EFFECT OF VITAMIN D_3 AND 25-HYDROXYCHOLECALCIFEROL ON BROILER BREEDER HEN NUTRITION AND THEIR INFLUENCE ON THE PERFORMANCE AND LEG ABNORMALITIES OF THE PROGENY

by

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(Under the Direction of GENE M. PESTI)

ABSTRACT

Experiments were conducted to determine the quantitative requirement of vitamin D_3 for the maximum performance of broiler breeder hens and to relate the effects of the various levels of vitamin D_3 in the maternal diet with the performance, tibia ash, tibial dyschondroplasia (TD) and Ca deficiency rickets incidence of the progeny fed a diet deficient in vitamin D_3 or diets supplemented with various levels of vitamin D_3, Ca, or 25-hydroxycholecalciferol. A second set of experiments were conducted to determine the relative biological value of 25- hydroxycholecalciferol in comparison to D_3 for broiler breeder hens and to relate the two D sources in the maternal diet with the performance, tibia ash and leg abnormalities of the progeny fed diets deficient in vitamin D_3 or supplemented with 1,100 IU of vitamin D_3 per kg of diet.

Analyses of the data indicate a requirement of approximately 1,400 and 2,800 IU of vitamin D_3 per kg of feed for the maximum performance of broiler breeder hens from 25 to 36 and 37 to 66 weeks of age, respectively. Increasing the level of vitamin D_3 in the maternal diet was effective in improving performance, tibia ash and in reducing TD and

Ca rickets incidence of the progeny fed diets deficient in vitamin D_3 or diets with various levels of vitamin D_3, Ca or 25- hydroxycholecalciferol. The results from these studies, although not always statistically significant for tibia ash, TD and Ca rickets incidence throughout the different experiments with the progeny, suggest that the requirement of broiler breeder hens to produce the heaviest and healthiest chicks is considerably higher than the requirement for maximum egg production of the hens and higher than the NRC (1994) or Scott et al. (1982) recommendations, especially when birds are raised in environments having almost no measurable UV light.

The relative biological value of 25- hydroxycholecalciferol in relation to vitamin D_3 depends on the level tested. When comparing vitamin D sources, 25-hydroxycholecalciferol has greater relative biological value than vitamin D_3 only at very low levels of supplementation.

INDEX WORDS: broiler breeders, requirement, progeny, vitamin D_3, 25-hydroxycholecalciferol, calcium, rickets, tibial dyschondroplasia

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DEDICATION

To the Lord, who has been a source of blessings.

To my parents, Marcelino and Maria, for their love.

To my sister, Anabel and my brothers, Abdiel, Ariel, Azael, Angel and Alibel for their continued encouragement.

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CHAPTER 1

INTRODUCTION

No work has been reported until the present on the vitamin D requirements of meat type hens for maximum egg production, hatchability, embryo mortality, egg quality and performance, tibia ash and leg abnormalities of the progeny. The NRC (1994) refers the reader to the requirement for white egg-type breeders which is 300 IU of cholecalciferol (D_3) per kg of feed in hens with a feed intake of 100g per day. Suggested levels for broiler breeders in books and commercial guidelines varies from 1,000 (Scott et al., 1982) to 3,500 IU of vitamin D_3/kg (Ross Feeding Program Summary, 2000)[1].

Effect of vitamin D_3 in the maternal diet on the performance and leg abnormalities of the progeny has been shown in laying type hens by Murphy et al. (1936), Bethke et al. (1936a,b), Griminger, (1966) and Edwards (1995) and in turkey poults by Robertson et al (1941), Stadelman et al, (1950) and Stevens et al (1984).

Similar to D_3, 25-hydroxycholecalciferol (25-OHD_3) is added to the diet of commercial broiler breeder hens even though no studies have been reported comparing the effect of 25-OHD_3 and D_3 on the same criteria mentioned above. Several papers have been published comparing the two D sources in commercial laying hens (Charles and Ernest, 1974; Charles et al., 1978; Marret et al. 1975; Roland and Harms, 1976; Abdulrahim et al. 1979; Keshavarz, 1996, 2003). However some researchers have observed higher potency of 25-OHD_3 than D_3 while others have observed no significant differences.

1 Ross Breeders, Inc. Huntsville, Alabama 35805

The first objective of this study was to determine the vitamin D_3 requirement of broiler breeder hens for maximum performance and to relate the effects of the various levels of vitamin D_3 in the maternal diet to the performance and leg abnormalities of the progeny fed various levels of vitamin D_3, 25-OHD_3 and calcium. The second objective of the study was to determine the relative biological value of 25-OHD_3 in comparison to vitamin D_3 for broiler breeder hens and to relate their levels in the maternal feed with the performance and leg abnormalities of the progeny fed a diet supplemented with vitamin D_3 or deficient in vitamin D_3.

CHAPTER 2

LITERATURE REVIEW

Ultraviolet light

The anti-rachitic properties of sun light have been documented for many years. By 1920, there was a collection of information indicating that rickets could be prevented or cured by exposure to direct sunlight or ultraviolet light. In 1919, Huldschinsky (cited by Lassiter and Edwards, 1982) showed that exposure of only a part of the body of a rackitic child to light from a mercury-vapor quartz lamp would cure rickets.

Vitamin D_3 is normally formed in the skin from 7-dehydrocholesterol in a photochemical reaction driven by the ultraviolet (UV) light (290-315 nm) of the sun or fluorescent lighting (Holick et al., 1980; MacLaughlin et al., 1982). The provitamin D_3 sterol, 7-dehydrocholesterol, is a precursor to and a product of cholesterol. 7-dehydrocholesterol is transformed to previtamin D_3 in the epidermis. In the skin, it isomerizes to the more stable molecule cholecalciferol, a reaction which is dependent on skin temperature (Holick et al., 1980). Cholecalciferol is then removed from the skin via vitamin-D binding protein and taken into the general circulation for further modification in the liver and kidney. Vitamin D_3 is not itself biologically actively, but is converted in the liver to 25-OHD_3 by the enzyme cholecalciferol 25-hydrozylase and then to 1,25-dihydroxycholecalciferol 1,25-$(OH)_2D_3$) in the kidney by the enzyme 25-OH-1α-hydroxylase (DeLuca, 1988; Norman, 1992). The most active form of vitamin D, 1,25-$(OH)_2D_3$ is involved in Ca and P absorption in the gut, bone

mineralization (bone formation) and demineralization (bone mobilization) and Ca and phosphate resorption by the kidney (Combs, 1998).

In contrast to all other vitamins, vitamin D_3 is produced endogenously in the skin of chicken exposed to UV light. However, the use of windowless houses with incandescent lights in commercial settings (broiler breeder and broiler chicks) does not allow the skin to produce endogenous vitamin D, therefore the vitamin most comes from the feed or from the yolk sac in the case of young chicks.

The effects of UV light on leg abnormalities in poultry have been reported for many years (Hughes et al., 1925). Bethke et al. (1936) conducted two experiments using White Leghorn hens to study the effect of sunlight and supplementation of vitamin D on hatchability, egg production and egg quality. The authors concluded that the exposure of birds to sunlight improved all criteria measured to levels comparable to D supplementation.

According to Koch and Koch (1941), the skin of the legs and feet of the chicken contains about eight times as much 7-dehydrocholesterol (pro-vitamin D_3) as the body skin. Tian et al. (1994) found highest concentration of 7-dehydrocholesterol in the leg of chicken (3524 ng cm^{-2}) than in the back (120 ng cm^{-2}). Whole body exposure of chickens to UV radiation resulted in the production of previtamin D_3 in the skin of the legs and feet (43 and 54 ng cm^{-2}, respectively), whereas no previtamin D_3 was detected in the back skin. Edwards (2003) conducted an experiment to determine whether the effectiveness of exposure of 1 day old chickens to UV radiation on bone growth and development was influenced by whether the radiation was from above or below the birds. Chickens that received UV light from below develop significantly less TD and had higher tibia ash than the ones expose from above. Edwards also reported that when the chickens were not exposed during the

first 24 hours of its life to radiation from fluorescent light, bone ash was significantly lower and the incidence of rickets and TD was higher at 16 days of age.

Edwards et al. (1992) found that exposure to UV light decreased the incidence of tibial dyschondroplasia in chicks which were fed diets containing 0, 200, or 2,000 international chick unit (ICU) per kg of vitamin D_3. Edwards et al. (1994) observed that when 800 to 1,600 ICU/kg of cholecalciferol were received from the diet, they grew and were comparable to those receiving UV light.

Using broiler chicks, Elliot and Edwards (1997) observed that the incidence and severity of TD was not completely reduced by dietary supplementation of vitamin D_3 in the absence of fluorescent lighting. At 50 µg/kg and no fluorescent lights, the incidence of TD was 50%, the TD score was 2.48. In the presence of fluorescent lighting, the corresponding values were 7% and 1.33.

Since high levels of vitamin D_3 have not been shown to be as effective in reducing the incidence of the TD as UV light, it is believed that the UV-light is more effective than vitamin D_3 (Edwards et al., 1992; Elliot and Edwards, 1997; Edwards 2003).

Vitamin D_3 in the Maternal Diet and its Effect on the Progeny

The nutritional status of broiler breeders is extremely important for the performance of the hens and crucial for the transfer of nutrients from the dam to the egg. Vitamin D_3 transfer from the hens to the eggs has been correlated with the amount of vitamin D_3 supply in the diet. Mattila et al. (1999) showed a positive correlation between the D_3 content of poultry feed and the D_3 and 25-OHD_3 contents of egg yolk. In their study three levels of vitamin D_3 were fed to laying hens (26.6, 62.4 and 216 µg/kg) and the amount of D_3 found in the eggs at 6 weeks after the

beginning of the experiment were 1.4, 3.4 and 23 μg/100 g of egg yolk, respectively. The corresponding values of 25-OHD_3 in the egg yolk were 0.5, 1.0 and 1.5 μg/100 g of egg yolk.

Higher concentrations of nutrients or vitamins in the egg tend to be associated with better hatchability, livability, good performance and healthier chickens. Several papers have shown the beneficial effect of increasing the level of vitamin D_3 in the diet of laying hens on the performance and general health of the progeny (Murphy et al., 1936; Bethke et al., 1936b; Griminger, 1966 and Edwards, 1995). However, no report exists on the vitamin D requirement of broiler breeder hens. The only vitamin requirement which has been studied in broiler breeders is biotin (Brewer and Edwards, 1972). Two experiments were conducted by Murphy et al. (1936) using Comb White Leghorn hens to study the relationship between the levels of vitamin D_3 fed to the hens with the bone ash of the progeny fed a vitamin D deficient diet. Hens were fed from day 1 to 76 weeks with the experimental diet in an environment that excluded UV-light. The levels of vitamin D_3 supplemented in the hens' diet, as cod liver oil, in U.S.P units per 100 g of feed were 1 to 135 in the first study and 17 to 270 in the second study. A difference in bone structure of the progeny was observed at 4 weeks, but not at 8 weeks of age between the chicken hatched from eggs laid by the different dam. In a subsequence study conducted by the same authors, higher bone ash was observed in chickens hatched from eggs laid by hens fed higher level of vitamin D_3 until two weeks of age, but not after two weeks of age. The authors indicated that vitamin D was transmitted from the hens to the eggs.

Besides the beneficial effect on the progeny, good nutrition of the mother will also result in a higher number of eggs and higher number of chicks hatched (Bethke et al., 1936a; Couch et al., 1947 and Abdulrahim et al., 1979). Bethke et al. (1936a)

using White Leghorn hens conducted two experiments to study the effect of sunlight and supplementation of vitamin D in the maternal diet on hatchability, egg production and egg quality. The sources of vitamin D used by the authors were cod liver oil or irradiated ergosterol. Egg production, egg shell quality, and hatchability of the eggs were greatly increased by the addition of vitamin D or exposure to UV-light. Microscopic examination of the liver and kidney showed areas of calcification in the kidneys of the hens fed the toxic level (54,000 units per 100 g of diet). With the egg and chicks produced by the hens used in the previous experiments, Bethke et al. (1936b) conducted several studies to better understand the effect of the maternal diet on the progeny. In their studies chicks from the groups of hens which received some source of the antirachitic factor had a significantly greater percentage bone ash and grew better than chicks from parent stock without vitamin D. Body ash of the embryo fallowed the same general pattern.

A comparable study was conducted by Griminger (1966) using leghorn hens. In his study hens were fed three level of vitamin D_3 (660, 6,600 and 66,000 IU/kg of diet) and their progeny were kept separate and fed a vitamin D deficient diet. No effect of the maternal diet was observed on the progeny during the two first weeks of life, but as the chickens got older the effect of the maternal diet became apparent. Body weight and tibia ash were higher in those chicks hatched from hens fed higher level of vitamin D. Another study was conducted several years later by Edwards (1995) using the progeny of laying hens fed various levels of vitamin D_3 (0 to 2000 IU/kg). He showed that when the maternal diet had 500 IU of vitamin D_3 per kg, the chicks could not reach maximum growth or bone ash when fed a diet supplemented with various levels of vitamin D_3 (0 to 200 IU/kg). But when the maternal diet

contained 2,000 IU/kg, higher growth and bone ash were achieved by chicks fed the diet supplemented with vitamin D_3 (0 to 200 IU/kg).

High egg production and hatchability and low embryo mortality was observed by Couch et al. (1947) by increasing the supplementation of vitamin D (19 to 36 IU/ 100 g of feed) in the diet of New Hampshire hens. In the same study the authors also observed higher requirements of vitamin D_3 at old age than at young age. In their experiment hens received sunlight during the summer and fall prior to the beginning of the experiment. Three different levels (19, 38 and 76 IU/ 100 g of diet) of vitamin D_3 were fed to the hens up to the first year of production and the same three levels for the second year of production. The authors point out that the requirement appears to be higher for the second year than for the first year. They also concluded that the D_3 required for the first year was 38 to 76 IU of vitamin D_3/100 g of feed and 76 IU of vitamin D_3/100 g of feed kg for the second year. It is reported in the literature that D_3 absorbed in the gut can be stored in adipose tissue from which it is slowly mobilized (Combs, 1992; Norman and DeLuca, 1963; Mawer et al, 1972).

An increase in egg production and hatchability was observed by Abdulrahim et al. (1979) by supplementing vitamin D_3 in the diet (0, 3, 9 and 18 μg/kg) of Single Comb White Leghorn hens (21 to 34 weeks of age)[2]. A difference in egg production and hatchability was observed between the treatment devoid of vitamin D_3 and the other three treatments, but no difference was observed between the three treatment supplemented with vitamin D_3. No effect of the vitamin supplementation was observed on egg quality. In a second phase of the study, after 34 weeks of age, when 1,100 IU of vitamin D_3/kg was supplemented to the basal diet it took 8 to 10 days for the hens to recover normal egg production and hatchability. In the same study 1α-

[2] One unit of vitamin D_3 (IU) is defined as the activity of 0.025 μg of vitamin D_3 (NRC, 1994).

OHD_3 and 1,25-$(OH)_2D_3$ were also fed to hens at 3 and 9 µg/kg, but they failed to maintain good hatchability at either level. Egg production increased when 3 µg/kg of both D metabolites were supplemented in the diet, but was reduced at the 9 µg/kg level.

Several studies conducted with turkeys have also shown the beneficial effects of the supplementation of vitamin D_3 in the maternal diet on the performance and general health of the progeny (Robertson et al., 1941; Stadelman et al., 1950; Griminger, 1966; Stevens et al., 1984a, 1984b).

Robertson et al. (1941) used poults that were hatched from eggs laid by hens fed 0, 100, 200 and 400 ICU of vitamin D_3 from cod liver oil per 100 g of feed to investigate the effects of the maternal diet on the progeny. The poults were fed a starting diet supplemented with 0 and 200 ICU of vitamin D_3 per 100 g of feed. Weekly body weights and percentage of bone ash indicated that the level of vitamin D in the hens' diet exerted an influence on the poults for the first two weeks; thereafter the influence of the vitamin D in the poults' diet was greater. In the same study the authors also observed that poults hatched from eggs laid by hens fed cod liver oil and exposed to sunlight attained greater weight at four weeks of age than poults hatched from hens fed the same or higher level of cod liver oil alone.

Similar to the previous work, Stadelman et al. (1950) fed various level of vitamin D to turkey breeder and to their progeny. The level of vitamin D supplied to the maternal diet had a direct influence on both body weight and bone calcification of the progeny that had received no vitamin D. As the vitamin D in the poults' diet increased, however, the effect of the maternal diet became less apparent. In this study the maternal diet had a direct influence on bone calcification in the progeny supplied with low level vitamin D, but not when the poults were fed high level of vitamin D.

Stevens et al. (1984a) conducted a study with large white turkey hens fed various levels of vitamin D_3 (300, 900 and 2,700 IU/kg of diet) from day 1 to 37 weeks of age. The authors observed low egg production, low hatchability, higher embryo mortality and lighter eggs with a thinner shell in the hens fed the lowest level of vitamin D_3. Using poults hatched from eggs laid by turkey hens used in the previous experiment, Stevens et al. (1984a) fed four levels of vitamin D_3 to determine the effect of the maternal diet on the performance and the activity of the enzyme 1-hydroxylase (cholecalciferol 25-hydrozylase). Low maternal D_3 increased kidney 1-hydroxylase activity in embryo and poults to at least 5 days of age. Low dietary vitamin D_3 increased the enzyme activity over that of poults fed higher levels. High levels of D_3 in the maternal diet did not affect plasma Ca, but improved body weight and tibia ash of the progeny.

D_3 and 25-OHD_3 on laying hens

No report has been made on the relative biological value of 25-OHD_3 in comparison to D_3 in broiler breeder hens and only a limited amount of research is available in laying hens. It is known that vitamin D deficiency in laying hens can result in low egg production, hatchability and production of eggs with thin shell. The affected hens can also present softening of beak, claws and long bones and bending of the sternum. In some cases inward turning of the ribs can also occur.

Both 25-OHD_3 and D_3 have been reported to be absorbed in the gut (Bar et al. 1980). However, the authors observed a higher absorption of 25-OHD_3 (74.9%) than D_3 (66.5%) in 14 day-old chicks. In experiments conducted with chickens, 25-OHD_3 has been shown to be more potent than D_3. McNutt et al. (1973) reported that 25-OHD_3 is twice as active as D_3 in supporting growth and 1.3 to 1.5 times as active as

D_3 in terms of increasing percentage of bone ash in chickens. Boris et al. (1977) observed that high levels of 25-OHD_3 and D_3 in the diet of chicks resulted in approximately the same tibia ash; however when the tibia ash from chicks fed low levels of D_3 or 25-OHD_3 were compared, 25-OHD_3 was twice as potent as D_3. Higher potency of 25-OHD_3 in comparison to D_3 has also been reported by Fritts and Waldroup (2003) when low levels are compare; however almost no difference was observed at high levels. It is known that when the level of a nutrient is far from meeting the animal requirement the response to every unit added in the diet is high. As the dietary concentration of a nutrient gets closer to the requirement, however, the response for each unit added decreases until it reach a plateau. This concept suggests that the relative biological value of some ingredients or nutrients change depending on the levels used in the study.

Several studies have been published comparing the effects of 25-OHD_3 and D_3 on commercial laying hens (Charles and Ernest, 1974; Charles et al., 1978; Marret et al. 1975; Roland and Harms, 1976; Abdulrahim et al. 1979; Keshavarz, 1996, 2003). Some researchers have observed no differences between the D-sources while others have observed significant differences. A mixture of factors such as levels of D metabolites used in the experimental diets, duration of the studies, exposure to UV light and presence of animal byproducts containing D activities may have contributed to these inconsistent results by different investigators. Vitamin D activity has been observed in several animal byproducts. Mattila (1995) found vitamin D_3 in fish, wild mushrooms, meat and milk. Atencio et al. (2003) also observed the presence of vitamin D activity in some samples of poultry fat.

In an experiment conducted for 28 days, Charles and Ernest (1974) reported an increase in specific gravity in old laying hens (144 weeks of age) fed 25-OHD_3 as

compared to vitamin D_3; however, such improvement was not observed with young laying hens (28 weeks of age). The same author also observed a lower egg production by the hens fed 25-OHD_3 when compared with the hens fed D_3. It is important to mention that the authors did not specify the levels of vitamin D sources used in their experiment. In a subsequent study, Charles et al. (1978) observed better shell quality in laying hens fed 25-OHD_3 at 200 IU/kg than those fed D_3 at the same level in a diet containing oyster shell and 3.5 % and 5 % of Ca. Marret et al. (1975) observed an increased of 3 to 4% in egg production by feeding laying hens after sexual maturity with 22 µg of 25-OHD_3/kg (880 IU/Kg) than with the same level of D_3. In a subsequent experiment conducted by the same authors, supplementing the diet with 12.54 µg of 25-OHD_3 per kg (500 IU/Kg) increased egg production by 10% when compared with hens fed D_3 at the same level. This study clearly shows higher potency at a low level than at a higher level of supplementation. Soares et al. (1976) reported that 25-(OH)D_3 was 2.5 times more effective in promoting eggshell and bone calcification than vitamin D_3.

No differences between 25-OHD_3 and D_3 were observed in egg production and hatchability by supplementing both vitamin D sources in the diet of 21 week-old leghorn hens at 360 IU/kg of diet (Abdulrahim et al., 1979). However a 5% difference was observed by feeding 25-OHD_3 when compared to D_3 at the 120 IU/kg level. It was noticed that animal byproduct (meat and bone meal) was used in the basal diet and no report was made whether UV-light was present or absent. Roland and Harm (1976) supplemented young and old laying hens with 2,200 ICU of vitamin D_3 per kg of diet and 1.1 mg of 25-OHD_3 kg of diet during 6 weeks. No differences between the two D sources were observed in egg production, serum calcium, egg weight and specific gravity.

In studies conducted by Keshavaraz (1996; 2003) no beneficial effect of supplementing 25-OHD_3 as compared to D_3 were found in performance, shell quality or bone ash in three different experiments using laying hens. In the 1996 experiment the level of both D sources was 500 IU/kg of diet while the two experiments of 2003 used 2,760 IU/kg of diet. The first experiment was carried out for 12 weeks whereas the second and third lasted for 20 and 16 weeks, respectively. Whether UV light was present or not in the study was not reported.

Effect of D Metabolites on Embryo Development

The effect of 25-OHD_3 on hatchability have been reported by several investigators. Hart and DeLuca (1985); Hart et al. (1986) and Abdulrahim et al. (1979) reported that hens fed 25-OHD_3 had normal hatchability and embryo development in the same proportion as hens fed D_3. Fraser and Emtage, (1976) and Mattila et al. (1999) reported higher D_3 transfer to egg than 25-OHD_3 when the hens were fed diet supplemented with D_3. Fraser and Emtage (1976) found that vitamin D_3 is transported into the yolk bound to a specific plasma protein (cholecalciferol-binding protein) which associates with yolk protein during its passage from liver to ovary. Although this binding protein has a higher affinity for D_3 than for 25-OHD_3, it will nevertheless effectively bind to the latter. In the same study was also observed that when large amount of 25-OHD_3 was fed to laying hens it was well incorporated in the yolk.

Unlike 25-OHD_3, 1,25-$(OH)_2D_3$ or 1α-OHD_3 fed to laying hens as their only source of vitamin D produced eggs that had high incidence of embryonic mortality prior to hatching (Sunde et al., 1978; Abdulrahim et al. ,1979 and Ameenuddin et al. 1983). The work of Abdulrahim et al. (1979) also showed that when hens fed 1,25-

$(OH)_2D_3$ or 1α-OHD_3 were switched to a diet supplemented with vitamin D_3 normal hatchability was restored. Sunde et al. (1978) and Ameenuddin et al. (1983) obtained an increase in hatchability of eggs by direct injection of 1,25-$(OH)_2D_3$ into eggs produced by deficient hens prior to incubation.

