.0048	0.8211	0.3480

Values are means of 12 pens per treatment with 22 male broiler chickens per pen.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's test.

Table 4.5. Effect of VitD sources on feed intake and feed conversion ratio (FCR) of Ross 708 male broilers

VitD	FI (g)			FCRadj*			
	0-7d	0-20d	0-41d	0-7d	0-20d	20-41d	0-41d
	-----------(g)--------			--------------------(g:g)---------------------			
Control	141^{b}	1,254	5,160	0.736^{b}	1.271^{a}	1.543^{b}	1.464^{b}
$1\alpha(OH)D_3$	145^{ab}	1,238	5,110	0.768^{a}	1.238^{b}	1.561^{b}	1.466^{b}
$25(OH)D_3$	147^{a}	1,245	5,097	0.769^{a}	1.255^{ab}	1.568^{b}	1.473^{b}
$1,25(OH)_2D_3$	147^{a}	1,236	5,168	0.760^{a}	1.264^{ab}	1.598^{a}	1.500^{a}
SEM	1.56	11.78	76.97	0.005	0.008	0.006	0.006
CV%	2.80	2.87	4.21	2.08	2.20	1.48	1.49
Source of variation	--------------------------------*P*-values ------------------------------------						
VitD Source	0.0042	0.6091	0.8112	<0.0001	0.0314	<0.0001	0.0008

Values are means of 12 pens per treatment with 22 male broiler chickens per pen.

[a-c] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's test.

*FCR was adjusted by mortality weights.

Table 4.6. Effect of VitD sources on incidence (0-1) of leg health issues, and foot pad dermatitis average score (0-9)[1] of Ross-708 male broilers at 42 d[2].

VitD Source	Straight	Valgus	Hock Burn	FPD
	------------------------(0 – 1) ------------------------			---- (0 – 9) ----
Control	0.12	0.88	0.41	2.05
$1\alpha(OH)D_3$	0.13	0.87	0.38	1.90
$25(OH)D_3$	0.17	0.80	0.34	1.76
$1,25(OH)_2D_3$	0.16	0.82	0.42	1.81
SEM	0.04	0.04	0.09	0.13
CV%	219.09	39.78	92.33	36.77
Source of variation	-----------------------------------*P*-values-----------------------------------			
VitD Source	0.8451	0.6752	0.9415	0.4594

[1] Allain et al. (2009)

[2]Values are means of 12 pens per treatment with 22 male broiler chickens per pen.

Table 4.7. Effect of VitD sources on tibia strength of Ross 708 male broilers at 42 d[1]

VitD source	Thickness	Inertia	Moment	Max Stress	Max Force
	---mm---	--m^4--	--Nm--	--MPa--	--Nm--
Control	1.53	3.37E-10	5.79	79.63	362.65
1α$(OH)D_3$	1.58	3.46E-10	5.79	78.94	364.611
$25(OH)D_3$	1.58	3.81E-10	6.28	78.16	389.52
$1,25(OH)_2D_3$	1.58	3.71E-10	6.08	78.16	382.459
SEM	0.04	1.61E-11	0.20	2.64	14.71
CV%	14.38	22.81	19.92	17.06	19.93
Source of variation	------------------------------------*P*-values--------------------------------------				
VitD source	0.8068	0.1891	0.4862	0.9770	0.4920

[1] Values are means ± SEM of 12 pens per treatment with three male broiler chickens per pen selected for processing.

Table 4.8. Effect of VitD sources on bone ash, relative weight and relative asymmetry in diets for Ross 708 male broilers at 42 days of age1

VitD sources	Tibia				Femur		
	Ash	Relative weight	RA. weight	RA. length	Relative weight	RA. weight	RA. Length
	---%--	----%---	--------g---------		----%----	--------g--------	
Control	43.25	1.37	0.14	0.08	0.90	0.14	0.11[a]
1α$(OH)D_3$	43.43	1.41	0.14	0.11	0.90	0.17	0.07[b]
$25(OH)D_3$	43.77	1.42	0.15	0.08	0.91	0.14	0.09[ab]
$1,25(OH)_2D_3$	43.25	1.43	0.15	0.08	0.94	0.17	0.08[ab]
SEM	0.36	0.02	0.01	0.01	0.02	0.01	0.01
Source of variation		-----------------------------*P*-values---------------------------------					
VitD source	0.6646	0.2292	0.9554	0.0572	0.3077	0.2032	0.0489

[1] Values are means ± SEM of 12 pens per treatment with three male broiler chickens per pen selected for processing.