As discussed previously in this review, hens transfer vitamin D_3 and 25-OHD_3 to their egg yolk before they are laid. The embryo then converts these compounds to 1,25-$(OH)_2D_3$ when is required for the active transport of Ca from the shell to the embryo across the chorioallantoic membrane (Hart and DeLuca, 1985). Responsible for the conversion of D_3 and 25-OHD_3 to 1,25-$(OH)_2D_3$, the enzymes D_3-25-hydroxylase and 25-OHD_3-1-hydroxylase are active in the liver and kidney of the chick embryo as early as day 18 (Moriuchi and DeLuca 1974; Kubota et al., 1981).

Hart and DeLuca (1985) fed different D metabolites to laying hens with no or low body D_3 reserved. Normal embryo development was found in eggs from hens given 25-hydroxyvitamin D_3, 24,25-difluoro-25-hydroxyvitamin D_3, whereas embryos from hens given 1,25-dihydroxyvitamin D_3, 24,25-dihydroxyvitamin D_3 or their combination failed to hatch. The embryos fed 1,25-dihydroxyvitamin D_3 and/or 24,25-dihydroxyvitamin D_3 had vitamin D deficiency exhibited by low bone ash, low plasma calcium, low total body calcium, and extremely high plasma phosphorus. Because the shell is the major source of calcium for the developing embryo, calcium transport from the shell to the embryo apparently fails, giving rise to the observed defects in embryonic development. It appears that either 1,25-$(OH)_2D_3$ is not transferred from hen to egg, or if it is transferred, it is metabolized early in embryonic life and it is no longer present when needed. The yolk and the shell are the two major sources of Ca for the chick embryo. The yolk, which contains low levels of Ca (28 mg) supplies most of the Ca during the first 10 to 12 days of development. After 12

days, the shell which contains higher levels (2 g) in the form of calcium carbonate becomes increasingly important in providing Ca to the embryo. In the same study conducted by Hart and DeLuca (1985) 20-day-old embryos from hens fed 25-hydroxyvitamin D_3 or 24,25-difluoro-25-hydroxyvitamin D_3 had two and three times more calcium than embryo from hens fed 24,25-dihydroxyvitamin D_3 and 1,25-dihydroxyvitamin, respectively.

Growth Plate and Bone Disease

Bone growth occurs at regions of specific cartilage, the growth plate situated at both ends of all long bones. The growth plate is found between the epiphysis and metaphysic of long bones (e.g. tibia). The growth plate is the name given to the cartilage matrix where the lengthening of long bone occurs. From the epiphysis to the metaphysis the growth plate can be divided in 5 histological zones: reserve zone (resting zone), zone of proliferation, zone of hypertrophy, zone of resorption and zone of ossification (Dellmann and Eurell, 1998; Farquharson and Jefferies, 2000). The cells present in the growth plate are called chondrocytes and are arrange in columns. These cells are responsible for the formation and maintenance of the cartilage matrix. The matrix is mineralized by hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. The type of bone formation that occurs in long bones is called endochondral ossification. In the resting zone the small chondrocytes are dispersed and do not participate in cell division. In the proliferating matrix, synthesis and cell division occurs to replace the one that disappears towards the metaphysis. In this zone the cells are in columns and their mitotic function provides new cells for longitudinal bone growth. Cells in the hypertrophyc zone increase in size and begin to accumulate calcium. The cellular calcium is released into the matrix of the deep hypertrophic zone and matrix vesicle

begin calcium uptake. Alkaline phosphatase produced in this zone causes a local increase of phosphate. Accumulation of phosphate and calcium leads to mineralization. In the zone of resorption, capillary loops and perivascular connective tissue invade the lacunae of the degenerating hypertrophic chondrocytes located at the base of the cell columns. In the zone of ossification, osteoblasts differentiate from cells accompanying the invading capillaries. This cell deposits bone on the remains of the calcified walls of the chondrocytic lacunae (Dellmann and Eurell, 1998). Proliferative chondrocytes of avian growth plate are arranged in columns, but, unlike mammals, they are not easily distinguishable due to a much higher density and less extracellular matrix (Howlett, 1979).

Disruption of this chondrocyte maturation sequence or lack of mineralization causes many skeletal abnormalities in poutry such as tibial dyschondroplasia and rickets, which are common causes of deformity and lameness in broiler chicken and significant economic loss in the poultry industry (Morris, 1993; Farquharson and Jefferies, 1998). The occurrence of tibial dyschondroplasia in rapidly growing broiler chicks was first described by Leach and Nesheim (1965). Tibial dyschondroplasia is macroscopically characterized by a mass of white, opaque, un-mineralized and un-vascularized cartilage in the growth plate of the tibiotarsus. Rickets is characterized by a generalized failure of endochondral ossification or a failure of mineralization of the growth plate. There are two types of rickets. Hypocalcemic rickets is characterized by an accumulation of proliferating chondrocytes (Jande and Dickson, 1980); and hypophosphataemic (phosphorus deficiency) rickets in which the hypertrophic chondrocytes accumulate with normal metaphyseal vessel invation. (Lacey and Huffer, 1982). Vitamin D_3 deficiency produces a similar lesion to calcium deficiency (Long et al. 1984c; Cheville and Horst, 1981). Subclinical cases of TD and rickets are

common in broilers. Birds affected with clinical rickets or TD show reluctance to move, and remain sitting on their hocks. They may use their winds to balance when they walk. The bone and beak are soft and bend easily.

The rate of bone growth during the first few weeks of age greatly exceeds that seen at any other period of the broiler's life (Bond et al., 1991). Additionally, broiler chicks severely affected with tibial dyscondroplasia (50% incidence) had lower concentration of 1,25-OH_2D_3 than chicks mildly affected (14% incidence) at 14 days of age (Vaiano et al., 1994). Lilburn (1994) reported that the incidence of TD reaches a maximum around 21 days of age, and proposed that the high rate of early growth was responsible.

The time taken for a chondrocyte to move from a proliferative to a terminally differentiated phenotype is species-dependent and is approximately 21 h in the broiler chick (Thorp, 1988). In contrast, the mean cycle time of proliferating cell within the growth plate of man and rat is 20 and 2 days, respectively (Kember and Sissons, 1976).

Edwards and Veltmann (1983) were able to induce TD by feeding birds a diet low in Ca and high in P. High incidence of TD has also been observed by Edwards (1984) in chicks fed low level of Ca (0.44) and low level of total phosphorus (0.52). Long et al. (1984a, 1984b and 1984c) in experiments conducted with broiler chickens fed a phosphorus rickets-inducing diet (low level of P and normal Ca or high level of Ca and normal P) and a Ca rickets inducing diet (vitamin D deficient diet or a Ca deficient diet) described the histological changes in the growth plate during Ca and P rickets.

Although the exact mechanism involved in the development of tibia dyschondroplasia is not yet completely understood, many studies have shown the

beneficial effects of supplemental 1-αOHD_3, 1,25-$(OH)_2D_3$, ultraviolet light exposure or high levels of supplemental vitamin D_3 and 25-OHD_3, on the reduction of TD and Ca rickets incidence in rapid growing broilers (Edwards, 1989, 1990; Thorp et al., 1993; Rennie and Whitehead, 1996; Xu et al., 1997; Mitchell et al., 1997a,b; Aburto et al., 1998; Edwards et al., 2004; McComark et al. 2004). Feed deprivation, which is the length of time each day that feed is not available to young broiler chicks, has also been shown to have an effect on reducing the development of tibial dyschondroplasia (Edwards and Sorensen, 1987; Elliot and Edwards, 1994; Su et al., 1999). The physiological changes involved in the reduction of TD by feed restriction have not yet been elucidated.

Studies have demonstrated that 1,25-$(OH)_2D_3$ is a potent regulator of cell proliferation and differentiation and may play an important role in cell maturation (Walters, 1992). It has also been reported that 1,25-$(OH)_2D_3$ promotes condrocytes maturation (Gerstenfeld, 1990). The chondrocytes in TD-associated growth plates remain hypertrophic and fail to reach complete maturation (Hargest et al., 1985). Mitchell et al. (1997a) fed two populations of chicks with high incidence or low incidence of TD, 1,25-$(OH)_2D_3$. He did not observe differences in the concentration of vitamin D receptors in the intestine or growth plate of the two populations nor were differences observed in plasma 1,25-$(OH)_2D_3$ levels.

Edwards (2000) and Bradshaw et al., (2002) in review papers discussed the studies conducted by several investigators relating nutritional factors on skeletal problems of poultry.

D_3 and 25-hydroxycholecalciferol in Broiler Nutrition

The antirachitic vitamin was named vitamin D by McCollum et al. (1922). Mellanby (1919) was the first to formally recognize rickets as a nutritional disease. He thought it was caused by a deficiency of vitamin A present in cod-liver oil; however McCollum et al. (1922) destroyed the vitamin A in cod-liver oil and found that the oil still possessed the antirachitic effect. The antirachitic substance was then named vitamin D. Vitamin D is the general term applied to a number of fat-soluble sterol derivatives which are active in the prevention of rickets in animals.

The NRC (1994) recommends 200 IU of vitamin D_3/kg of diet for starting broilers (1 to 21-day of age). However, studies conducted in environments excluding UV-light indicate that the vitamin D_3 requirement in young broiler chickens (Mitchell et al., 1997; Elliot et al., 1997; Kasim and Edwards, 2000; Fritts and Waldroup, 2003; McCormack et al., 2004) is higher than the NRC (1994) recommendation.

Several factors such as presence of animal byproduct in the basal diet (Mattila, 1995; Atencio et al., 2003), UV-light (Edwards et al., 1992, 1994; Edwards, 2003), amount of cholecalciferol present in the chick at hatching (Murphy et al., 1936; Bethke et al., 1936; Griminger, 1966; Edwards, 1995) and level of other nutrients (Aburto and Britton, 1998; Aburto et al., 1998; Baker et al., 1998; Waldroup et al., 1965) may be influencing the growing chicks' quantitative requirement for vitamin D_3 for the maximum performance, maximum bone ash and minimum leg abnormalities. The quantitative requirement of vitamin D_3 varies according to the criteria under evaluation. Usually the requirement is based on bone ash or incidence of TD and rickets which have been found to be more sensitive indicators than growth rate. Edwards et al. (1994) using a corn-soybean diet containing 1.1% Ca and 0.72% total P and an environment excluding UV light found requirement in ICU per kg of diet of

275 for growth, 503 for bone ash, 552 for blood plasma Ca and 904 for rickets prevention.

Waldroup et al. (1965) showed that the requirement of vitamin D depended on the dietary Ca and phosphorus levels. The authors report that with levels of 1.00 % Ca and 0.70% P, maximum body weight and bone ash were obtained with 198 ICU/kg of diet; however with a dietary Ca level of 1.00% and total P of 0.50%, almost 8,000 ICU/kg were needed for optimum body weight gain and bone ash. When Ca level were reduced (50%) the requirement of vitamin D_3 was also reduced (1,600 ICU/kg).

Aburto and Britton (1998a,b) and Aburto et al. (1998) reported that high levels of vitamin A and E negatively affected the utilization of vitamin D_3 or 25-OHD_3 or increased the requirement for vitamin D_3. The authors observed that 45,000 IU/kg of vitamin A reduced bone ash when vitamin D_3 was supplemented at 500 IU/kg to chicks in a UV-light free environment; however no negative effect was observed when D_3 was at 2,000 IU/kg. Similarly, vitamin E at 10,000 IU/kg reduced body weight, bone ash, plasma Ca and increased rickets incidence when vitamin D_3 was supplemented at 500 IU/kg whereas no negative effect was observed when vitamin D_3 was supplemented at 2,500 IU/kg.

Kasim and Edwards (2000) and Edwards et al. (2002) conducted experiments to compare different sources of vitamin D_3 or the potency of vitamin D_3 against 1α-OHD_3. The authors observed an increase in body weight gain, tibia ash and a reduction in rickets incidence as the dietary vitamin D_3 supplementation increased in the diet. The maximum levels of vitamin D_3 used by the authors were 1,200 and 1,600 IU/kg, respectively.

Fritts and Waldroup (2003) observed a decrease in TD incidence and an even greater decrease in severity by supplementing vitamin D_3 up to 4,000 IU/kg in the diet

of broiler chicks. More recently, McCormack et al. (2004) using vitamin D_3 at concentrations of 200, 800, 5,000 and 10,000 IU/kg reported that 10,000 IU can prevent TD almost completely. It is not known, however, if a lower level of vitamin D_3 would have been equally as effective as 10,000 IU since the next lower increment was 5,000 IU. Baker et al. (1998) conducted an experiment in a UV-light free environment and observed that chicks were able to tolerate high levels of vitamin D_3 in diet (50,000 IU/kg).

25-hydroxycholecalciferol (25-OHD_3) is currently used as a commercial feed additive. Supplementation of the diet with 25-OHD_3 can significantly reduce the incidence and severity of TD, but not as efficiently as 1-αOHD_3, 1-25$(OH)_2D_3$ (Boris et al., 1977; Edwards et al., 1989). Yarger et al. (1995) indicated that 25-OHD_3 could safely be used in place of cholecalciferol in commercial broiler rations. The authors conducted two experiments using high levels of 25-OHD_3 (69, 207 and 690 µg/kg, experiment 1 and 69 to 13,800 µg/kg, experiment 2). No toxic effects or improvement in performance were observed by increasing the level of 25-OHD_3 in experiment 1. In the second experiment, however, there was some evidence of renal calcification in birds fed 25-OHD_3 at 10 times the basal level, whereas dietary levels of vitamin D_3 at 50 times the basal level were required to show some evidence of renal calcification. The authors concluded that 25-OHD_3 is 5 to 10 times more toxic than vitamin D_3.

25-OHD_3 has been shown to improve tibia ash and lessen the incidence and severity of TD and Ca rickets in broiler chicks (Mitchell et al., 1997b and Zhang et al., 1997; Ledwaba and Roberson, 2003; Fritts and Waldroup, 2003); however other studies have shown no reduction in TD incidence in broiler chicks selected for high incidence of TD by supplementing 25-OHD_3 in the diet (Mitchell et al., 1997b; Zhang et al., 1997). Saunders-Bladewes and Korver (2004) supplemented broiler diets with

25-OHD_3 at 69µg/kg or vitamin D_3 at 3,000 IU /kg and observed faster body gain and greater breast and leg portions with 25-OHD_3. Even though when high levels of D sources were supplied the birds still developed rickets at 11 days of age.

Several studies have shown that 25-OHD_3 is more potent than D_3 (McNutt et al., 1973; Boris et al. 1977; Fritts and Waldroup, 2003); however when compared to vitamin D_3, its potency depends on the levels tested.

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CHAPTER 3

THE VITAMIN D_3 REQUIREMENT OF BROILER BREEDER HENS[1]

[1] Atencio, A., H.M. Edwards Jr., G.M. Pesti and G.O. Ware. Submitted to Poultry Science.

ABSTRACT

An experiment was conducted with 25-to-66-wk-old-Ross broiler breeders in an environment excluding UV light, to determine the D_3 requirements for hen day egg production (HDEP), hatchability, body weight of the progeny at 1-d (BW), embryo mortality during the early (1-10-d of incubation EEM), middle (11-15-d MEM) and late stages (16-21-d LEM), egg weight (EW), specific gravity (SG) and body ash of the progeny at 1-d of age (BAP). Five levels of D_3 (125, 250, 500, 1,000 and 2,000 IU/kg of diet) were fed to hens from 25-to-66-wk of age. One additional group was fed no supplemental D_3 until 36-wk of age and was then changed to 4,000 IU/kg. Separate regression analyses were performed for wk 27-to-36 (peak original design) and for wk 37-to-66 (post-peak production modified design). The D_3 levels for the predicted maximum HDEP during peak and post peak production were 1,424 IU/kg and 2,804 IU/kg, respectively. The D_3 levels for the predicted maximum hatchability were 1,390 IU/kg (peak) and 2,708 IU/kg (post peak). The level of D_3 that resulted in the minimum EEM was 1,288 IU/kg at peak; however no significant effect was observed at post-peak. The D_3 levels for minimum MEM were 1,130 IU/kg (peak) and 2,568 IU/kg (post-peak) and for LEM were 1,393 IU/kg (peak) and 2,756 IU/kg (post-peak). The D_3 level for maximum EW was 1,182 IU/kg (peak) and for SG were 1,337 IU/kg (peak) and >2,000 IU/kg (post-peak). The D_3 level for maximum BAP was >2,000 IU/kg. Analysis of the data from the original design of the experiment (treatment 0, 125, 250, 500, 1,000 and 2,000 D_3/kg for the 27-36-wk of age) indicate a requirement of approximately 1,400 IU of D_3/kg of feed for broiler breeder hens. When the data from the modified experiment 37-66-wk of age includes the conversion of the 0 IU of D_3/kg treatment to the 4,000 IU of D_3/kg treatment, the requirement may be approximately 2,800 IU of D_3/kg.

Key words: broiler breeders, requirement, vitamin D_3

INTRODUCTION

Vitamin D, or its more active metabolite (1,25-dihydroxycholecalciferol) is involved in Ca and P absorption in the gut, bone mineralization (bone formation) and demineralization (bone mobilization) and Ca and phosphate resorption by the kidney (Combs, 1998). The most common problems of economic importance in laying hens from a deficiency of vitamin D_3 quoted in the literature are decreases in egg production and hatchability (Bethke et al., 1936; Couch et al., 1947 and Abdulrahim et al., 1979).

Since no work has been reported until the present on the vitamin D requirements of meat type hens, the NRC (1994) refers the reader to the requirement for egg-type breeders. The NRC (1994) vitamin D_3 requirement for egg-type white egg breeders with feed intake of 100 g of feed/hen/day is 300 IU of vitamin D_3/kg. This translates into a daily requirement of 30 IU of vitamin D_3/hen/day. Brown egg layers with a feed intake of 110 g/hen/day are estimated to require 33 IU of vitamin D_3/hen/day. Suggested levels of use for vitamin D_3 for broiler breeder rations in books and commercial guidelines vary from 1,000 (Scott et al., 1982) to 3,500 IU of vitamin D_3/kg of diet (Ross Feeding Program Summary, 2000)[1]. Studies indicate that birds can obtain vitamin D_3 from vitamin premix, endogenous production (UV light radiation) and animal byproduct.

Couch et al. (1947) using New Hampshires hens observed an increase in egg production, hatchability and egg quality, and a decrease in embryo mortality by increasing the D_3 level in the diet (19, 38 and 76 IU of vitamin D_3/kg). An increase in egg production and hatchability was also observed by Abdulrahim et al. (1979) in an experiment conducted with 26-34 wk old Leghorn laying hens by supplementing

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vitamin D_3 (0, 360 and 720 IU/kg) to a vitamin D_3 deficient diet. It is important to point out that animal byproducts were used in the basal diets by both researchers. Vitamin D is found naturally in fish, wild mushrooms, meat and milk (Mattila, 1995). Vitamin D activities were also observed by Atencio et al. (2003) in some samples of poultry fat.

In contrast to all other vitamins, vitamin D_3 is produced endogenously in skin exposed to UV light (Tian et al., 1994). Several studies indicate that UV light is effective in reducing the development of tibial dyschondroplasia (TD) and rickets in chickens (Edwards et al., 1994; Elliot and Edwards, 1997; Edwards, 2000, 2003; Ledwaba and Roberson, 2003). However, husbandry methods that have evolved during the last part of the 20th century have created environments where breeder hens receive almost no measurable UV light. Thus, most of the D_3 present in the egg must come from the feed. The presence of animal byproducts in the diet with D activity or the exposure of the birds to UV light can result in a lower dietary D_3 requirement.

It has also been documented that D_3 present in the chick at hatch affects the D_3 requirement in the diet of broilers. Studies conducted by Mattila et al. (1999) showed a positive correlation between the D_3 content of hen diets and the D_3 and 25-OHD_3 contents of egg yolk. Murphy et al. (1936) reported that D_3 and sunlight given to hens affected bone calcification of the progeny. The effect of vitamin D_3 levels in the hens diets on the body weight and bone ash of the progeny reared on corn soybean meal diets containing 50, 100, 150, and 200 IU of vitamin D_3/kg was reported by Edwards (1995). These results show that when the maternal diet contains only 500 IU of vitamin D_3/kg, the chick cannot reach maximum growth or bone ash when the chick's diet contains 200 IU/kg. But when the maternal diet contains 2,000 IU/kg, high levels

of growth and bone ash are achieved by chicks fed diets with much lower levels of D_3.

The objective of this experiment were to determine the vitamin D_3 requirement of broiler breeders for maximum performance and to relate D_3 levels in the maternal feed with the performance of the progeny, incidence of TD and general health.

MATERIALS AND METHODS

Five hundred and ten female broiler breeders (Ross[2] - 508) and sixty two males (Ross 344) were raised using corn soybean meal diets containing 1,100 IU D_3/kg to 23 wk of age. From wk 3 to 22 the birds had 8-h light per day, from 22 to 23 wk 14-h and from 24 to 25 wk 15-h and from 26 wk to the termination of the experiment (66 wk) 16-h. At 23 wk of age, three hundred and sixty pullets and thirty six males were randomly allocated to 18 floor pens (2.4 m wide x 3.6 m length). The experiment was conducted in a house where incandescent light bulbs that emit no UV light were used and the outside light was excluded by baffles on the ventilation system. Room temperature was maintained at approximately 22.72 ºC. The maximum temperature in the summer was 30.5ºC and the minimum in the winter was 9.4ºC. All birds were vaccinated according to a vaccination program recommended by the breeders**.**

Six levels of D_3 (0, 125, 250, 500, 1,000 and 2,000 IU/kg of diets) were fed to the hens from 25 to 66 wk of age, except for the treatment with 0 level of D_3. The hens receiving the 0 level of D_3 were switched to a diet containing 4,000 IU/kg at 36

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wk of age. Three repetitions of twenty hens and two males per pen were used for each treatment group.

No D_3 or other vitamin D source was added to the vitamin premix and no animal byproducts were used in the corn, wheat middling and soybean meal based basal diet to guarantee that no cholecalciferol metabolites were present in the diets (Table 1**)**. The same basal diet was used throughout the experiment. Feed was mixed at approximately monthly intervals for all treatments.

Hens and males were fed every day in separate feeders with the experimental diets to meet their daily requirement for energy and protein according to the breeder's guideline recommendations[3]. To make sure that all males received vitamin D_3 to meet their requirements, they were given 18,380 IU D_3/bird orally once per month.

Eggs were collected every day and stored in a cooler at 17 ºC. Eggs collected during 7 days were grouped and set in the incubator by treatment and pen. Total incubation time was 21.5 day. Temperature and relative humidity settings from 0-18 and 19-21 day of incubation were 37.8 °C and 37.2°C, respectively.

All incubated eggs were candled after 10 days of incubation, and clear eggs were removed, opened and classified as early embryo mortalities or infertile eggs. Clear eggs were classified as early dead embryos if the opaca and pellucida areas could be differentiated or if positive development was observed. At 18 days of incubation the eggs were transferred to the hatcher. All eggs cracked prior to incubation or at transfer were removed from the data set. Percentage of hatchability was calculated based on the number of fertile eggs. Those eggs that failed to hatch were broken out to determine percentages of middle embryo mortality (11-15 d), late embryo mortality (16-21 d) and pips. Embryos completely covered with feathers were

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considered late embryo mortality other wise it was considered middle embryo mortality. Pips were any embryos that were completely covered with feathers and had the shell of the egg broken. There were 40 hatches in this study.

Two one-d-old chicks per pen, hatched at three different times (wk 42, 58 and 65) were killed, placed in individual crucibles, dried and ashed (500 ºC) in a muffle furnace overnight for the determination of body ash. Egg weight and specific gravity were measured every two wk on one hundred percent of the eggs collected during one particular day.

Statistical Analysis

The experiment consisted of a split plot design with vitamin D_3 as the whole plot with subplot measurement taken over time at different ages. The analyses were conducted using the mean of each repetition per week as an observation. Possible patterns of correlation and changes in variation of the mean pen responses over time were investigated. The condition required for validity in meeting the assumptions for utilizing multiple regression analyses in modeling the responses over time is called the Huynh-Fedt (H-F) condition. PROC GLM with the REPEATED statement was utilized to test whether the measurements over time met the H-F condition (Littell et al., 1998). It appeared that the measurements over longer periods of time did meet the H-F condition, but a few measurements for shorter periods of time did not.

PROC MIXED was then utilized to compare the results from modeling the repeated measures using three different variance-covariance structures. They included l) the compound symmetry (CS), the autoregressive structure [AR(l)], and unstructured. The models were compared based on the -2 Res Log Likelihood values as part of the output Fit Statistics of PROC MIXED. The more complex structure of

AR(1), and unstructured did not provide a significant improvement in fit based on the log likelihood values. There were some computational convergence problems with the unstructured model for some variables. As indicated, the variance-covariance structure, CS that assumes equal variances and equal correlations over time was selected as the preferred fit. Probably the main reason that the more complex covariance structures did not provide a significant improvement in fit is because individual birds were not tracked with repeated measurements recorded for each bird. Instead pen averages for the response measures of adult birds were computed for each pen at each age. There was no strong statistical evidence of unequal population variances or generally increasing and decreasing trends in variances. Therefore, the assumptions required for utilizing multiple regression analyses were met based on the CS variance-covariance structure.

Regression analyses were performed to fit a reduced quadratic regression equation using the General Linear Models procedure in SAS (SAS Institute, 1996). The probability values for the regression coefficients, D_3 and D_3^2, were found using an F value calculated by dividing their mean square by the mean square of the pen within vitamin D_3 treatment. All other probabilities values were calculated using an F-value found using the residual. In order to calculate the level of D_3 that resulted in the maximum or minimum response for the different criteria and different ages, two methods were used: multiple factor regression (2^{nd} order) with D_3 and age as predictor variables and simple factor regression (1^{st} (linear) and 2^{nd} (quadratic) order) with D_3 as the predictor variable. The multiple factor regression was used when the regression coefficient for the linear by linear (D_3 by age) interaction was significant ($P<0.10$). Contour plots and response surface plots were generated from the regression equation only when the regression coefficient for the interaction (D_3 by age) was significant

($P<0.10$). The simple factor regression was used when the multiple factor regression could not be used and when a significant linear or quadratic effect was observed to the different D_3 levels. Different from the multiple factor regression, the simple factor regression did not allow the calculation of the age when the maximum or minimum response to D_3 level was reached.

The coefficient of determination (r^2) was calculated using the sum of squares of the regression divided by the sum of squares of the total.

Since the hens in one treatment were switched from 0 to 4,000 IU of vitamin D_3/kg of diet the data were analyzed in four different ways: first, the 7 D_3 levels (0, 125, 250, 500, 1000, 2000 and 4000 IU/kg) were analyzed from 27 to 66 wk of age (complete data) ; second, 6 levels of D_3 (0, 125, 250, 500, 1,000 and 2,000 IU/kg) were analyzed from 27 to 36 wk of age (peak production); Third, 6 levels of D_3 (125, 250, 500, 1,000, 2,000 and 4,000 IU/kg) were analyzed from 37 to 66 wk of age (post peak production) and fourth, 5 levels of D_3 (125, 250, 500, 1,000 and 2,000 IU/kg) were analyzed from 27 to 66 wk of age. The second and third analyses will be discussed in this chapter. The first and the fourth analyzes are presented for the reader to have a different perspective of the results.

RESULTS

The hens fed no D_3 were switched to 4,000 IU D_3/kg at 36 wk because no eggs were being produced, feed consumption was dramatically reduced and the mortality rate of the hens was very high (15%). The number of hens that died per treatment were 9, 2, 2, 1, 0 and 0 for 0, 125, 250, 500, 1,000 and 2,000 IU of vitamin D_3/kg of diet, respectively. Switching the diet from 0 to 4,000 IU/kg resulted in a decrease in

mortality, increase in feed consumption and increase in performance in less than two wks.

Hen day egg production (HDEP)

The highest and the lowest percentage of HDEP throughout the experiment were observed in the treatments that received the highest and the lowest supplementation of D_3 in the diet, respectively (Table 2 and Figure 1). All hens reached maximum HDEP at approximately 31 wk of age, except the hens receiving no D_3 supplementation in the diet which reached the maximum HDEP at an earlier age. At 31 wk of age, HDEP responded linearly ($P<0.0001$) to the different D_3 levels (Figure 2). No effect of D_3 on HDEP was observed before 31 wk of age. This indicated that it took 6 wk for the hens fed the diet with low vitamin D_3 supplementation to use their D_3 body reserves and become deficient in vitamin D up to a point that egg production rate was influenced. Changing the diet from 0 to 4,000 IU/kg at 36 wk of age increased HDEP to the highest levels in less than 2 wk. The decrease in HDEP occurred at a faster rate after 56 wk of age in the treatments with the lowest D_3 levels compared to treatments with the highest D_3 levels (figure 1).

Significant interactions in the regression coefficient of D_3 by age were observed during peak production. This interaction indicated that different amounts of D_3 in the diet were necessary to obtain maximum HDEP as the experiment progressed. Figure 3 and 4 show contour plots (two dimensions) and surface plots (three dimensions), respectively, for vitamin D_3 and age. These plots illustrate the interaction between D_3 and age. The peak of the response surface (Figure 4) which is the predicted maximum HDEP (stationary point) correspond to the center of the ellipse in figure 3. This point is reached when D_3 is at 1,424 for 32 wk old hens,

therefore the D_3 level required at peak production for the predicted maximum HDEP (91%) was 1,424 IU/kg at 32 wk of age by using the multiple factor regression. During post peak production no interaction was observed in the regression coefficient D_3 by age. In this case a simple factor regression was used, 2^{nd} order, to find the D_3 level for the predicted maximum response. The D_3 level that resulted in the maximum HDEP was 2,804 IU/kg (Figure 5). Even though the D_3 by age regression coefficient was not significant, the D_3 level for the predicted maximum HDEP was 2,811 IU/kg at 38 wk of age by using the multiple factor regression. The D_3 level required for the predicted maximum during post peak is almost identical in both models.

In addition, the results showed that D_3 level for the maximum response was lower at peak than post peak. The HDEP of four different wk were plotted to illustrate these results (Figure 6). At wk 34 and 36 the predicted maximum HDEP is reached at lower levels of D_3 than at wk 40 and 50. Figure 6 also shows the interaction between D_3 and age because the lines are not parallel.

Hatchability

The percentage hatchability followed the same general pattern as the HDEP. Once again, the highest and the lowest percentages of hatchability were observed in the hens that received the highest and the lowest supplementation of D_3 in the diet, respectively (Table 3 and Figure 7). At 29 wk of age, hatchability responded linearly ($P<0.0004$) to the D_3 levels in the diet (Figure 8). This result indicates that hatchability was affected by the different D_3 levels earlier than HDEP. Changing the diet from 0 to 4,000 IU/kg at 36 wk of age increased hatchability to the highest levels in less than 2 wk. The decrease in hatchability occurred at a faster rate at the end of

the experiment in the treatments with the two lowest D_3 levels (125 and 250) than in the other treatments (Figure 7).

Significant interactions in the regression coefficient D_3 by age were observed during peak and post peak production. Figures 9 and 10 show contour and surface plots, respectively, for D_3 and age during peak production. The plots illustrate that the predicted inflection is at a saddle point not a true maximum. A saddle point does not have a unique maximum or minimum for the fitted model because the maximum is in a region rather then in a specific point (stationary point). A saddle point is reminiscent of a horse saddle-rising up from front to back but sloping down from side to side. When the maximum or minimum is at a saddle point, further research should be performed in order to find a unique maximum. In order to have an idea of the D_3 level for the predicted maximum response, a simple factor regression, 2^{nd} order was used. The D_3 level for the maximum hatchability found was 1,390 IU/kg (Figure 11). Figure 12 and 13 shows the interaction and the combination of D_3 and age at which the maximum hatchability was reached at post peak. The D_3 level for the maximum hatchability was 2,708 IU/kg at 52 wk of age.

Treatments with the lowest D_3 levels had higher variation between repetitions than treatments with the highest D_3 levels. The higher standard errors of the mean in Table 3 (hatchability) and the points spread around the curves in Figure 8 (hatchability, during peak) and in Figure 11 (hatchability, during post peak) illustrate differences in variation between treatments.

Early embryo mortality (EEM)

A significant interaction in the regression coefficient D_3 by age was observed during peak production for EEM; however no effect of D_3 was observed during post

peak production (Table 4). The highest EEM was observed in the hens fed the lowest D_3 levels from 32 to 36 wk of age (Figure 14). Figures 15 and 16 show that minimum EEM was at a saddle point. By using a second order simple regression the D_3 level for the minimum early embryo mortality was 1,288 IU/kg (Figure 17).

Middle embryo mortality (MEM)

Significant interactions in the regression coefficient D_3 by age were observed during peak and post peak production for MEM (Table 5 and Figure 18). The highest middle embryo mortality was observed in the hens fed with no D_3 supplementation and 125 IU/kg, especially from 31 to 41 wk (Figure 19). Contour plots (Figure 20 and 21) illustrate the interactions for peak and post peak production. The predicted D_3 levels for MEM were 1,130 (at 29 week of age) and 2,568 IU/kg (at 52 week of age) during peak and post peak production, respectively. In addition to the multiple factor regression analyses, a simple factor regression was also performed. The D_3 levels for the lowest MEM were 1393 and 2,759 IU/kg during the peak and post peak production, respectively.

Late embryo mortality (LEM)

Again, hens fed diets containing the lowest and the highest D_3 levels produced the highest and the lowest LEM, respectively, except for the period from 51 to around 58 wk of age in which almost no effect of D_3 was observed (Table 6 and Figure 22). Interaction in the regression coefficient D_3 by age was observed in LEM during peak production. However, during post peak production the regression coefficient for the interaction (D_3 by age) was not significant (P=0.5115). Contour plots (Figure 23 and 24) illustrate the responses during peak and post peak production. The minimum

LEM during peak production was at a saddle point. By using the simple factor regression the D_3 level for the minimum LEM was 1,393 IU of D_3/kg (Figure 25). During post peak production, the requirements were 2,756 and 2,759 IU/kg by using multiple and simple factor regression (2^{nd} order), respectively (Figure 26). Hens fed with the lowest D_3 levels produced a higher variation between repetitions in the same treatments than hens fed with the highest D_3 levels (standard errors of the mean in Table 6 and points distributed around the curves in figures 25 and 26 illustrate this observation).

Body weight of the progeny (BW)

No interaction in the regression coefficient of D_3 by age for BW at 1 day of age was observed during peak or post peak production (Table 7). The main effect of D_3 was not significant for either period. Figure 27 and the horizontal line with no significant slope in figure 28 illustrate the lack of D_3 effect. Age was the only significant factor. As hens got older the body weight of the progeny increased.

Egg weight (EW)

Significant interaction was observed in the regression coefficient of D_3 by age during peak production. However, the predicted inflexion is at a saddle point. By using the simple regression, 2^{nd} order, the predicted D_3 level for the maximum EW was 1,182 IU D_3/kg (Table 8 and Figure 29). No interaction in the regression coefficient D_3 by age and no linear or quadratic effect for the different D_3 levels were observed during post peak production.

Specific gravity (SG)

In general, eggs from hens fed with the lowest D_3 levels had lower SG than those from hens fed with the highest D_3 levels (Table 9 and Figure 30). A significant interaction in the regression coefficient of D_3 by age was observed during peak; however the maximum was at a saddle point. By using the simple factor regression, 2^{nd} order the D_3 level for the maximum SG was 1,337 IU/kg of diet (Figure 31). During post peak production the regression coefficient for D_3 by age, was not significant. The main effect of D_3 was highly significant from 37 to 66 wk of age. Specific gravity responded linear to the different D_3 levels. A linear effect (simple factor regression, 1^{st} order) indicate that a value between 2,000 and 4,000 IU/kg is required for maximum specific gravity (Figure 32).

Body ash of the progeny (BAP)

Significant interaction in the regression coefficient of D_3 by age was observed for BAP; however, the predicted inflection is at a saddle point not a true maximum (Table 10). Chicks hatched from eggs laid by hens fed the lowest vitamin D_3 levels had lower body ash than chicks hatched from eggs laid by hens fed the highest D_3 levels (Figure 33). Body ash increased linearly as the D_3 levels in the diet increased (Figure 34). Plotting all three wk separately, a linear increase ($P<0.01$) in BAP was observed as the D_3 levels in the diet increases, for wk 42 and 48 (Figure 35). The slope of the lines decreased as the week increased. This observation indicates that at older ages the effects of D_3 decreased. Body ash of the progeny increased linearly ($P<0.0016$) as the hens got older (Figure 36). At 42 wk the body ash was 779 mg, at 58 wk was 781 mg and at 65 wk was 820 mg. At older ages of the hens the eggs got bigger and the egg production was reduced. Lower egg production may allow the hens

to concentrate more nutrients in the eggs and produce a stronger chick. Chicks hatched from hens fed the lowest D_3 levels had higher variation in BAP between repetitions of the same treatment than chicks with the highest D_3 levels (Figure 34 and 35). The D_3 level for maximum BAP is between 2,000 and 4,000 IU/kg.

No D_3 effect was observed on fertility and on the number of pipped eggs (values not presented).

Table 11 presents a summary of the estimated vitamin D_3 requirement for the different characteristics and for the two different periods, during peak (27 to 36) and post peak production (37 to 66 wk of age).

DISCUSSION

No effects of the different vitamin D_3 levels in the hens' diet were observed on fertility, pipped eggs, body weight of the progeny at any time or on early embryo mortality and egg weight after peak production. These results indicate that all vitamin D_3 levels met the requirements for fertility, pipped eggs and body weight of the progeny.

A significant interaction in the regression coefficient of D_3 by age, were observed for hatchability, middle embryo mortality and body ash of the progeny during peak and post peak production. However, for hen day egg production, early embryo mortality, late embryo mortality, egg weight and specific gravity a significant interaction was observed only during peak production. These interactions indicated that different amounts of vitamin D_3 in the hens' diet were necessary to obtain maximum or minimum responses as the experiment progressed.

In general hens fed high D_3 levels in the diet had higher egg production, hatchability, egg weight, specific gravity, body ash of the progeny and lower embryo mortality than hens fed low D_3 levels. The results obtained in this experiment show the important role of vitamin D_3 in improving broiler breeder hen performance. These results are in agreement with Couch et al. (1947) and Abdulrahim, et al. (1979) who observed an increase in egg production, hatchability and egg quality and a reduction in embryo mortality by feeding hens with increasing levels of D_3 in the diet.

No eggs with soft shells were seen during the experiment. Hens reduced their egg production to the lowest levels in the treatments with low D_3 levels, but they still produced eggs suitable for hatching. Figure 29 shows that the heaviest eggs at 62 wk were produced by hens receiving 125 IU of D_3/kg of diet (74, 69, 76g/egg). It is important to note that only 3 eggs were laid that day out of a total of 52 hens. Hens fed the highest levels of vitamin D_3 were not producing the largest eggs, but they were producing the largest number of eggs. During the experiment it was casually observed but not documented that hens fed diets with no vitamin D_3 or with 125 IU of D_3/kg, often ate their own eggs.

When eggs from the hens receiving low levels of vitamin D_3 were broken after been incubated to determine at which stage the embryo died, it was noticed that the shell was extremely soft when compared with the eggs from hens receiving high D_3 levels.

Some criteria were affected at an earlier age than others. Hatchability was more sensitive (affected at 29 wk of age) than egg production (affected at 31 wk of age) to the vitamin D_3 levels in the diet. This indicates that it took about 5 to 6 wk after the hens were fed the low vitamin D_3 experimental diets for them to deplete their body D_3 reserves and become deficient. It is important to emphasize that the hens

were fed 1,100 IU of vitamin D_3/kg during the first 24 wk of life prior to the beginning of the experiment. Figures 1 and 7 show that after 56 wk of age egg production and hatchability were decreasing at a faster rate in hens fed with low D_3 levels than in hens fed with high D_3 levels.

A reduced effect of vitamin D_3 was observed around wk 51 to 60 for hatchability, middle embryo mortality, late embryo mortality and specific gravity. There is no obvious explanation for these results. In the northern hemisphere the longest day of the year is June 21st. Week 54 coincided exactly with the longest day of the year. Even though the experiment was conducted in an environment excluding UV light, the data suggests that UV light got into the house during these long days of the year. Several authors have shown the effect of UV light in the synthesis of endogenous D_3 by chickens (Murphy et al., 1936; Tian et al., 1994; Edwards, et al., 1994; Edwards, 2000, 2003; Elliot and Edwards, 1997; Ledwaba and Roberson, 2003).

In general, for most of the criteria, studied hens fed diet with the lowest vitamin D_3 levels had a higher variation among repetitions in the same treatment than hens fed with the highest vitamin D_3 levels. The higher standard errors of the mean in most of the tables and the spread of values around the quadratic and linear graphs for treatments with the lowest vitamin D_3 levels illustrate this observation. This indicates that the hens receiving the treatments with the lowest vitamin D_3 levels were not as consistent in their response. Although they reduced their egg production they always managed to produce eggs with vitamin D_3 reserves sufficient to hatch. As the hens got older, egg production decreased and body ash of the progeny increased. This result reinforces the observation that the hens always try to produce an egg that could result in a good chick.

Different vitamin D_3 requirements were observed for maximum or minimum response and for peak and post peak production (Table 11). The differences in the requirement during peak and post peak production is probably related to the D_3 reserves in the body, the D_3 output in the egg, the absence of the 4,000 IU/kg level in the first period and the duration of each period (10 wk during peak production versus 30 wk during post peak production). At the beginning of the experiment, 25 wk of age, we assumed that all the hens had the same D_3 reserves in their bodies. The D_3 absorbed in the gut can be stored in adipose tissue from which it is slowly mobilized (Combs, 1992; Norman and DeLuca, 1963; Mawer et al, 1972). It appears that the estimated requirement was lower because the hens had higher D_3 reserves in their bodies during peak production than during post peak production. During the first wk of the experiment no differences were observed in egg production or hatchability because the hens were using their D_3 body reserves, but as egg production increased, a D_3 effect was observed because the D_3 was being removed by the eggs at a faster rate. Another possible reason for the lower D_3 level estimated during peak production was the absence of the 4,000 IU of vitamin D_3/kg treatment. Hens receiving the 4,000 IU/kg after 36 wk of age may have performed even better if they had not been subjected to a deficient diet during the first 11 wk of the experiment. Therefore, the estimated D_3 level for maximum performance during post peak production could have been even higher.

Higher requirements for egg production, hatchability and embryo mortality were also observed by Couch et al. (1947) in New Hampshire hens at a later age than at earlier age. In their experiment hens received sunlight during the summer and fall previous to the beginning of the experiment. Three different levels (19, 38 and 76 IU/100 g of diet) of vitamin D_3 were fed to the hens up to the first year of production

and the same three levels for the second year of production. The authors point out that the requirement appears to be higher for the second year than for the first year. They also concluded that the D_3 required for the first year was 38 to 76 IU of vitamin D-$_3$/100 g of diet and 76 IU of vitamin D_3/100 g of diet for the second year.

When broiler breeder hens are exposed to sunlight, the requirement may be lower than the one found in this study. Broiler breeder hens with egg production higher or lower than the one used in this study may also have slightly higher or lower vitamin D_3 requirements, respectively. Mattila et al. (1999) showed that vitamin D_3 in the diet is transferred to the eggs; therefore, when more eggs are being produce it is expected that the hens will have higher output of vitamin D_3 into the eggs and consequently higher vitamin D_3 requirement.

Analysis of the data from the original design of the experiment (treatment 0, 125, 250, 500, 1,000 and 2,000 D_3/kg for the 27 to 36 wk of age) indicate a requirement of approximately 1,400 IU of D_3 per kg of feed for broiler breeder hens. When the data from the modified experiment 37 to 66 wk of age includes the conversion of the 0 IU of D_3/kg treatment to the 4,000 IU of D_3/kg treatment, the requirement may be approximately 2,800 IU of D_3/kg.

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Table 1 – Composition of the experimental diet

Ingredient	%
Corn, ground	43.060
Wheat middling	30.000
Soybean meal (48.5 % CP)	16.788
Limestone	6.759
Defluorinated phosphate	1.376
Vegetable oil	1.000
Salt (NaCl)	0.400
Vitamin Premix[1]	0.250
Mineral Premix[2]	0.075
Dl-Methionine	0.237
Coccidiostat (Coban 60)[3]	0.050
Total	100
Calculated composition [4]	
Crude Protein, %	16.868
ME, kcal/kg	2489
Ca, %	3.10
Total Phosphorus, %	0.742
Non-phytate phosphorus, %	0.400

[1] Vitamin premix provides the following (per kilogram of diet): Vitamin A (trans-retinyl acetate), 5500 IU; vitamin E(all-rac-tocopherol acetate), 11 IU; riboflavin (B_2), 4.4 mg; Ca pantothenate, 12 mg; pyridoxine HCl (B_6), 4.0 mg; nicotinic acid 44 mg; folic acid 3 mg; biotin 0.3 mg, thiamine mononitrate (B_1) 2.2 mg; vitamin B_{12} (cobalamin), 0.01 mg; menadione sodium bisulfate complex, 1.1 mg; choline chloride added to meet the requirement 220 mg; ethoxyquin, 125 mg.

[2] Mineral premix provides the following (per kilogram of diet): Manganese ($MnSO_4H_2O$), 60 mg; Iron ($FeSO_47H_2O$), 30 mg; Zinc (ZnO), 50 mg; copper ($CuSO_45H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide; 1.5 mg Selenium, 0.3 mg.

[3] 66.15 mg of monensin sodium/kg

[4] NRC (1994).

Table 2 – Mean of hen day egg production (%)[1], and regression coefficients in broiler breeders from 27 to 66 wk of age, according to the vitamin D_3 levels in the diet

Age	Vitamin D_3 Levels (IU/kg of diet)						
(wk)	0	125	250	500	1,000	2,000	4,000
27-31	58.6±5.4 [15]	66.4±4.8 [15]	63.7±5.5 [15]	62.6±6.0 [15]	65.3±5.6 [15]	70.4±5.3 [15]	
32-36	20.4±3.1 [15]	39.3±3.5 [15]	71.2±1.9 [15]	78.4±1.0 [15]	74.8±1.7 [15]	76.9±2.5 [15]	
37-41		18.4±2.5 [15]	62.0±2.8 [15]	70.4±1.5 [15]	68.6±1.9 [15]	71.2±1.9 [15]	73.5±1.8 [15]
42-46		16.5±3.8 [15]	48.7±3.0 [15]	60.5±1.4 [15]	59.9±1.4 [15]	63.7±2.1 [15]	70.0±1.2 [15]
47-51		17.5±2.9 [15]	51.6±2.9 [15]	59.5±1.2 [15]	58.5±1.0 [15]	63.2±2.1 [15]	63.5±1.9 [15]
52-56		22.1±4.1 [15]	55.4±1.7 [15]	58.2±1.1 [15]	57.8±1.6 [15]	58.3±1.8 [15]	65.9±1.9 [15]
57-61		16.6±4.4 [15]	43.5±2.8 [15]	48.6±1.7 [15]	47.9±2.1 [15]	50.6±1.7 [15]	58.6±1.3 [15]
62-66		5.2±1.5 [15]	22.8±1.3 [15]	34.3±1.7 [15]	38.0±1.3 [15]	46.5±2.0 [15]	48.0±1.9 [15]
Mean	39.5±4.7 [30]	25.3±2.1 [120]	52.4±1.6 [120]	59.1±1.4 [120]	58.8±1.31 [120]	62.6±1.3 [120]	63.2±1.1 [90]

---D_3 levels (Hens age)---	---0–4,000[2] (27–66)---		---0–2,000[3] (27–36)---		---125–4,000[4] (37–66)---		---125–2,000[5] (27–66)---	
---Regression Analysis---	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-
Intercept	38.0980	0.0003	-1252.6630	0.0001	-15.076	0.5188	39.6991	0.0004
Vit D	0.0205	0.0018	-0.0279	0.2427	0.0273	0.0032	0.0473	0.0041
Vit D*vit D	-0.000004	0.0001	-0.00001	0.0004	-0.000004	0.0004	-0.00002	0.0008
Age	1.0560	0.0258	84.0927	0.0001	2.8411	0.0020	0.7770	0.1111
Age*age	-0.0220	0.0001	-1.3483	0.0001	-0.0367	0.0001	-0.0201	0.0001
Vit D*age	0.0001	0.0293	0.0023	0.0002	-0.000007	0.8821	0.0001	0.0177
R^2	0.45		0.46		0.53		0.52	
Age at maximum response	30		32		38		25	
Vit D at maximum response	2,527		1,424		2,811		1,300	
Predicted maximum	79.97		91.68		77.83		80.23	

---Period (wk)---	Pr>F	---Simple factor regression equation---	D_3 Level	R^2
27-36	0.0009	$\hat{Y}$= 39.40744330 + 0.02092364x-0.00000419x^2	1,359	0.22
37-66	0.0001	$\hat{Y}$ = 31.06301246+0.02691811x-0.00000480x^2	2,804	0.35

[1] Observed mean and observed SEM with their respective number of observations in superscripts.
[2] The analysis includes all levels of D_3.
[3] The analysis does not include hens fed 4,000 IU of vitamin D_3.
[4] The analysis does not include the data from wk 27 to 36.
[5] The analysis does not include hens fed D_3 level 0 and 4,000.
X D_3 level.
$\hat{Y}$ Hen day egg production.