Table 4.9. Effect of VitD source on carcass and cut-up part yields of Ross-708 male broilers at 42d[1]

VitD source	Hot carcass yield	Cold carcass yield	Cut – up parts relative to carcass weight				
			Wings	Leg quarters	Skin	Breast meat	Rack
	---------- (%) ----------						
Control	76.30	76.22	9.30	29.81	2.46	41.29	17.11
$1\alpha(OH)D_3$	76.64	76.60	9.25	29.84	2.49	41.02	17.27
$25(OH)D_3$	76.46	76.53	9.27	29.57	2.52	41.56	16.81
$1,25(OH)_2D_3$	76.51	76.51	9.26	29.66	2.53	41.36	17.20
SEM	0.18	0.25	0.08	0.25	0.19	0.24	0.48
CV%	1.32	1.46	4.38	3.94	12.58	3.88	5.20
Source of variation	---------- *P*-values ----------						
VitD source	0.5205	0.4243	0.9821	0.8148	0.9041	0.5717	0.2563

[1]Values are means ± SEM of 12 pens per treatment with three male broiler chickens per pen selected for processing.

Table 4.10. Effect of VitD sources on carcass and cut-up parts weights of Ross-708 male broilers at 42 d[1]

VitD source	Live weight	Hot Carcass	Carcass yield	Cut – up parts relative to carcass weight				
				Wings	Leg Quarters	Skin	Breast meat	Rack
	(g)							
Control	3,463^{a}	2,642	2,638	245	786	64.2	1089	451
$1\alpha(OH)D_3$	3,417^{ab}	2,616	2,616	242	777	65.1	1078	452
$25(OH)D_3$	3,431^{ab}	2,627	2,626	242	773	66.1	1090	439
$1,25(OH)_2D_3$	3,386^{b}	2,595	2,601	242	769	65.5	1079	446
SEM	20	14	16	3	10	5.2	10	11
CV%	2.96	3.18	3.24	4.49	5.03	13.76	5.58	5.82
Source of variation	*P*-values							
VitD source	0.0175	0.1532	0.3514	0.7677	0.4252	0.9184	0.7674	0.2705

[1].Values are means ± SEM of 12 pens per treatment with three male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different (P < 0.05) by Tukey's test.

Table 4.11. Effect of VitD sources on breast meat quality and color parameters of Ross-708 male broilers at 42d[1]

VitD Source	pH post – processing 6 h	pH post – processing 24 h	Cook loss	Drip loss	Color L* (Lightness)	Color a* (Redness)	Color b* (Yellowness)
	----------- pH ------		---------- % -------				
Control	5.95	6.01	20.31^{b}	0.94	55.02	3.43	8.57
$1\alpha(OH)D_3$	5.96	5.98	20.87^{b}	0.91	54.17	3.51	8.88
$25(OH)D_3$	6.01	6.04	22.94^{a}	1.00	55.57	3.57	9.72
$1,25(OH)_2D_3$	5.98	6.02	22.23^{ab}	0.97	54.69	3.49	8.99
SEM	0.03	0.03	1.43	0.07	0.61	0.22	0.48
CV%	1.87	1.75	13.82	23.91	6.52	33.96	23.54
Source of variation	---------- *P*-values ----------						
VitD Source	0.2340	0.3132	0.0367	0.5245	0.4045	0.9743	0.2147

[1]Values are means of 12 pens per treatment with 3 male broiler chickens per pen selected for processing.
[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's test.