Table 3 – Mean of hatchability (%)[1], and regression coefficients in broiler breeders from 27 to 66 wk of age, according to the vitamin D_3 levels in the diet

Age (wk)	Vitamin D_3 Levels (IU/kg of diet)						
	0	125	250	500	1,000	2,000	4,000
27-31	65.8±7.8 [15]	82.5±3.4 [15]	90.7±1.2 [15]	92.7±1.2 [15]	89.7±2.3 [14]	95.6±1.4 [15]	
32-36	17.7±6.7 [13]	42.1±5.7 [15]	74.6±2.5 [15]	77.5±2.1 [15]	85.4±2.0 [15]	90.3±1.5 [15]	
37-41		17.5±3.4 [15]	78.2±2.5 [15]	79.4±2.7 [15]	85.4±1.7 [15]	89.9±1.7 [15]	88.4±1.9 [15]
42-46		53.7±6.5 [12]	76.5±2.7 [7]	84.4±2.1 [15]	90.0±1.5 [15]	91.4±0.8 [15]	95.2±0.7 [15]
47-51		48.3±9.6 [7]	82.5±1.5 [15]	85.1±2.7 [7]	88.8±1.1 [8]	91.3±1.0 [8]	92.4±1.8 [7]
52-56		80.±3.5 [9]	90.1±1.3 [15]	89.1±1.4 [15]	88.1±1.0 [15]	91.9±1.4 [15]	93.1±0.7 [15]
57-61		66.4±6.5 [10]	88.4±1.8 [15]	87.2±2.2 [15]	87.2±1.6 [15]	90.0±1.1 [15]	95.3±1.0 [15]
62-66		21.3±11.8 [6]	60.0±5.1 [15]	88.3±2.4 [15]	91.3±1.6 [15]	95.1±1.4 [15]	93.5±1.2 [15]
Mean	43.5±6.9 [28]	55.4±3.2 [89]	79.9±1.3 [112]	85.6±0.9 [112]	88.2±0.6 [112]	92.0±0.5 [113]	93.1±0.6 [82]

---D_3 levels (Hens age)---	---0 - 4,000[2] (27 – 66)---		---0 - 2,000[3] (27 – 36)---		---125 - 4,000[4] (37 – 66)---		---125 - 2,000[5] (27 – 66)---	
---Regression Analysis---	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-
Intercept	79.3391	0.0001	431.3432	0.0177	-123.8833	0.0001	83.9355	0.0001
Vit D	0.0293	0.0001	-0.0226	0.3422	0.030	0.0001	0.0522	0.0001
Vit D*vit D	-0.00005	0.0001	-0.00002	0.0001	-0.000004	0.0001	-0.00001	0.0001
Age	-0.8886	0.1275	-18.5348	0.1081	7.1436	0.0001	-1.1598	0.0473
Age*age	0.0113	0.0730	0.2061	0.2583	-0.0655	0.0001	0.0128	0.0395
Vit D*age	-0.00004	0.4058	0.0028	0.0001	-0.00009	0.0914	-0.000008	0.9254
R^2	.28		0.55		0.38		0.29	
Age at Maximum response	SP[6]		SP		52		SP	
Vit D at maximum response	SP		SP		2,708		SP	
Predicted maximum	SP		SP		105		SP	

---Period (wk)---	Pr>F	---Simple factor regression equation---	D_3 level	R^2
27-36	0.0001	$\hat{Y}=54.39250075+0.06566813x-0.00002363x^2$	1,390	0.33
37-66	0.0001	$\hat{Y}=1.51056530+0.02478978x-0.00000457x^2$	2,712	0.27

[1] Observed mean and observed SEM with their respective number of observations in superscripts.
[2] The analysis includes all levels of D_3.
[3] The analysis does not include hens fed 4,000 IU of vitamin D_3.
[4] The analysis does not include the data from wk 27 to 36.
[5] The analysis does not include hens fed D_3 level 0 and 4,000.

[6] SP (Saddle Point): Optimum not found.
X D_3 level.
$\hat{Y}$ Hatchability.

Table 4 – Mean of early embryo mortality (%)[1], and regression coefficients in broiler breeders from 27 to 66 wk of age, according to the vitamin D_3 levels in the diet

Age	Vitamin D_3 Levels (IU/kg of diet)						
(wk)	0	125	250	500	1,000	2,000	4,000
27-31	2.38±0.62 [15†]	2.68±0.58 [15]	1.99±0.60 [15]	1.78±0.41 [15]	0.89±0.35 [15]	1.6±0.56 [15]	
32-36	23.58±5.31 [14]	13.02±2.66 [15]	6.96±1.61 [15]	4.35±0.85 [15]	4.06±0.6 [15]	4.24±0.74 [15]	
37-41		14.52±3.06 [15]	5.78±0.69 [15]	4.98±0.93 [15]	7.26±1.27 [15]	4.50±0.62 [15]	6.18±0.91 [15]
42-46		7.81±1.93 [13]	5.35±0.84 [15]	5.00±0.90 [15]	7.85±1.38 [15]	6.98±1.79 [15]	4.18±0.51 [15]
47-51		4.21±1.68 [7]	5.83±1.61 [7]	3.72±1.12 [7]	7.81±1.75 [8]	6.51±1.91 [8]	3.72±0.89 [7]
52-56		6.18±2.30 [14]	4.72±0.68 [15]	3.79±0.52 [15]	5.87±0.49 [15]	5.51±0.94 [15]	3.88±0.77 [15]
57-61		2.50±1.17 [12]	2.92±0.64 [15]	4.58±0.65 [15]	7.60±1.24 [15]	8.6±2.20 [15]	4.32±0.61 [15]
62-66		8.73±4.04 [10]	5.57±1.39 [15]	9.71±3.07 [15]	10.93±1.15 [15]	10.72±1.75 [15]	6.22±1.19 [15]
Mean	12.62±3.23 [29]	7.80±0.93 [101]	4.82±0.38 [112]	4.81±0.51 [112]	6.45±0.45[113]	6.06±0.55 [113]	4.85±0.36 [82]

---D_3 levels (Hens age)---	---0-4,000[2] (27–66)---		---0-2,000[3] (27–36)---		---125-4,000[4] (37–66)---		---125-2,000[5] (27–66)---	
----Regression Analysis---	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-
Intercept	-3.6339	0.4402	-54.3329	0.4812	47.1733	0.0001	-0.6254	0.8927
Vit D	-0.0035	0.0530	0.0143	0.1849	-0.00007	0.9756	-0.0079	0.0601
Vit D*vit D	-.00000001	0.9699	0.000006	0.0026	-0.0000004	0.1455	0.0000007	0.5594
Age	0.4807	0.0253	2.2724	0.6437	-1.6307	0.0001	0.3428	0.0918
Age*age	-0.0051	0.0279	-0.0068	0.9294	0.0155	0.0001	-0.0038	0.0739
Vit D*age	0.00005	0.0063	-0.0009	0.0010	0.00003	0.1104	0.0001	0.0001
R^2	0.03		0.32		0.05		0.06	
Age at minimum response	SP		SP		SP		SP	
Vit D at minimum response	SP[6]		SP		SP		SP	
Predicted minimum	SP		SP		SP		SP	
---Period (wk)---	Pr>F		---Simple factor regression equation---				D_3 level	R^2
27-36	0.0095		$\hat{Y}=10.26706470-0.01566331x+0.00000608x^2$				1,288	0.13

[1] Observed mean and observed SEM with their respective number of observations in superscripts.
[2] The analysis includes all levels of D_3.
[3] The analysis does not include hens fed 4,000 IU of vitamin D_3.
[4] The analysis does not include the data from wk 27 to 36.
[5] The analysis does not include hens fed D_3 level 0 and 4,000.
[6] SP (Saddle Point): Optimum not found.
X D_3 level.
$\hat{Y}$ Early embryo mortality.

Table 5 – Mean of middle embryo mortality (%)[1], and regression coefficients in broiler breeders from 27 to 66 wk of age, according to the vitamin D_3 levels in the diet

Age	Vitamin D_3 Levels (IU/kg of diet)						
(wk)	0	125	250	500	1,000	2,000	4,000
27-31	3.97±1.11 [15†]	1.84±0.45 [15]	1.67±0.66 [15]	0.80±0.28 [15]	2.59±0.84 [14]	1.30±0.51 [15]	
32-36	25.22±4.59 [9]	16.56±3.99 [14]	6.81±1.37 [15]	2.92±0.54 [15]	2.93±0.52 [15]	1.01±0.33 [15]	
37-41		22.17±4.39 [14]	3.80±0.83 [15]	1.20±0.30 [15]	0.91±0.25 [15]	0.71±0.23 [15]	0.84±0.30 [15]
42-46		7.79±2.91 [12]	2.54±0.69 [15]	1.14±0.41 [15]	0.61±0.23 [15]	0.82±0.25 [15]	0.28±0.15 [15]
47-51		5.27±2.38 [6]	2.98±0.92 [7]	0.98±0.35 [7]	0.52±0.34 [8]	0.83±0.55 [8]	0.20±0.20 [7]
52-56		4.83±2.40 [14]	1.08±0.36 [15]	1.72±0.56 [15]	1.84±0.48 [15]	1.41±0.41 [15]	1.40±0.31 [15]
57-61		2.67±0.93 [12]	0.67±0.40 [15]	1.55±0.43 [15]	0.76±0.30 [15]	1.23±0.35 [15]	0.67±0.27 [15]
62-66		4.29±2.02 [7]	1.64±0.77 [15]	1.20±0.57 [15]	1.15±0.40 [15]	0.14±0.14 [15]	0.84±0.41 [15]
Mean	11.94±2.80 [24]	8.77±1.27 [94]	2.63±0.33 [112]	1.47±0.17 [112]	1.46±0.18 [112]	0.94±0.13 [113]	0.75±0.13 [82]

---D_3 levels (Hens age)---	---0-4,000[2] (27–66)---		---0-2,000[3] (27–36)---		---125-4,000[4] (37 – 66)---		---125-2,000[5] (27–66)---	
---Regression Analysis---	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-
Intercept	1.2249	0.8447	110.8710	0.2744	47.3976	0.0003	3.6989	0.5448
Vit D	-0.0109	0.0003	0.0409	0.0052	-0.0100	0.0038	-0.0180	0.0080
Vit D*vit D	0.000001	0.0034	0.000009	0.0002	0.000001	0.0131	0.000005	0.0156
Age	0.5089	0.0747	-9.3189	0.1498	-1.4003	0.0073	0.3885	0.1473
Age*age	-0.0073	0.0188	0.1972	0.0549	0.0113	0.0244	-0.0055	0.0534
Vit D*age	0.00007	0.0100	-0.0021	0.0001	0.00008	0.0034	0.00006	0.0956
R^2	0.13		0.44		0.13		0.13	
Age at minimum response	SP[6]		29		52		SP	
Vit D at minimum response	SP		1,130		2,568		SP	
Predicted minimum	SP		-4.38		-2.09		SP	

---Period (wk)---	Pr>F	---Simple factor regression equation---	D_3 Level	R^2
27-36	0.0010	$\hat{Y}=14.37193470-0.02511071x+0.00000944x^2$	1,330	0.17
37-66	0.0001	$\hat{Y}=6.468229607-0.005953437x+0.000001153x^2$	2,583	0.08

[1] Observed mean and observed SEM with their respective number of observations in superscripts.
[2] The analysis includes all levels of D_3.
[3] The analysis does not include hens fed 4,000 IU of vitamin D_3.
[4] The analysis does not include the data from wk 27 to 36.
[5] The analysis does not include hens fed D_3 level 0 and 4,000.

[6] SP (Saddle Point): Optimum not found.
X D_3 level.
$\hat{Y}$ Middle embryo mortality.

Table 6 – Mean of late embryo mortality (%)[1], and regression coefficients in broiler breeders from 27 to 66 wk of age, according to the vitamin D_3 levels in the diet

Age (wk)	Vitamin D_3 Levels (IU/kg of diet)						
	0	125	250	500	1,000	2,000	4,000
27-31	24.9±7.16 [15]	10.9±3.4 [15]	5.4±0.9 [15]	3.3±1.0 [15]	5.5±1.5 [14]	2.5±0.8 [15]	
32-36	38.9±7.79 [13]	34.0±4.2 [15]	11.6±1.4 [15]	14.4v1.6 [15]	6.4±1.2 [15]	4.2±0.8 [15]	
37-41		55.1±3.5 [15]	14.1±1.7 [15]	16.3±2.2 [15]	9.8±1.3 [15]	7.5±1.4 [15]	7.2±1.2 [15]
42-46		38.2±7.1 [12]	17.8±2.6 [15]	12.0±1.6 [15]	7.1±1.3 [15]	6.1±0.8 [15]	3.1±0.4 [15]
47-51		28. ±57.7 [7]	10.6±1.1 [7]	11.3±2.4 [7]	8.2±0.9 [8]	6.0±0.7 [8]	6.2±1.5 [7]
52-56		6.7±12.0 [14]	6.8±1.2 [15]	7.9±1.2 [15]	7.5±0.8 [15]	5.1±0.9 [15]	3.9±0.6 [15]
57-61		23.1±6.5 [12]	9.8±1.9 [15]	9.9±2.0 [15]	10.3±1.3 [15]	7.1±1.1 [15]	2.5±0.7 [15]
62-66		53.8±13.4 [8]	35.3±5.5 [15]	8.4±2.1 [15]	5.7±1.2 [15]	2.9±0.9 [15]	3.6±0.7 [15]
Mean	31.9±7.5 [28]	31.3±5.9 [98]	13.9±2.0 [112]	10.4±1.7 [112]	7.5±1.2 [112]	5.2±0.9 [113]	4.4±0.8 [82]

---D_3 levels (Hens age)---	---0-4,000[2] (27–66)---		---0-2,000[3] (27–36)---		---125-4,000[4] (37–66)---		---125-2,000[5] (27–66)---	
---Regression Analysis---	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-
Intercept	6.7083	0.4966	-502.0145	0.0005	164.6576	0.0001	1.4355	0.8926
Vit D	-0.0169	0.0001	-0.0024	0.8253	-0.0197	0.0003	-0.0326	0.0003
Vit D*vit D	0.00003	0.0001	0.00001	0.0001	0.000003	0.0001	0.00001	0.0001
Age	0.7314	0.1044	31.3153	0.0006	-5.3913	0.0001	1.0508	0.0245
Age*age	-0.0071	0.1433	-0.4580	0.0015	0.0504	0.0005	-0.0096	0.0530
Vit D*age	-0.00003	0.4916	-0.0011	0.0259	0.00003	0.5115	-0.00007	0.2711
R^2	0.22		0.39		0.30		0.24	
Age at minimum response	SP[6]		SP		52		SP	
Vit D at minimum response	SP		SP		2756		SP	
Predicted minimum	SP		SP		-4.14		SP	

---Period (wk) ---	Pr>F	Simple factor regression equation	D_3 level	R^2
27-36	0.0020	$\hat{Y}=25.77461510-0.03808949x+0.00001367x^2$	1,393	0.26
37-66	0.0001	$\hat{Y}=24.96169925-0.01816007x+0.00000329x^2$	2,759	0.24

[1] Observed mean and observed SEM with their respective number of observations in superscripts.
[2] The analysis includes all levels of D_3.
[3] The analysis does not include hens fed 4,000 IU of vitamin D_3.
[4] The analysis does not include the data from wk 27 to 36.
[5] The analysis does not include hens fed D_3 level 0 and 4,000.
[6] SP (Saddle Point): Optimum not found.

X D_3 level.
$\hat{Y}$ Late embryo mortality.

Table 7 – Mean of body weight of the progeny (g)[1], and regression coefficients in broiler breeders from 27 to 66 wk of age, according to the vitamin D_3 levels in the diet

Age	Vitamin D_3 Levels (IU/kg of diet)						
(wk)	0	125	250	500	1,000	2,000	4,000
27-31	37.53±0.57 [14]	36.08±0.40 [15]	36.88±0.42 [15]	37.15±0.52 [15]	38.00±0.51 [14]	37.21±0.44 [15]	
32-36	40.65±0.68 [8]	40.07±0.69 [15]	40.21±0.43 [15]	40.36±0.30 [15]	40.93±1.07 [15]	42.15±1.31 [15]	
37-41		41.11±0.64 [12]	41.64±0.27 [15]	42.14±0.25 [15]	41.56±0.33 [15]	41.74±0.30 [15]	41.94±0.33 [15]
42-46		43.56±0.83 [12]	44.16±0.53 [15]	43.62±0.18 [15]	43.56±0.31 [15]	43.55±0.25 [15]	43.94±0.15 [15]
47-51		44.78±1.64 [6]	43.69±0.65 [7]	43.23±0.70 [15]	43.25±0.68 [8]	43.37±0.50 [8]	43.79±0.70 [8]
52-56		43.80±0.40 [14]	44.27±0.35 [15]	44.07±0.21 [7]	44.33±0.34 [15]	44.46±0.50 [15]	44.49±0.26 [15]
57-61		44.88±0.47 [12]	44.13±0.71 [15]	44.48±0.37 [15]	44.74±0.17 [15]	44.95±0.41 [15]	44.03±0.33 [15]
62-66		41.78±1.15 [6]	44.31±0.44 [15]	44.63±0.43 [15]	44.25±0.38 [15]	43.38±0.44 [15]	43.48±0.24 [15]
Mean	38.66±0.54 [22]	41.63±0.39 [92]	42.32±0.30 [112]	42.40±0.27 [112]	42.58±0.28 [112]	42.55±0.31 [113]	43.60±0.15 [83]

---D_3 levels (Hens age) ---	---0-4,000[2] (27–66) ---		---0-2,000[2] (27–36)---		---125 - 4,000[3] (37–66) ---		---125-2,000[4] (27–66) ---	
---Regression Analysis---	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-
Intercept	12.9367	0.0001	-28.2822	0.2868	17.4507	0.0001	11.8787	0.0001
Vit D	0.0008	0.2985	-0.0004	0.9035	0.0004	0.6938	0.0018	0.3475
Vit D*Vit D	-.00000007	0.5958	-0.00000003	0.9628	-.00000002	0.9098	-0.0000003	0.6259
Age	1.1350	0.0001	3.4570	0.0288	0.9545	0.0001	1.1643	0.0001
Age*age	-0.0102	0.0001	-0.0440	0.0775	-0.0084	0.0001	-0.0104	0.0001
Vit D*age	-0.00001	0.0925	0.00003	0.6956	-0.000006	0.2861	-0.00001	0.0578
R^2	0.63		0.45		0.24		0.62	
Age at maximum response	54		48		56		54	
Vit D at maximum response	1,880		2191		1038		1168	
Predicted maximum	44		51		44		44	

---Period (wk) ---	Pr>F	---Simple factor regression equation---	D_3 level	R^2
27-36	0.0624L	$\hat{Y}$= 38.87	-	-
37-66	0.2780Q	$\hat{Y}$=43.61	-	-

[1] Observed mean and observed SEM with their respective number of observations in superscripts.
[2] The analysis includes all levels of D_3.
[3] The analysis does not include hens fed 4,000 IU of vitamin D_3.
[4] The analysis does not include the data from wk 27 to 36.
[5] The analysis does not include hens fed D_3 level 0 and 4,000.
X D_3 level.

Ŷ Body weight.
L Linear effect.
Q Quadratic effect.

Table 8 – Mean of egg weight (g)[1], and regression coefficient in broiler breeders from 27 to 66 wk of age, according to the vitamin D_3 levels in the diet

Age	Vitamin D_3 Levels (IU/kg of diet)						
(wk)	0	125	250	500	1,000	2,000	4,000
27-31	57.84±0.66 [41]	57.29±0.67 [41]	59.25±0.59 [50]	59.36±0.50 [57]	58.36±0.58 [52]	57.76±0.66 [50]	
32-36	54.40±1.77 [8]	58.24±0.80 [42]	60.64±0.52 [83]	62.28±0.46 [90]	61.53±0.48 [81]	60.95±0.54 [93]	
37-41		58.77±0.85 [18]	64.80±0.63 [65]	65.65±0.54 [75]	64.45±0.68 [77]	63.87±0.56 [88]	63.02±0.51 [70]
42-46		63.83±0.90 [28]	65.35±0.51 [75]	66.55±0.51 [101]	66.19±0.52 [109]	65.37±0.43 [116]	65.67±0.39 [103]
47-51		65.91±1.39 [16]	67.59±0.69 [57]	68.42±0.64 [66]	67.88±0.66 [67]	67.21±0.52 [71]	67.64±0.60 [54]
52-56		66.75±0.86 [32]	69.32±0.58 [78]	69.02±0.51 [95]	69.21±0.50 [90]	69.41±0.48 [100]	68.91±0.44 [94]
57-61		67.71±1.62 [17]	69.25±0.67 [51]	69.91±0.83 [47]	69.52±0.87 [50]	69.58±0.87 [45]	68.80±0.56 [55]
62-66		67.67±2.62 [6]	65.12±1.64 [17]	69.68±0.91 [22]	69.07±0.89 [43]	68.30±0. 68 [47]	67.90±0.66 [51]
Mean	57.28±0.65 [49]	61.94±0.46 [200]	65.14±0.28 [476]	66.05±0.25 [553]	65.76±0.26 [569]	65.27±0.25 [610]	66.87±0.23 [427]

---D_3 levels (Hens age) ---	---0-4,000[2] (27–66) ---		---0-2,000[3] (27–36) ---		---125-4,000[4] (37–66) ---		---125-2,000[5] (27–66) ---	
---Regression Analysis---	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-
Intercept	15.8607	0.0001	411.5262	0.1350	12.2299	0.0486	17.6770	0.0001
Vit D	0.0016	0.1645	-0.0071	0.3795	0.0012	0.4090	0.0043	0.1297
Vit D*Vit D	-0.0000004	0.0255	-0.000003	0.0003	-0.0000003	0.0955	-0.000002	0.0315
Age	1.8003	0.0001	-21.7939	0.1928	1.9508	0.0001	1.7166	0.0001
Age*age	-0.0155	0.0001	0.3337	0.1894	-0.0170	0.0001	-0.0149	0.0001
Vit D*age	0.000004	0.6963	0.0004	0.1059	0.000006	0.6254	0.00001	0.4110
R^2	0.68		0.41		0.43		0.69	
Age at maximum response	58		SP[6]		57		58	
Vit D at maximum response	2,261		SP		2,289		1,268	
Predicted maximum	70		SP		69		70	

---Period (wk) ---	Pr>F	---Simple factor regression equation---	D_3 level	R^2
27-36	0.0008	$\hat{Y}= 56.93344229+0.00858217x-0.00000363x^2$	1,182	0.28

[1] Observed mean and observed SEM with their respective number of eggs in superscripts.
[2] The analysis includes all levels of D_3.
[3] The analysis does not include hens fed 4,000 IU of vitamin D_3.
[4] The analysis does not include the data from wk 27 to 36.
[5] The analysis does not include hens fed D_3 level 0 and 4,000.
[6] SP (Saddle Point): Optimum not found.
X D_3 level.
$\hat{Y}$ Egg weight.

Table 9 – Mean of specific gravity of eggs[1], and regression coefficients in broiler breeders from 27 to 66 wk of age, according to the vitamin D_3 levels in the diet

Age	Vitamin D_3 Levels (IU/kg of diet)						
(wk)	0	125	250	500	1,000	2,000	4,000
27-31	1.08268±.0009 [41]	1.08354±.0009 [41]	1.08620±.0006 [50]	1.08632±.0007 [57]	1.08529±.0005 [52]	1.08390±.0008 [50]	
32-36	1.07375±.0024 [8]	1.07607±.0011 [42]	1.08127±.0007 [83]	1.08400±.0005 [90]	1.08191±.0005 [81]	1.08258±.0005 [93]	
37-41		1.07056±.0015 [18]	1.07923±.0007 [65]	1.08273±.0005 [75]	1.08091±.0005 [77]	1.08085±.0006 [88]	1.08420±.0006 [69]
42-46		1.07643±.0011 [28]	1.08013±.0007 [75]	1.08238±.0005 [101]	1.08078±.0004 [109]	1.08116±.0005 [116]	1.08325±.0004 [103]
47-51		1.07906±.0018 [16]	1.08114±.0007 [57]	1.08265±.0006 [66]	1.07993±.0007 [67]	1.08092±.0006 [71]	1.08093±.0006 [54]
52-56		1.07953±.0012 [32]	1.08122±.0007 [78]	1.08258±.0005 [95]	1.07967±.0005 [90]	1.08055±.0005 [100]	1.08181±.0005 [94]
57-61		1.07676±.0017 [17]	1.08000±.0007 [51]	1.07936±.0007 [47]	1.07840±.0007 [50]	1.07989±.0006 [45]	1.08109±.0007 [55]
62-66		1.07250±.0036 [6]	1.07765±.0014 [17]	1.07977±.0010 [22]	1.07953±.0009 [43]	1.07989±.0007 [47]	1.08137±.0007 [51]
Mean	1.0782±.0009 [49]	1.0779±.0005 [216]	1.0810±.0003 [479]	1.0828±.0002 [554]	1.0808±.0002 [569]	1.0812±.0002 [610]	1.0823±.0002 [427]

---D_3 levels (Hens age) ---	---0–4,000[2] (27–66) ---		---0–2,000[3] (27–36) ---		---125–4,000[4] (37–66) ---		---125–2,000[5] (27–66) ---	
---Regression Analysis---	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-	-Coefficient-	-Pr>|t|-
Intercept	1.0827	0.0001	1.6899	0.0001	1.0548	0.0001	1.0832	0.0001
Vit D	0.000003	0.0315	-0.00001	0.2429	0.000002	0.1403	0.000007	0.0306
Vit D*vit D	-0.0000	0.0616	-0.000000006	0.0011	-0.0000	0.2616	-.0000	0.0121
Age	-0.0001	0.6207	-0.0353	0.1385	0.0009	0.0029	-0.0001	0.5128
Age*age	0.0000002	0.8945	0.0005	0.1607	-0.000009	0.0019	0.0000003	0.8647
Vit D*age	-.00000001	0.5147	0.0000009	0.0278	-.00000001	0.3707	.000000001	0.9654
R^2	0.14		0.55		0.16		0.17	
Age at maximum response	SP[6]		SP		46		SP	
Vit D at maximum response	SP		SP		3,680		SP	
Predicted maximum	SP		SP		1.0829		SP	

---Period (wk) ---	Pr>F	---Simple factor regression equation---	D_3 Level	R^2
27-36	0.0040	$\hat{Y}= 1.077447738+0.000013367x-0.000000005x^2$	1,337	0.28
37-66	0.0001	$\hat{Y}=1.079043787+0.000000798x$	>2,000	0.10

[1] Observed mean and observed SEM with their respective number of eggs in superscripts.
[2] The analysis includes all levels of D_3.
[3] The analysis does not include hens fed 4,000 IU of vitamin D_3.
[4] The analysis does not include the data from wk 27 to 36.
[5] The analysis does not include hens fed D_3 level 0 and 4,000.
[6] SP (Saddle Point): Optimum not found.

X D_3 level.
$\hat{Y}$ Specific gravity.

Table 10 – Mean of body ash progeny (mg)[1], and regression coefficients in one day old chick, according to the vitamin D_3 levels in the maternal diet

Age (wk)	Vitamin D_3 Levels (IU/kg of diet)					
	125	250	500	1,000	2,000	4,000
42	649 ± 49^{3}	710 ± 33^{5}	794 ± 15^{5}	798 ± 21^{5}	839 ± 26^{6}	821 ± 10^{5}
58	686 ± 33^{4}	793 ± 14^{6}	774 ± 24^{5}	776 ± 18^{6}	795 ± 13^{6}	829 ± 14^{6}
65	809 ± 21^{4}	840 ± 15^{6}	825 ± 22^{6}	785 ± 27^{6}	811 ± 13^{6}	846 ± 18^{6}
Mean	721 ± 28^{11}	785 ± 17^{17}	799 ± 12^{16}	786 ± 12^{17}	815 ± 11^{18}	833 ± 8^{17}

---Regression Analysis---	-Coefficient-	-Pr>|t|-
Intercept	1240.227712	0.0009
Vit D	0.101955	0.0016
Vit D*vit D	-0.000007	0.0570
Age	-22.369230	0.1045
Age*age	0.238445	0.0671
Vit D*age	-0.000970	0.0584
R^2	0.38	
Age at Maximum response	SP[2]	
Vit D at maximum response	SP	
Predicted maximum	SP	

---Period (wk) ---	Pr>F	---Simple factor regression equation---	D_3 level	R^2
42-65	0.0001	$\hat{Y}=768.7535079+0.0181072x$	>2,000	0.19
42	0.0002L	$\hat{Y}=737.6427279 + 0.0297053x$	>2,000	0.31
58	0.0014 L	$\hat{Y}=753.0807789 + 0.0200905x$	>2,000	0.33
65	0.6345 L	$\hat{Y}=820$	-	-

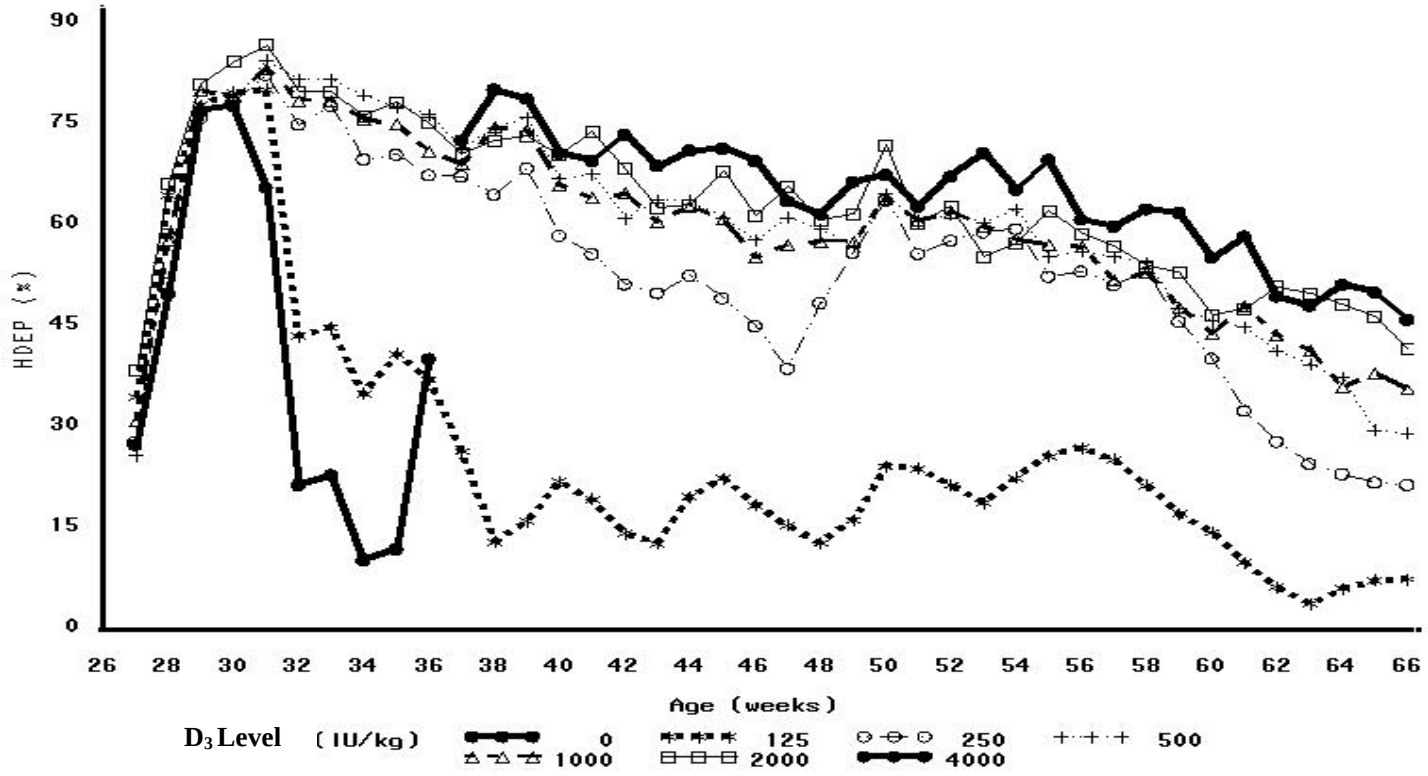
[1] Observed mean and observed SEM with their respective number of chicks in superscripts.
[2] SP (Saddle Point): Optimum not found.
X D_3 level.
$\hat{Y}$ Body ash of the progeny.
L Linear effect.

Table 11 - Estimated vitamin D_3 requirements for the different characteristics

Characteristic (%)	Vitamin D_3 requirements IU/kg of diet	
---Hens age---	---26 to 36 wk---	---37 to 66 wk---
---D_3 levels---	---0-2,000---	125-4,000---
Hen day egg production	1,424 [1]	2,804 [2]
Hatchability	1,390 [2]	2,708 [1]
Early mortality	1,288 [2]	◊
Middle mortality	1,130 [1]	2,568 [1]
Late mortality	1,393 [2]	2,759 [2]
Egg weight	1,182 [2]	2,269 [2]
Specific gravity	1,337 [2]	>2,000 [3]
Progeny whole body ash	○	>2,000 [3]

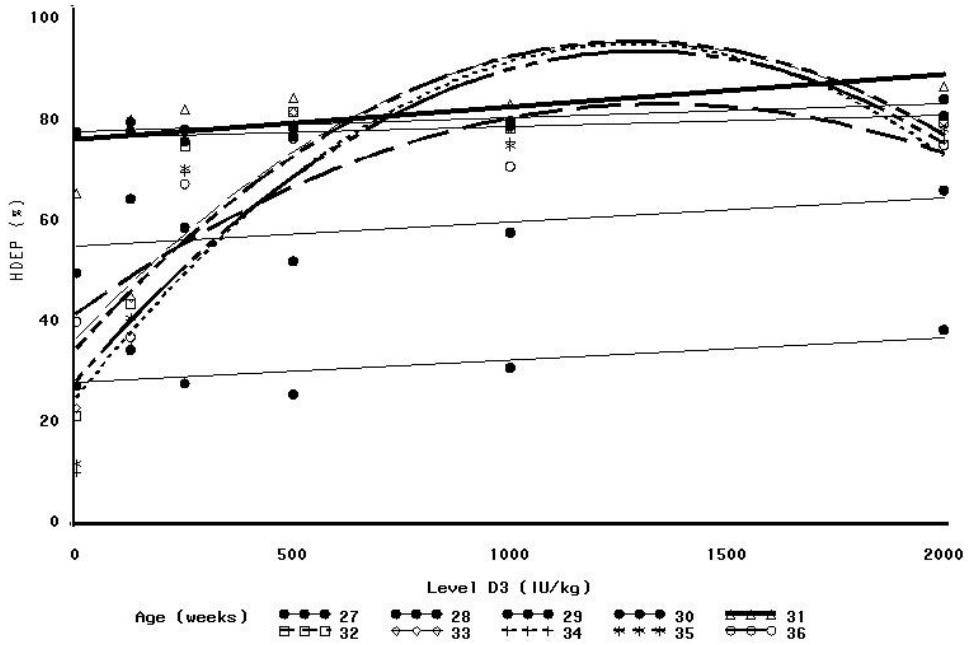
[1] Multiple regression techniques.
[2] 2nd order regression.
[3] 1st order regression.
◊ All levels were adequate.
○ No samples were collected at these ages.

Figure 1 – Effect of D_3 level on hen day egg production (HDEP) of broiler breeders from 27 to 66 wk of age



Every symbol represents the mean of 3 repetitions of the same treatment

Figure 2 – Effect of D_3 level on hen day egg production (HDEP) of broiler breeders for weeks 27 to 36 of age



31 wk: first week that an effect of D_3 on HDEP was observed.

Figure 3 – Contour plot of D_3 by age on hen day egg production (HDEP) during peak production

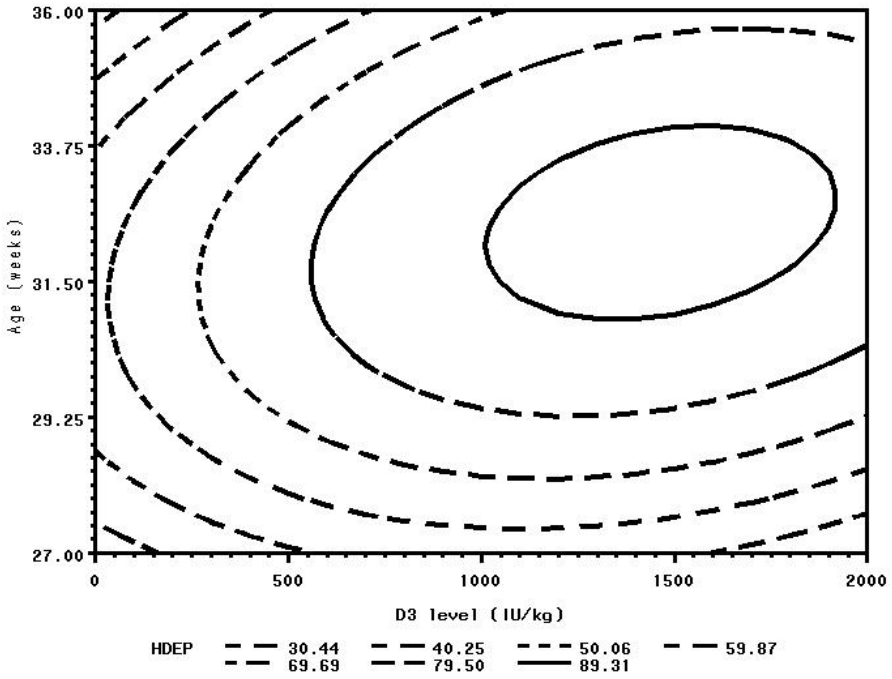


Figure 4 – Response surface of D_3 by age on hen day egg production (HDEP) from 27 to 36 wk of age.

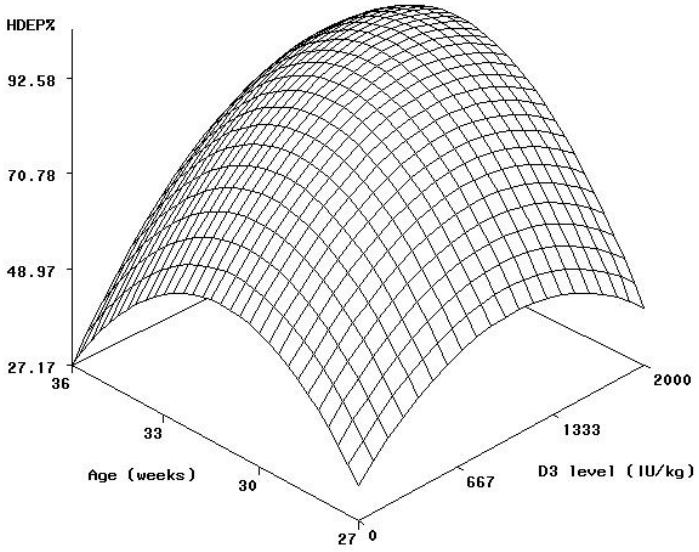
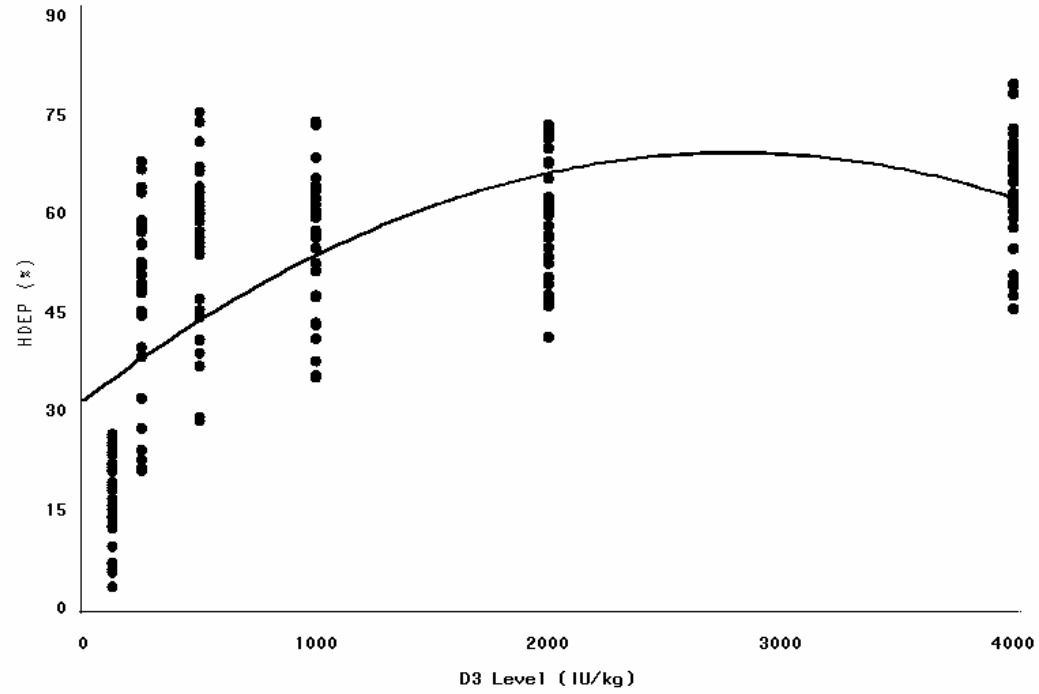


Figure 5 – Effect of D_3 level on hen day egg production (HDEP) of broiler breeders during post peak production



● Represent the mean of 3 repetitions of the same treatment

Figure 6 – Effect of D_3 levels on hen day egg production (HDEP) of broiler breeders at 34, 36, 40 and 50 wk of age

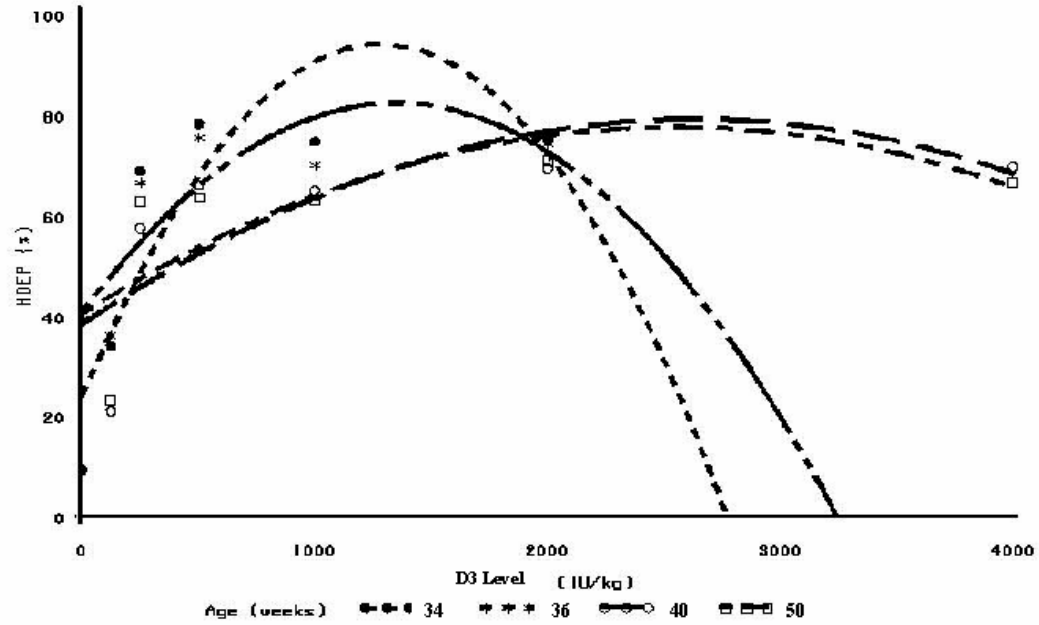
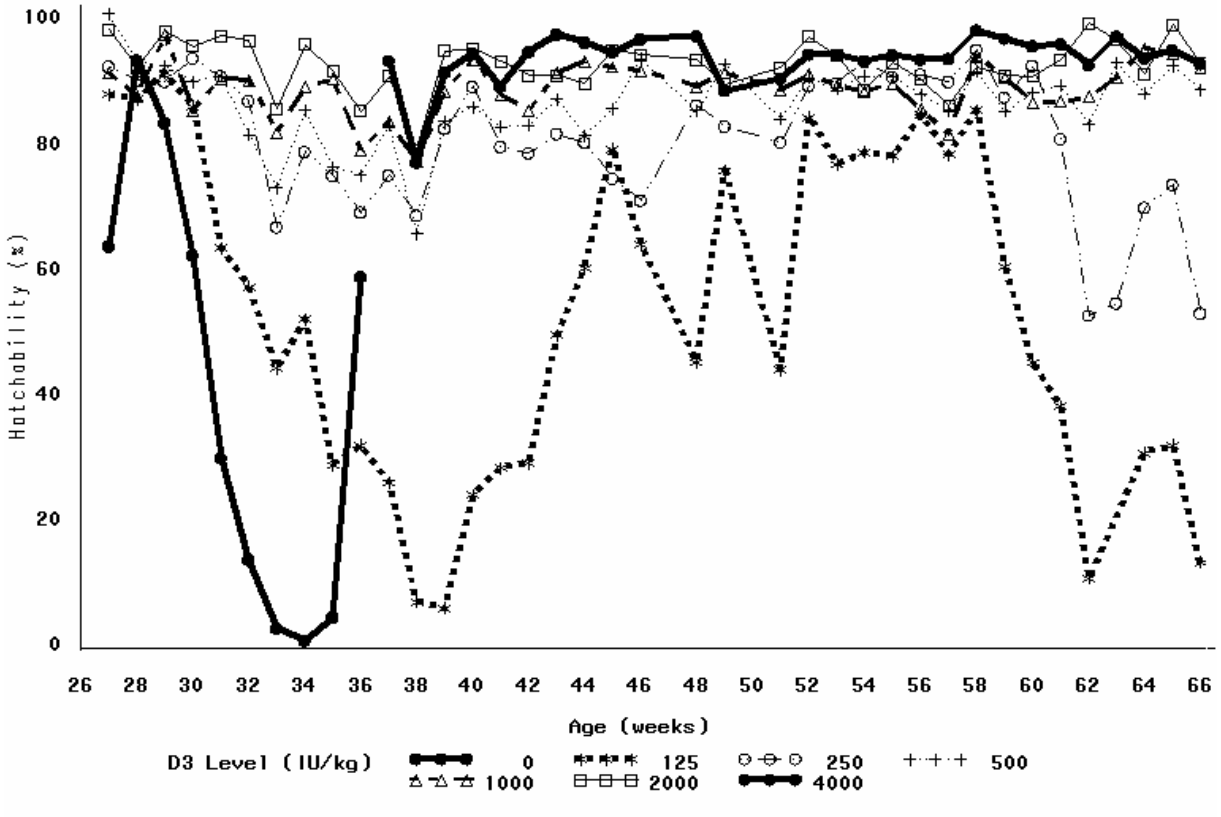
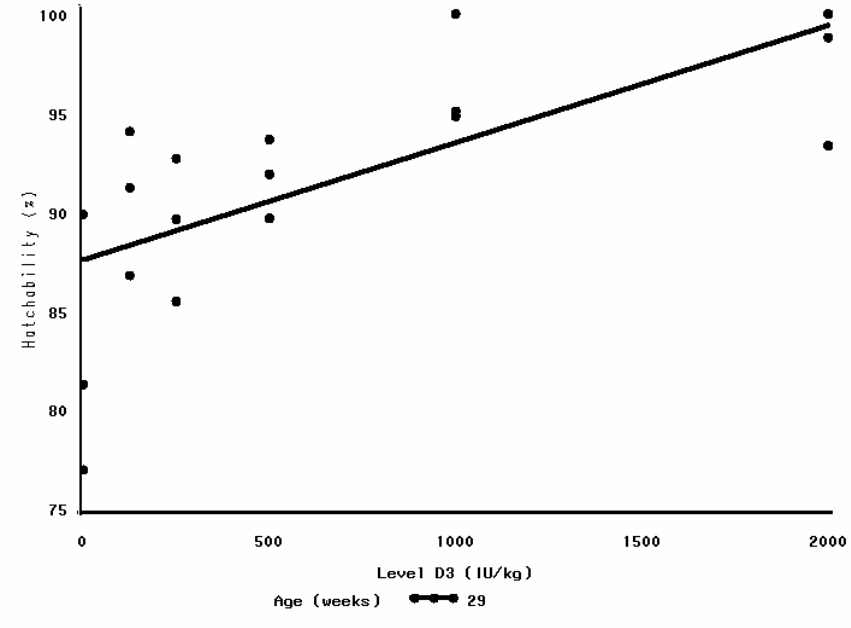


Figure 7 – Effect of D_3 levels on hatchability of broiler breeders from 27 to 66 wk of age



Every symbol represents the mean of 3 repetitions of the same treatment

Figure 8 – Effect of D_3 levels on hatchability of broiler breeders at 29 wk of age.