Table 4.12. Effect of VitD sources on breast meat quality and on wooden breast score distribution of Ross-708 male broilers at 42d[1]

VitD Source	Sensorial Scores		WB Score distribution			
	White striping	Wooden Breast	1	2	3	4
	----(1-3)----	---- (1-4) ----	----------------- (0.0 – 1.0) -----------------			
Control	2.04	2.91[ab]	0.06	0.32	0.29	0.34
$1\alpha(OH)D_3$	2.06	2.64[b]	0.08	0.36	0.39	0.17
$25(OH)D_3$	2.03	3.22[a]	0.00	0.19	0.39	0.42
$1,25(OH)_2D_3$	2.22	2.91[ab]	0.06	0.26	0.39	0.28
SEM	0.09	0.13	0.03	0.07	0.08	0.08
CV%	33.34	29.82	450.62	161.36	127.94	146.31
Source of variation	--------------------------------*P* – values ------ --------------------------					
VitD Source	0.5265	0.0553	0.3905	0.4383	0.8025	0.1870

[1] Values are means of 12 pens per treatment with three male broiler chickens per pen selected for processing.

[a-b] Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Tukey's test.

SUMMARY

The objective of the present study was to determine the effect of $1\alpha(OH)D_3$ under different dietary Ca levels, at parameters that are known to be affected by VitD metabolism. Noteworthy results were obtained where interactions effects of Ca levels and $1\alpha(OH)D_3$ were detected. In starter diets, it was noticed an improvement in performance with the supplementation of $1\alpha(OH)D_3$. However, this effect is lost in diets with high Ca levels. During the grower phase no significant effect was observed.

$1\alpha(OH)D_3$ affected mineral retention in started and grower phases. $1\alpha(OH)D_3$ increased Ca and P retention in the starter phase with diets under 1.40%Ca, and Mn and Zn retention in diets over 0.95% Ca. In grower diets P digestibility was also improved when compared to diets without supplementation. Also, in grower diets $1\alpha(OH)D_3$ supplementation improved Ca digestibility in broilers fed diets with more than 0.70% Ca compared to diets without supplementation.

Interactions were detected of Ca levels and $1\alpha(OH)D_3$ supplementation tibia ash and breast meat ph. It was detected and interaction on tibia Ash in starter phase and improvement of ash with 1 alpha in grower phase were detected. Interesting the effect of $1\alpha(OH)D_3$ resulted in improved RA of bones and this effect was consistent in the 3 experiments conducted. These results remark the effect of $1\alpha(OH)D_3$ in the generation of homogenous bone development.

It can be concluded that the effect of $1\alpha(OH)D_3$ when combined with cholecalciferol was found beneficial on bone development and mineral digestibility at adequate dietary Ca levels. Furthermore, it is very important to manage and guarantee feed Ca level. Any imbalance in Ca%, as well as its relationship with the P, can affect $1\alpha(OH)D_3$ activity disturbing the adequate intestinal absorption of Ca, mineral performance, bone development and mineralization.

APPENDIX

Appendix A. Mineral analysis of experimental treatments (Experiment 2)[1,2]

		Ca		P total		Cu		Mn		Zn	
Ca	VitD	F[3]	R[4]	F	R	F	R	F	R	F	R
		------------------ (%)------------------				---------------------------- (g/ton) ------------------------					
0.80	VitD	0.68	0.67	0.565	0.70	20	18.9	155	175.0	160	144.7
	VitD + 1α(OH)D_3	0.68	0.70	0.565	0.71	20	21.6	155	208.8	160	161.8
0.95	VitD	0.83	0.85	0.565	0.73	20	24.0	155	210.3	160	153.6
	VitD + 1α(OH)D_3	0.83	0.80	0.565	0.77	20	19.2	155	197.8	160	158.0
1.10	VitD	0.98	1.03	0.565	0.74	20	17.4	155	197.8	160	148.6
	VitD + 1α(OH)D_3	0.98	0.94	0.565	0.71	20	16.2	155	180.3	160	143.4
1.25	VitD	1.13	1.06	0.565	0.73	20	23.5	155	187.1	160	149.2
	VitD + 1α(OH)D_3	1.13	0.99	0.565	0.73	20	18.9	155	194.7	160	150.6
1.40	VitD	1.28	1.15	0.565	0.71	20	18.9	155	201.0	160	156.1
	VitD + 1α(OH)D_3	1.28	1.22	0.565	0.76	20	18.5	155	195.3	160	149.4

[1] Analyzed values are means of two replicates and corrected for dry matter.
[2] Formulated values did not consider 0.12% of Ca and 0.12% avP from phytase (Natuphos E®).
[3] F= Formulated.
[4] R=Recovered in analysis.