● Represent the observation of every repetition

Figure 9 – Contour plot of D_3 by age on hatchability during peak production

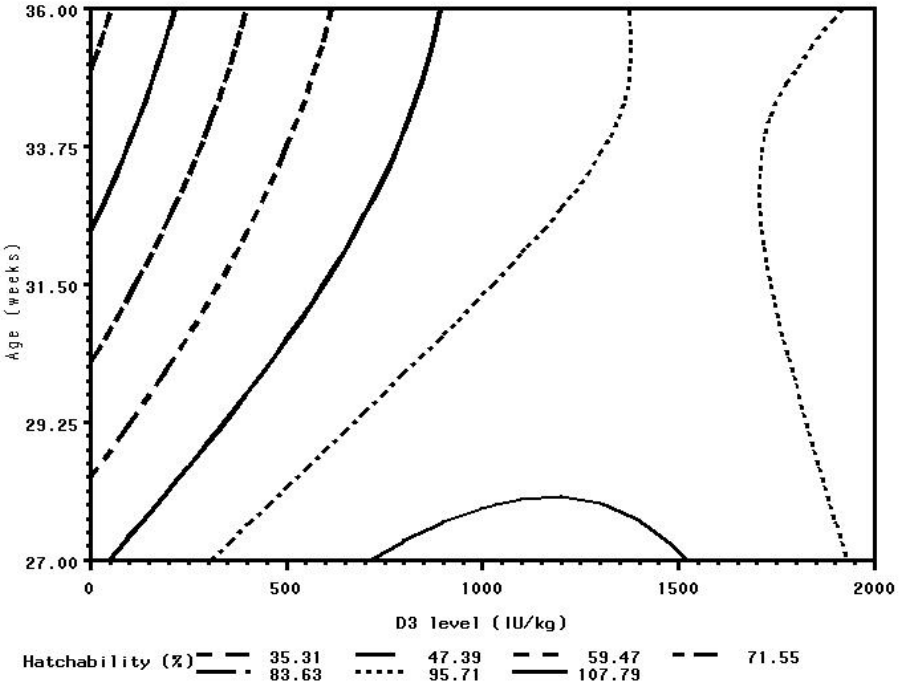


Figure 10 – Response surface of D_3 by age on hatchability during peak production

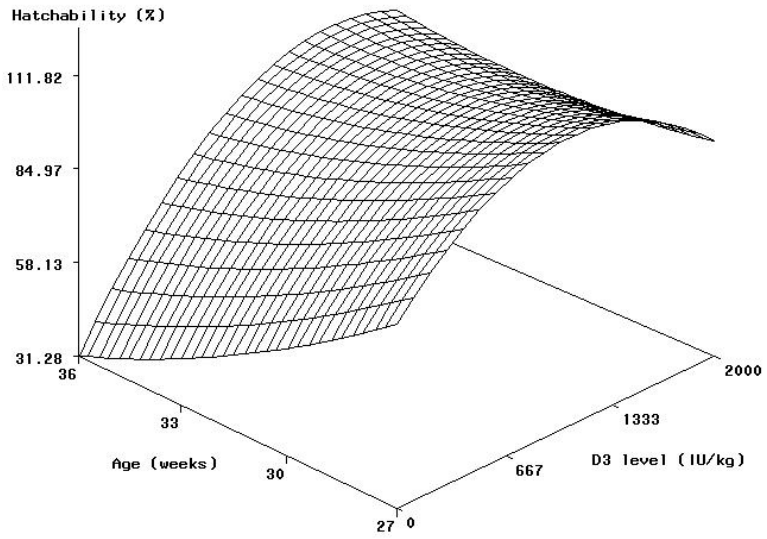
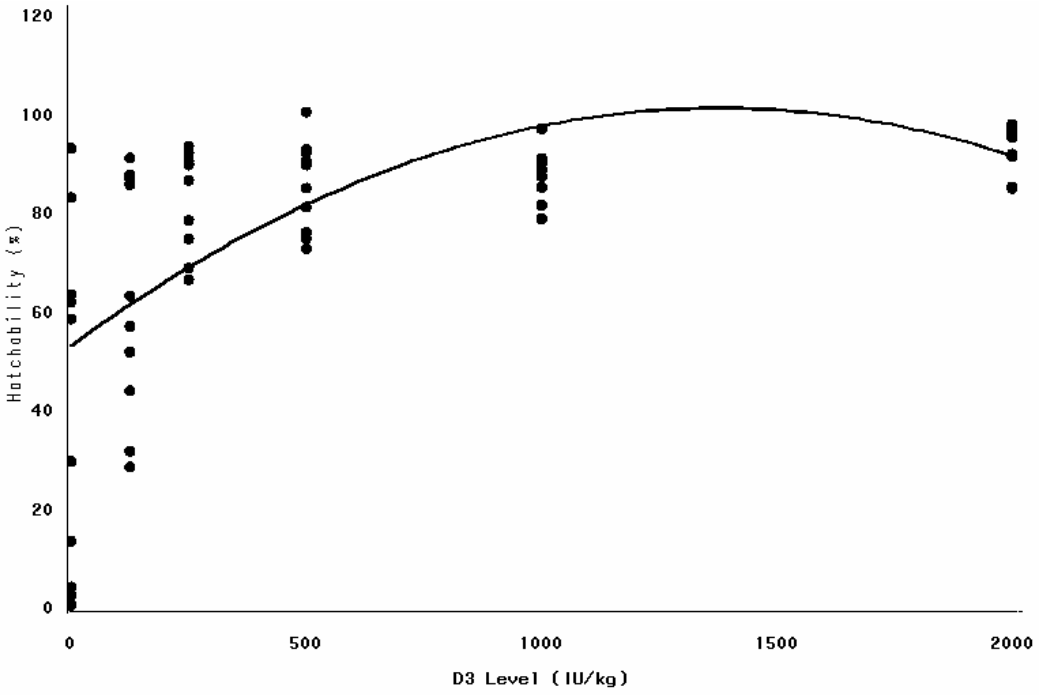


Figure 11 – Effect of D_3 levels on Hatchability of broiler breeders during peak production



● Represent the mean of 3 repetitions of the same treatment

Figure 12 – Contour plot of D_3 by age on hatchability during post peak production

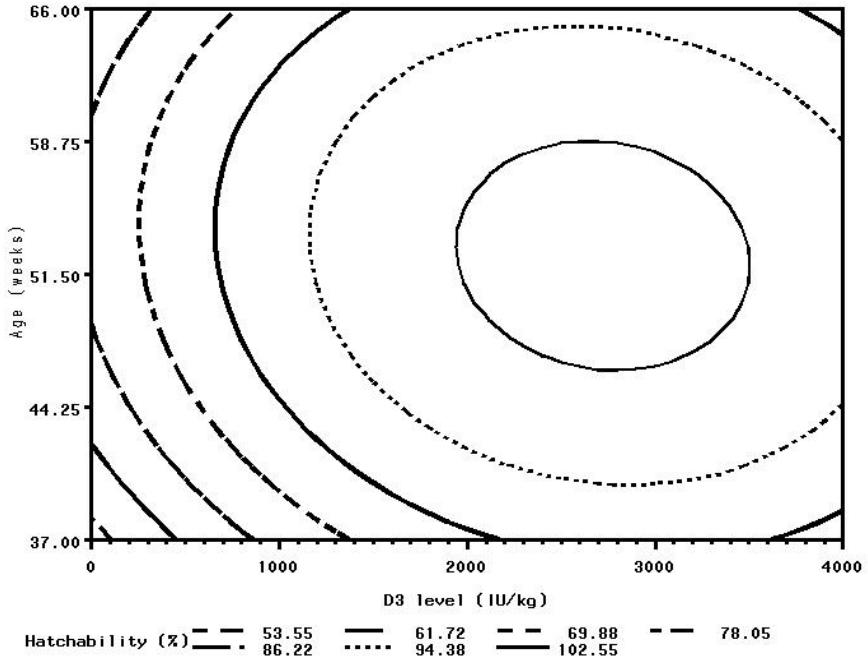


Figure 13 – Response surface of D_3 by age on hatchability during post peak production

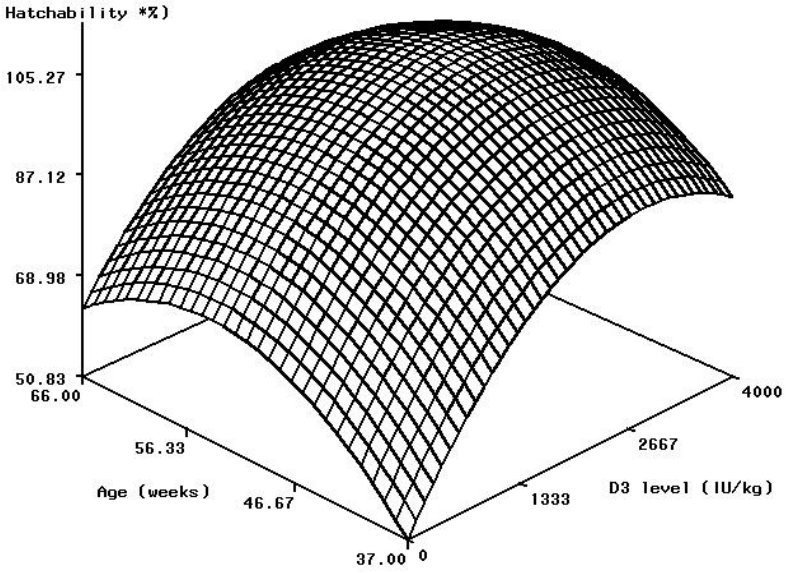
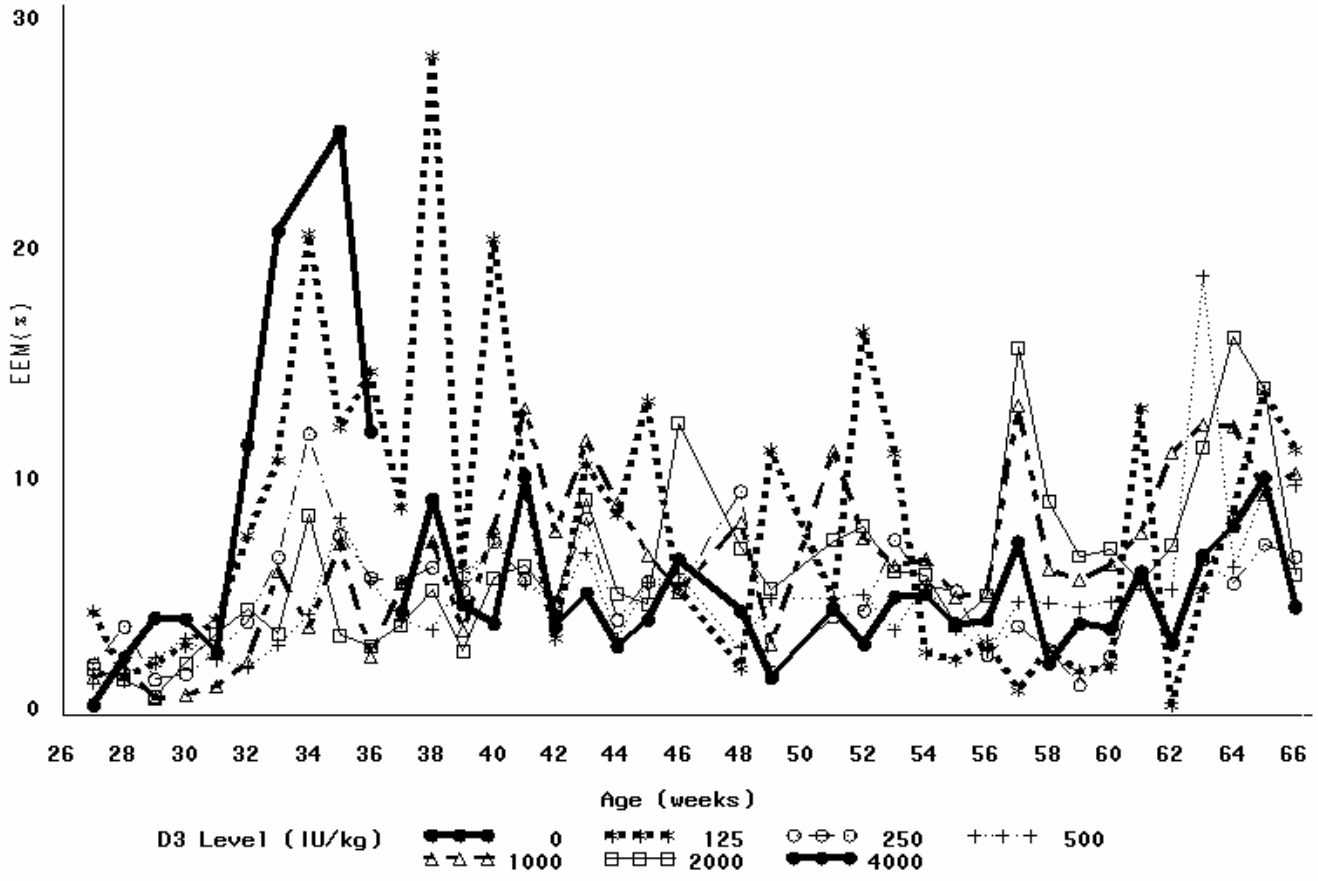


Figure 14 – Effect of D_3 levels on early embryo mortality (%) of broiler breeders from 27 to 66 wk of age



Every symbol represents the mean of 3 repetitions of the same treatment

Figure 15 – Contour plot of D_3 by age on early embryo mortality (EEM) during peak production

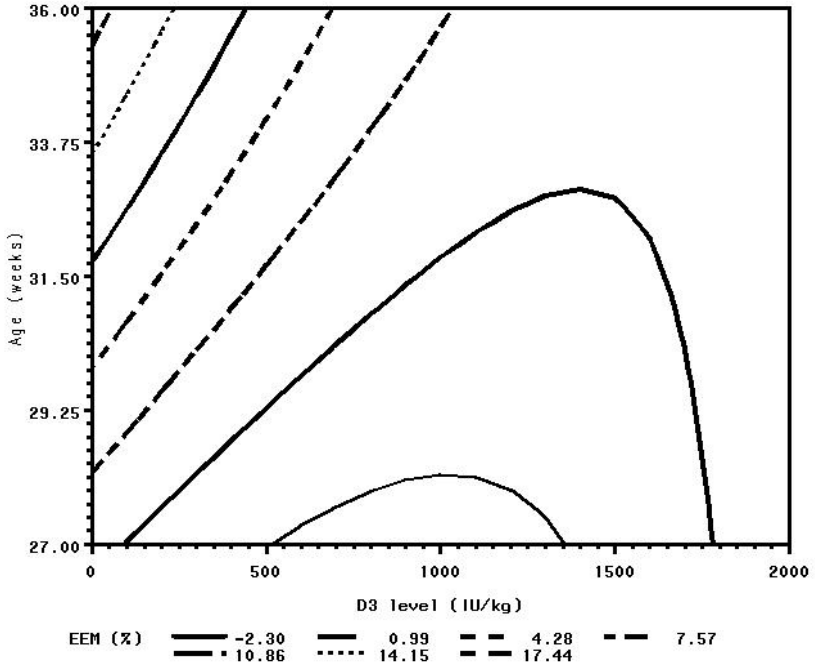


Figure 16 – Response surface of D_3 by age on early embryo mortality during peak production

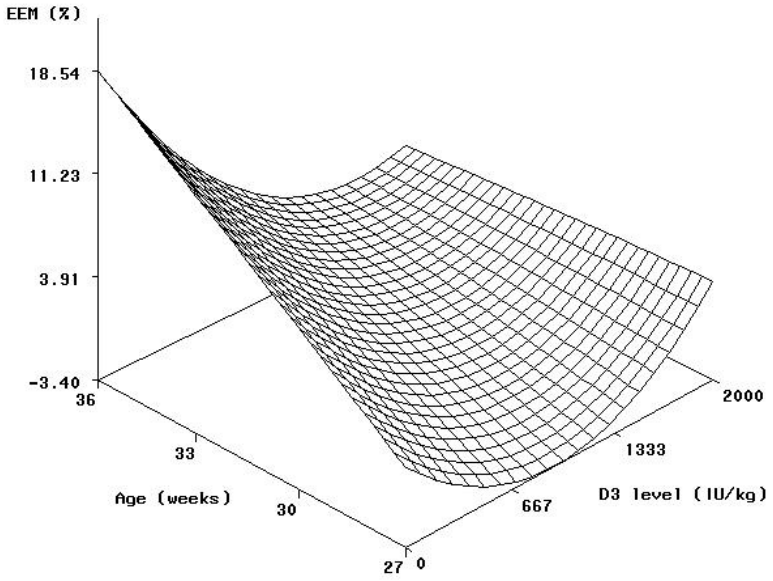
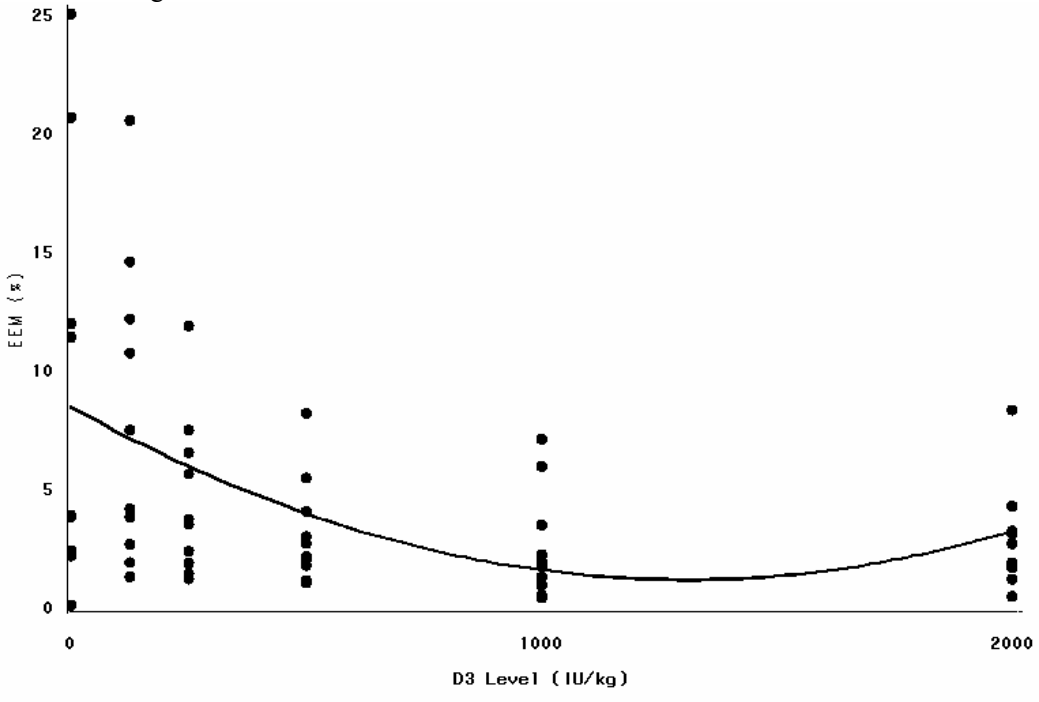
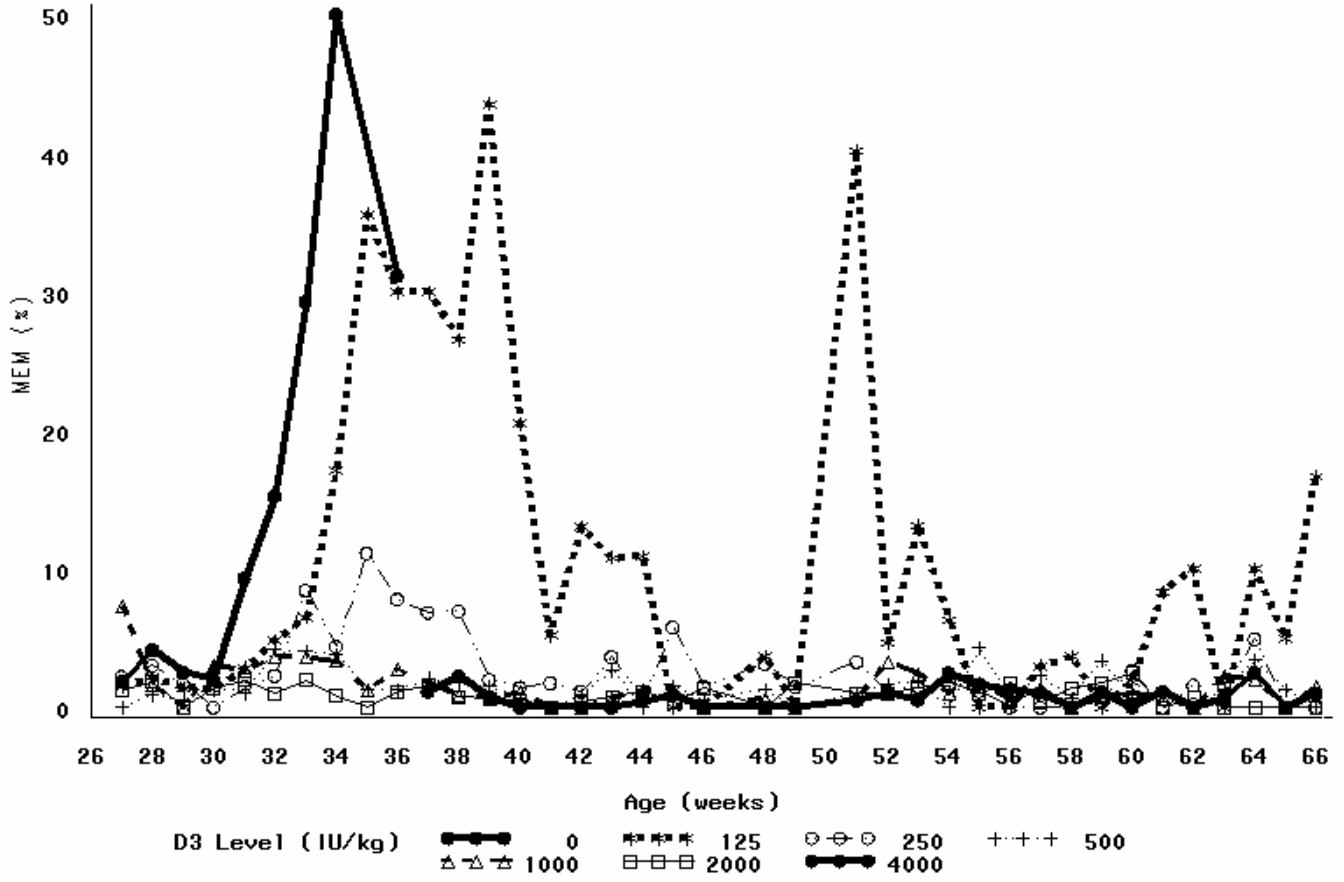


Figure 17 – Effect of D_3 levels on early embryo mortality (EEM) of broiler breeders from 27-36 wk of age.



● Represent the mean of 3 repetitions of the same treatment

Figure 18 – Effect of D_3 levels on middle embryo mortality (MEM) of broiler breeders from 27 to 66 wk of age



Every symbol represents the mean of 3 repetitions of the same treatment

Figure 20 – Contour plot of D_3 by age on middle embryo mortality (MEM) during peak production

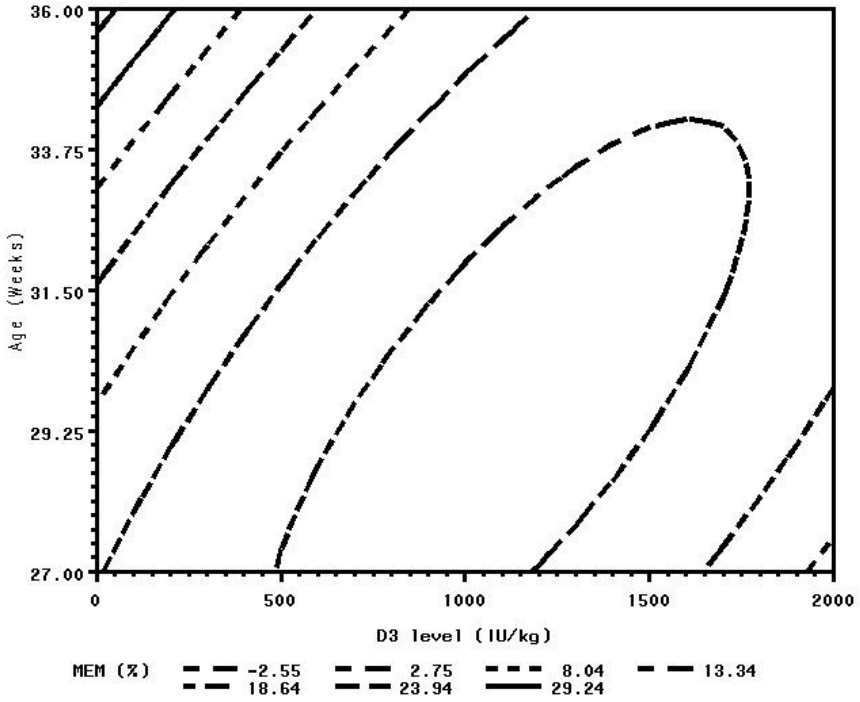


Figure 21 – Contour plot of D_3 and age on MEM from 36 to 66 wk of age

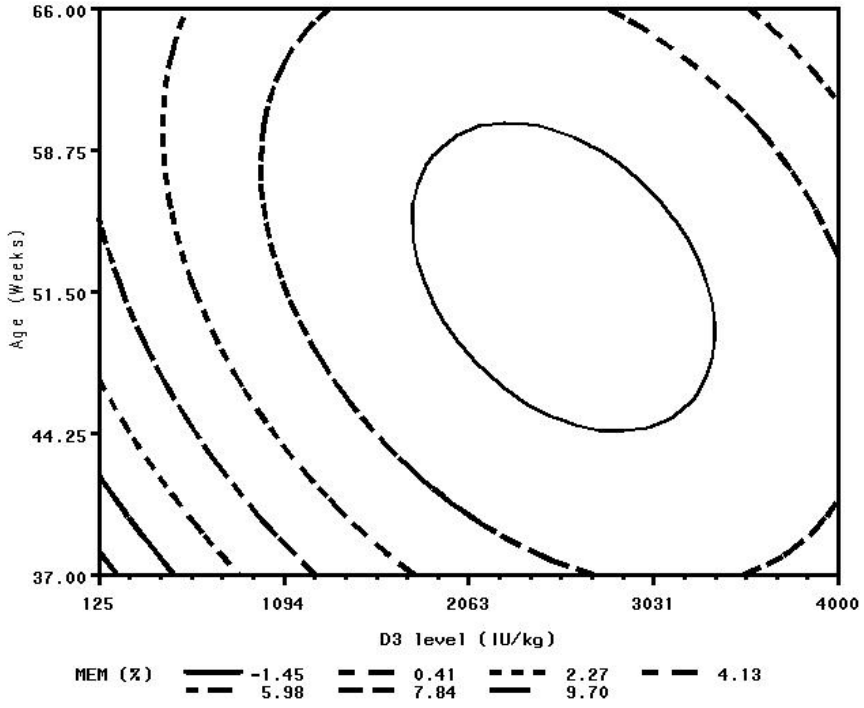
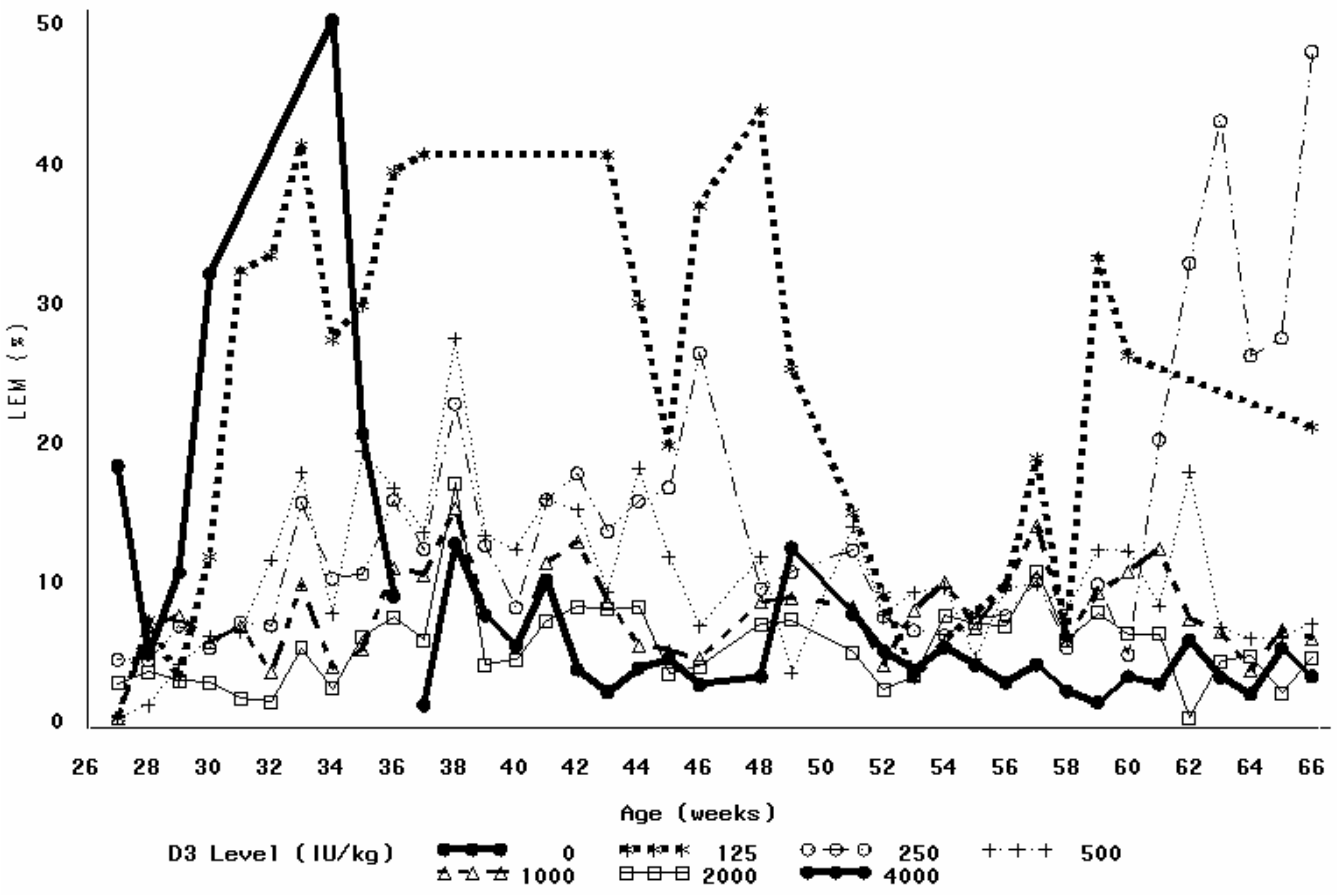


Figure 22 – Effect of D_3 levels on late embryo mortality (LEM) of broiler breeders from 27 to 66 wk of age



Every symbol represents the mean of 3 repetitions of the same treatment.

Figure 23 – Contour plot of D_3 by age on late embryo mortality (LEM) during peak production

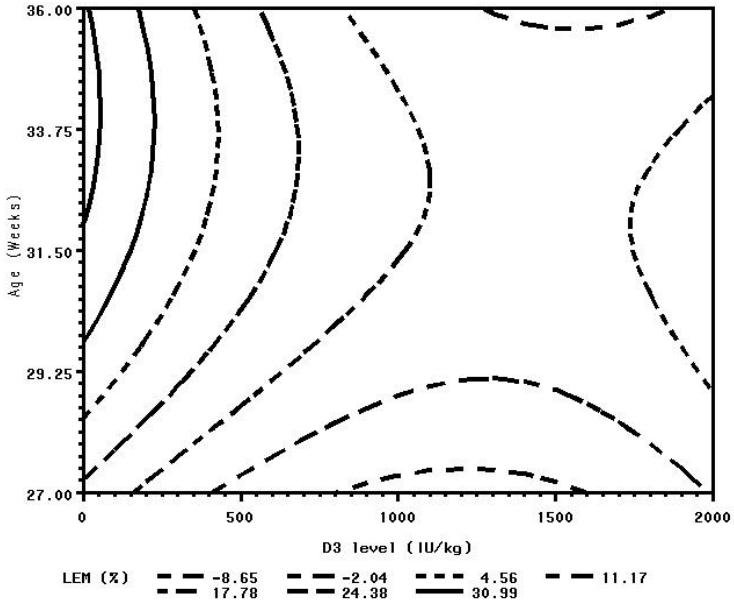


Figure 24 – Contour plot of D_3 by age on late embryo mortality (LEM) during post peak production

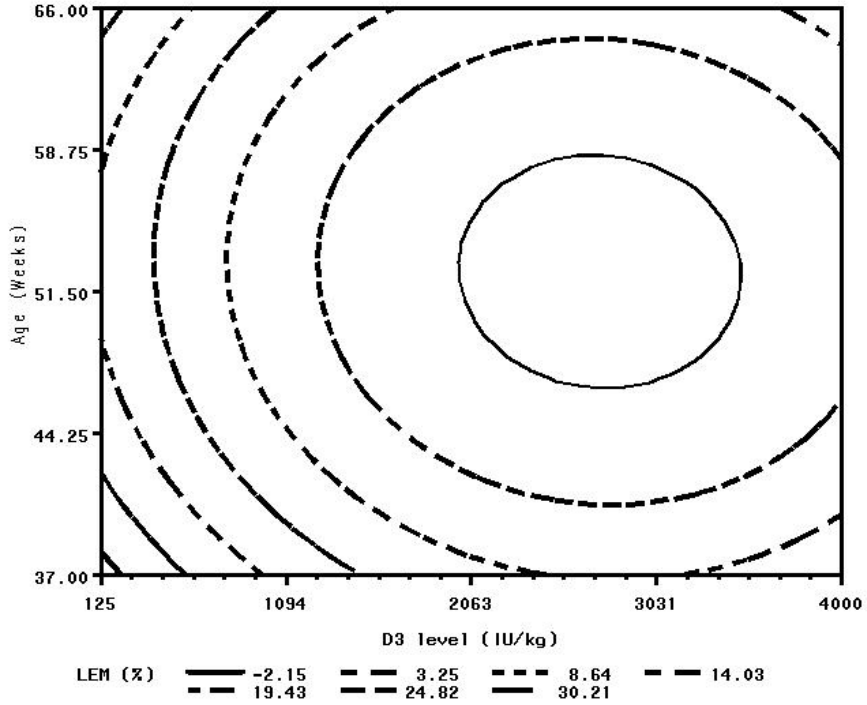
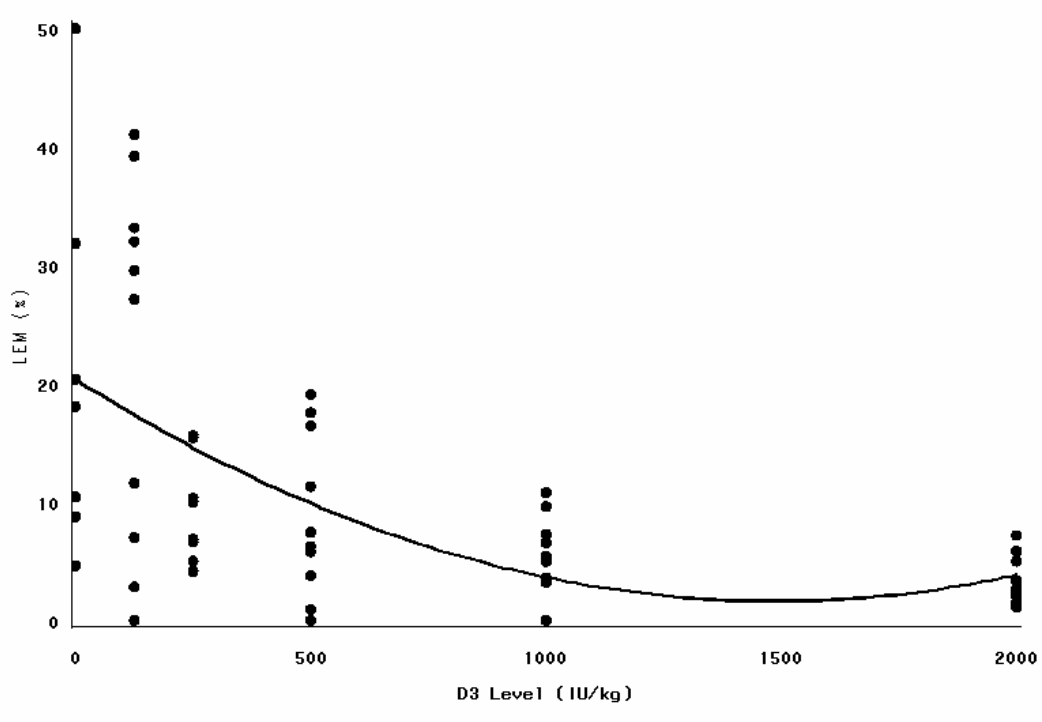
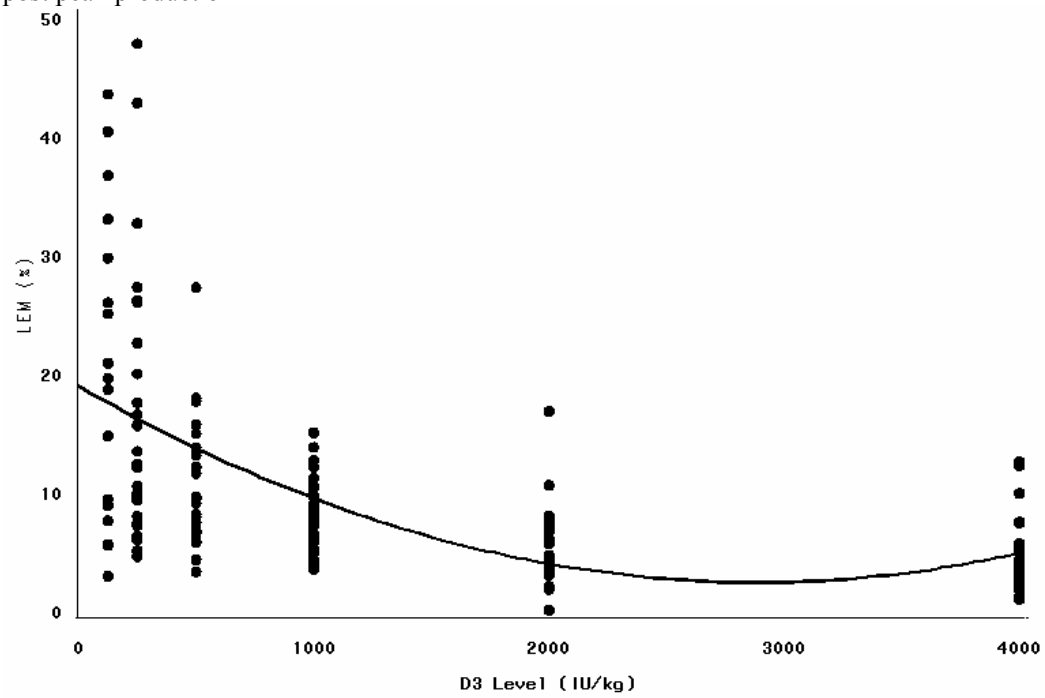


Figure 25 – Effect of D_3 levels on late embryo mortality (LEM) of broiler breeders during peak production



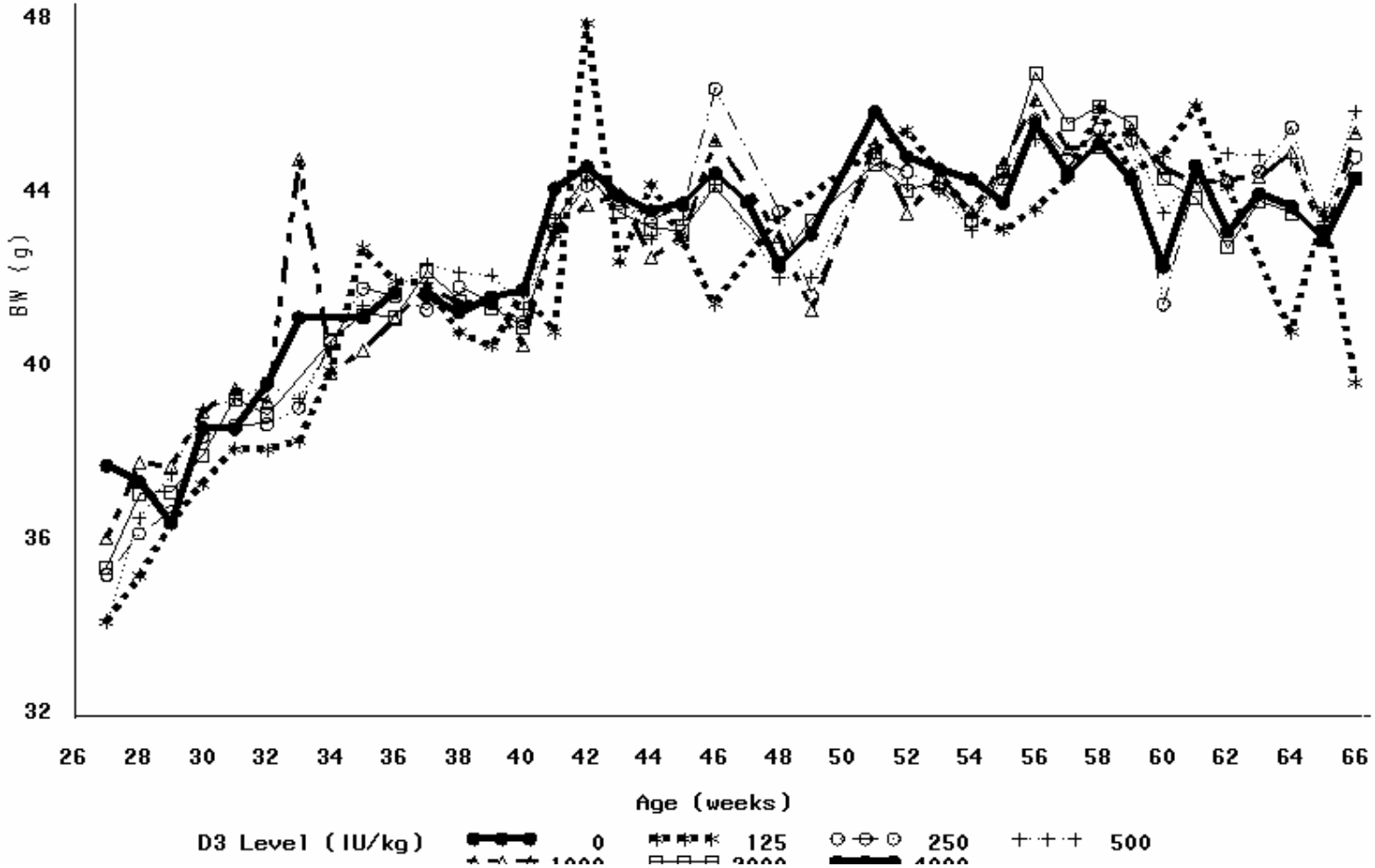
● Represent the mean of 3 repetitions of the same treatment

Figure 26 – Effect of D_3 levels on late embryo mortality (LEM) of broiler breeders during post peak production



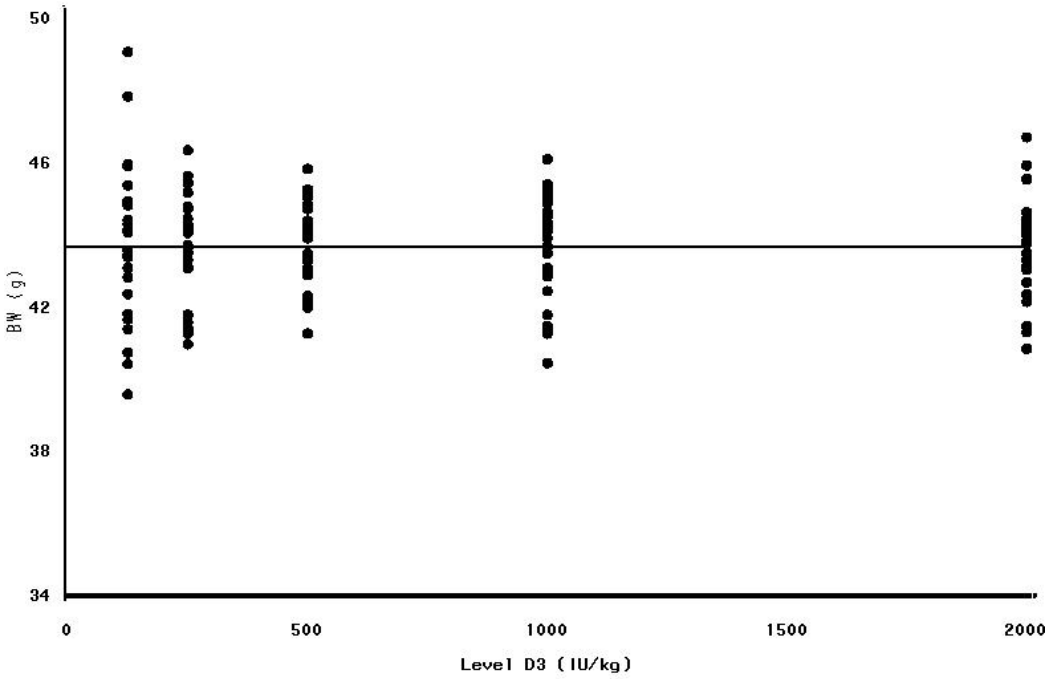
● Represent the mean of 3 repetitions of the same treatment

Figure 27 – Effect of vitamin D_3 levels on body weight of the progeny (g) of broiler breeders from 27 to 66 wk of age



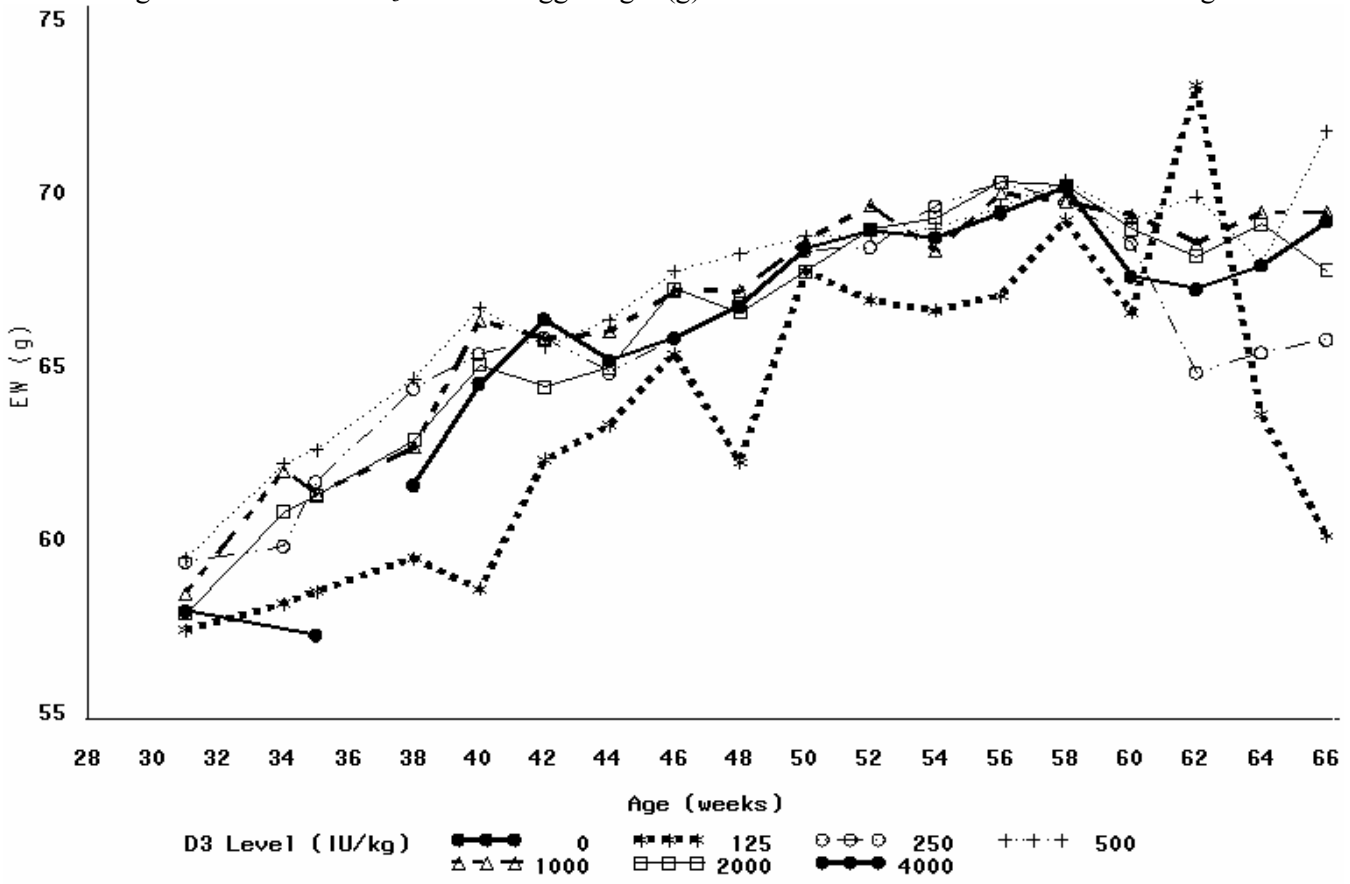
Every symbol represents the mean of 3 repetitions of the same treatment.

Figure 28 – Effect of D_3 levels on body weight (g) of broiler breeders during peak production



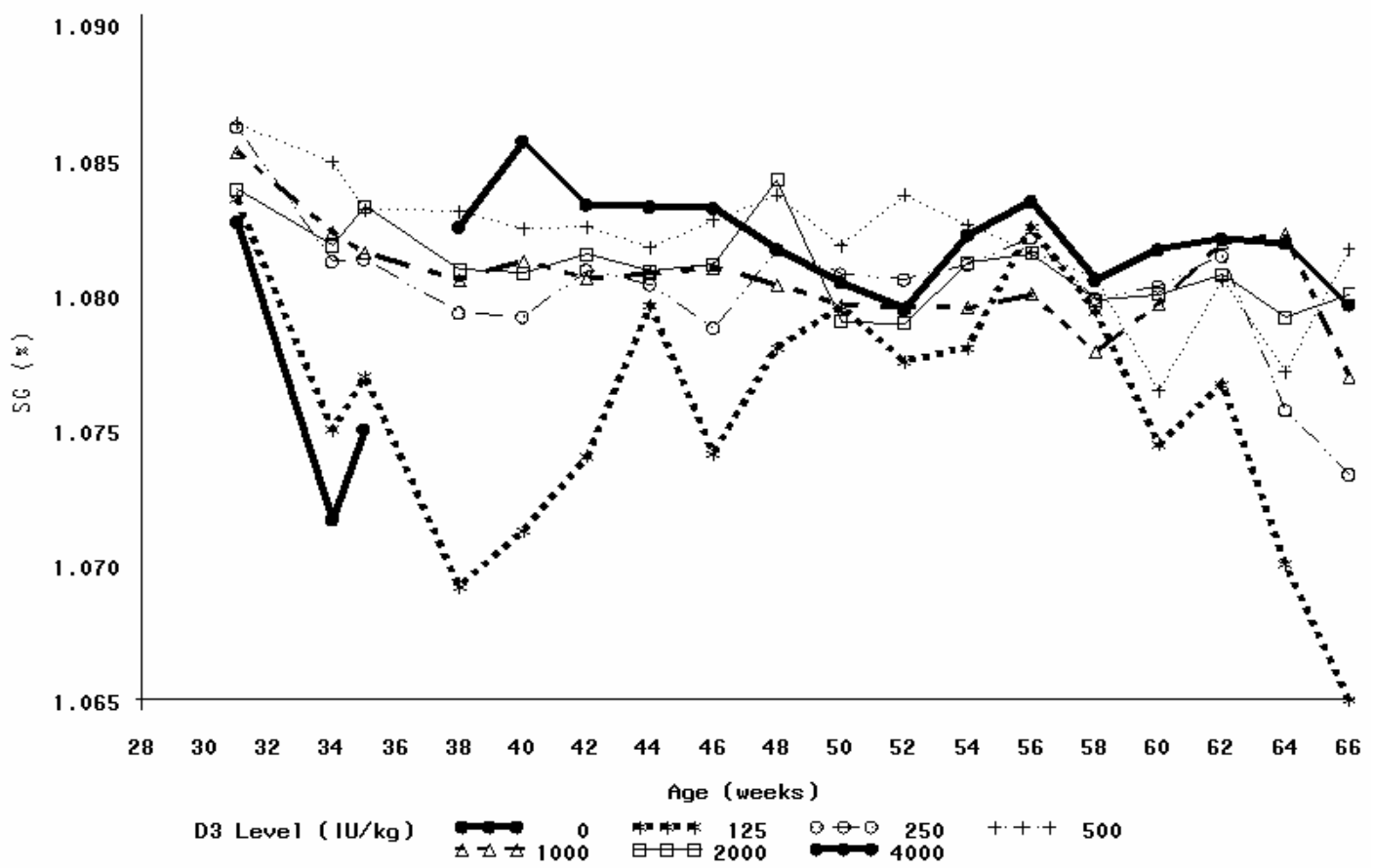
● Represent the mean of 3 repetitions of the same treatment.

Figure 29 – Effect of D_3 levels on egg weight (g) of broiler breeders from 27 to 66 wk of age.



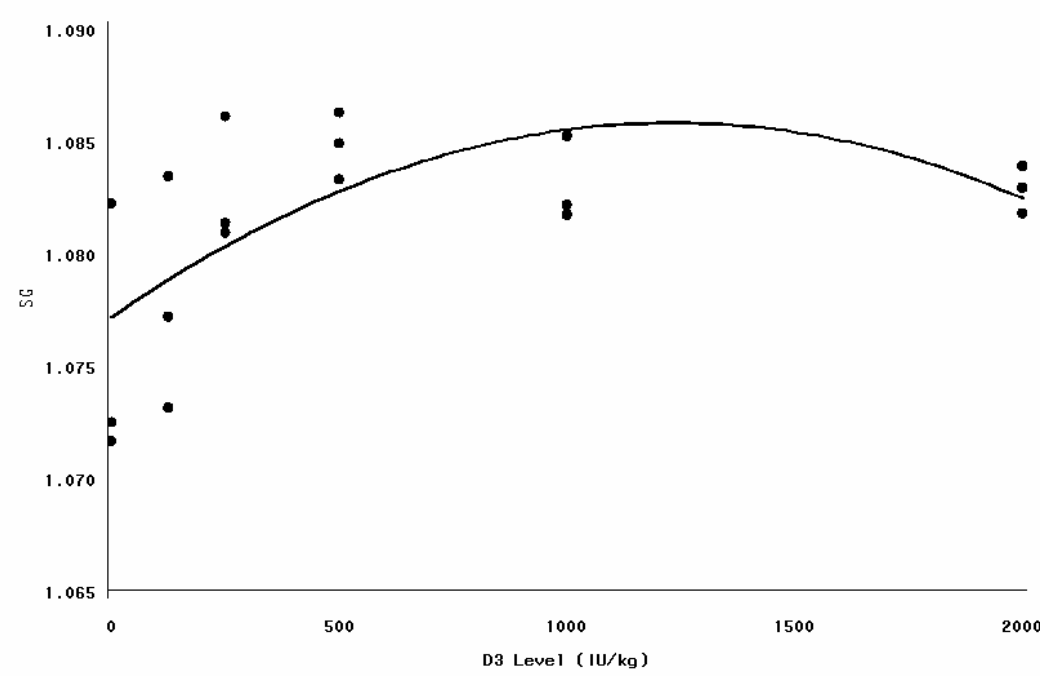
Every symbol represents the mean of 3 repetitions of the same treatment.

Figure 30 – Effect of D_3 levels on egg specific gravity (SG) of broiler breeders from 27 to 66 wk of age



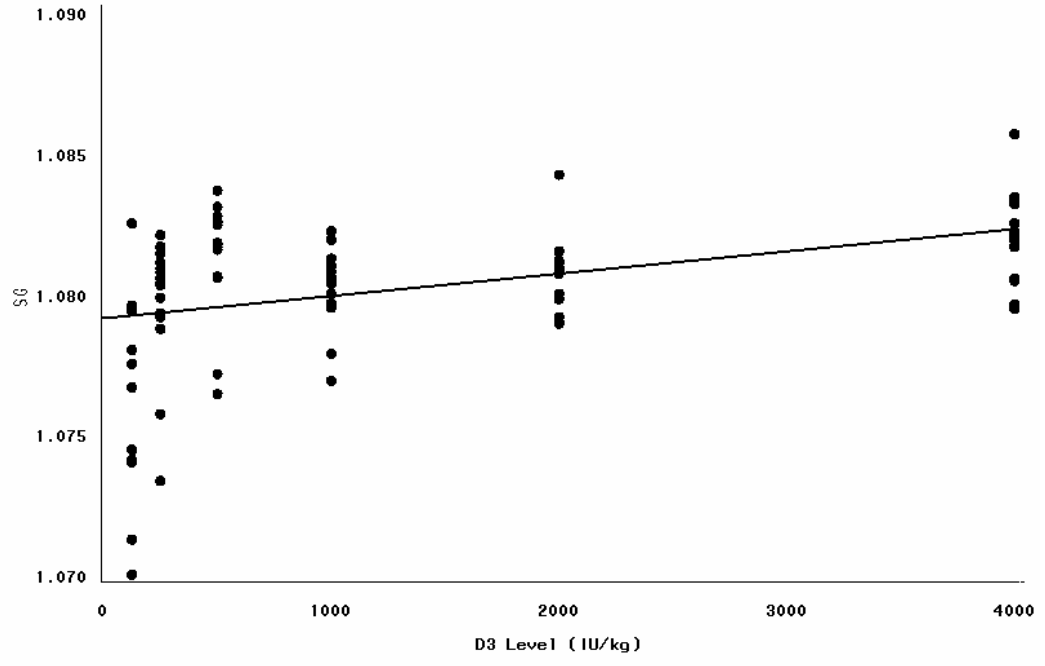
Every symbol represents the mean of 3 repetitions of the same treatment.

Figure 31 – Effect of D_3 levels on egg specific gravity (SG) of broiler breeders during peak production



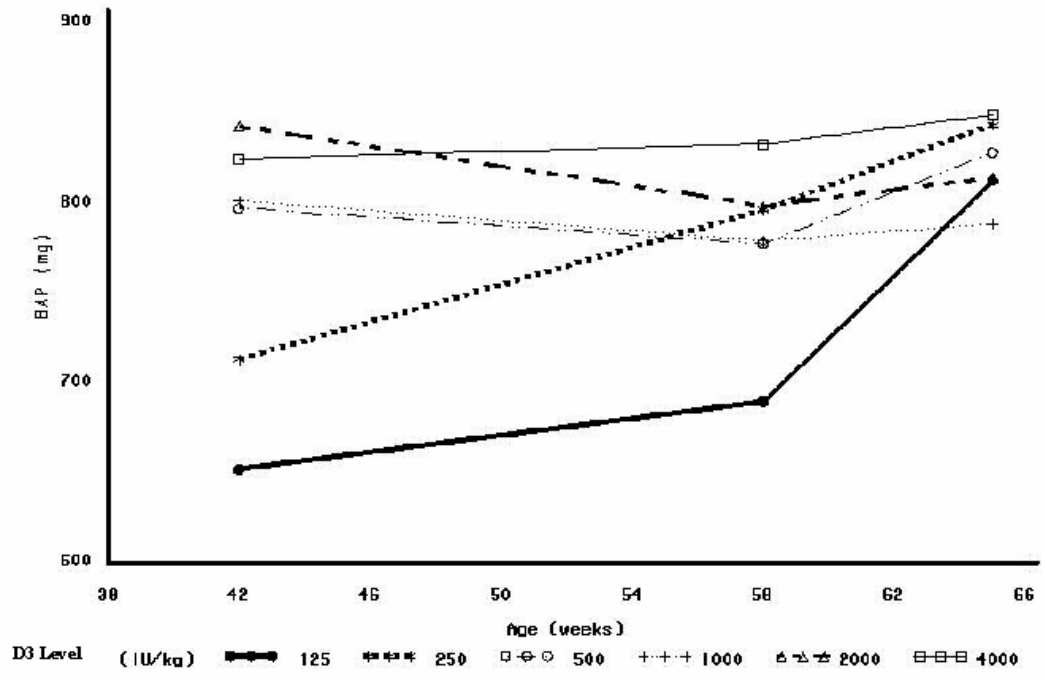
● Represent the mean of 3 repetitions of the same treatment

Figure 32 – Effect of D_3 levels on egg specific gravity (SG) of broiler breeders during post peak production



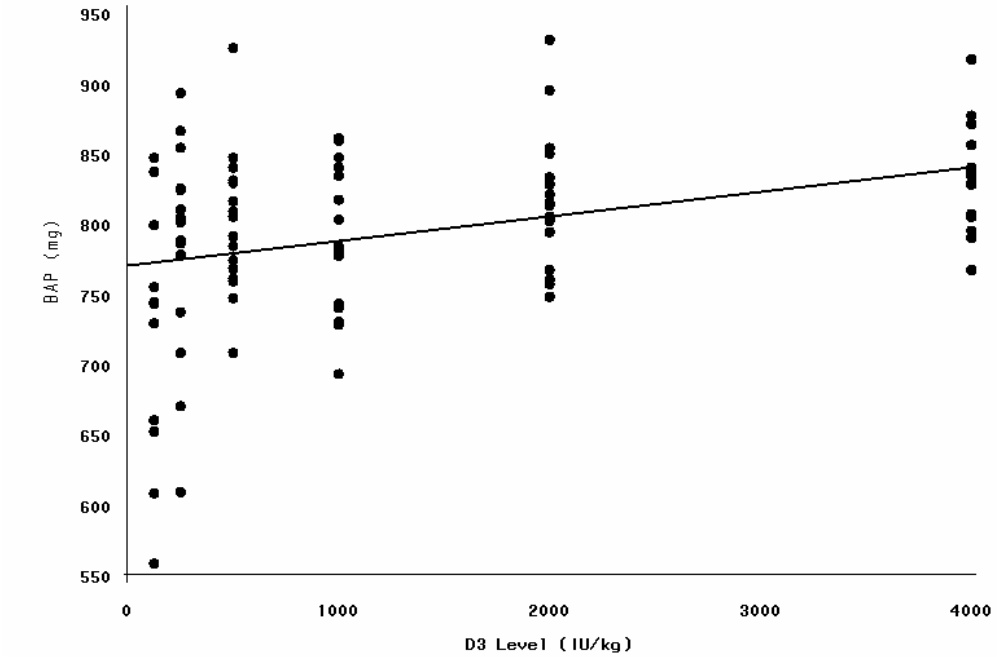
● Represent the mean of 3 repetitions of the same treatment

Figure 33 – Effect of D_3 levels on body ash of the progeny (BAP)



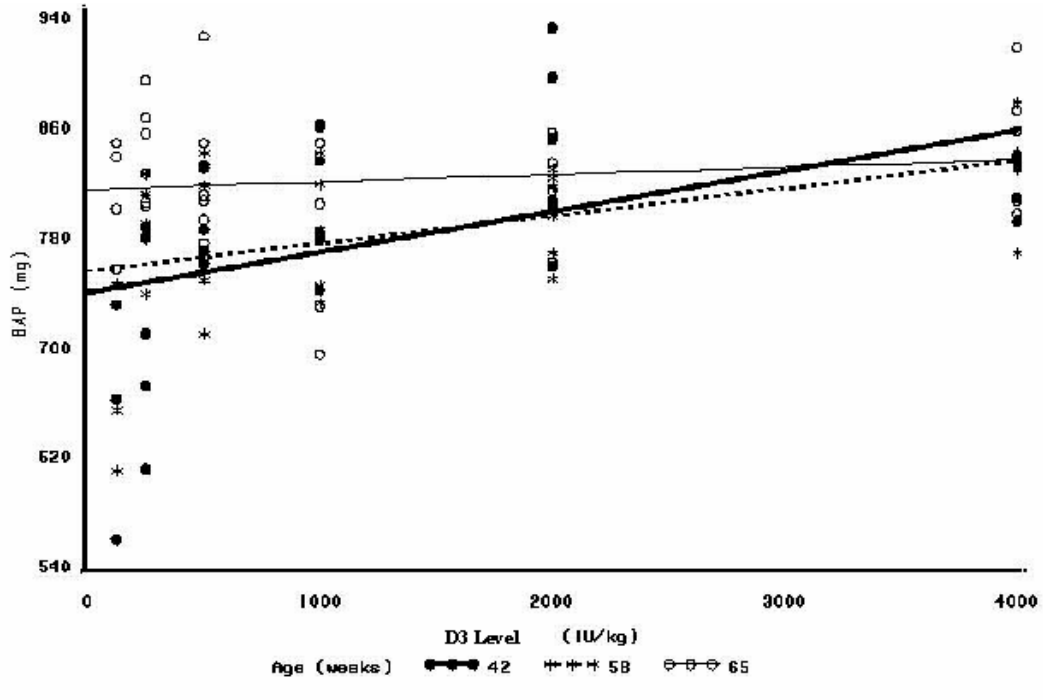
Every Symbol represents the mean of 3 repetitions of the same treatment.

Figure 34 – Effect of D_3 levels on body ash of the progeny (BAP) at 1 day of age.



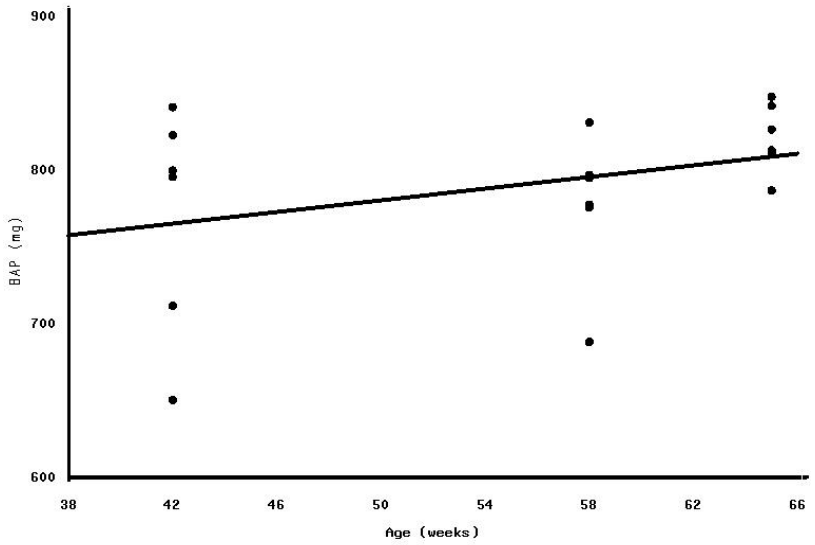
● Represent every chick ashed.

Figure 35 – Effect of D_3 levels on body ash of the progeny (BAP) at 42, 58 and 65 wk.



● Represent every bird ashed.

Figure 36 – Effect of age on body ash of the progeny (BAP)



● Represent the mean of 3 repetitions of the same treatment

CHAPTER 4

EFFECT OF THE VITAMIN D_3 DIETARY SUPPLEMENTATION OF BROILER BREEDER HENS ON THE PERFORMANCE AND BONE ABNORMALITIES OF THE PROGENY[1]

[1] Atencio, A. H.M. Edwards Jr., and G.M. Pesti. To be Submitted to Poultry Science.

ABSTRACT

Six experiments were conducted using Ross x Ross chicks hatched from eggs laid by broiler breeder hens fed various levels of vitamin D_3 to determine the effect of the vitamin D_3 level in the maternal diet on the performance and leg abnormalities of the progeny. Six levels of vitamin D_3 (0, 125, 250, 500, 1,000 and 2,000 IU/kg of diets) were fed to the hens from 25 to 66 weeks of age, however, at 36 weeks of age hens that were receiving the 0 level of vitamin D_3 were switched to a diet containing 4,000 IU/kg. Chicks hatched from eggs laid when the hens were 27, 41, 29, 36, 45 and 52 weeks of age were used in Experiments 1, 2, 3, 4, 5 and 6, respectively. The studies were conducted in a UV-light-free environment. Experiments 1 and 2 were conducted as complete randomized design with the maternal diets as the treatments and Experiments 3, 4, 5 and 6 were conducted as a split plot design, with vitamin D_3 in the chicks' diet as the whole plot and vitamin D_3 in the maternal diet as a subplot. Chicks in Experiments 1 and 2 were fed a vitamin-D_3 deficient diet while chicks in Experiments 3 and 4 were fed four levels of vitamin D_3 (0, 100, 200 and 400 IU/kg of diet) and chicks in Experiments 5 and 6 were fed six levels of vitamin D_3 (0, 200, 400, 800, 1,600 and 3,200 IU/kg of D_3). The highest body weight gains and tibia ash were observed in chicks hatched from hens fed the highest levels of D_3 in all experiments. Reductions in the incidence of Ca rickets were observed in Experiments 3 and 6 while increases in tibia ash were observed in Experiments 2 and 6 as the level of D_3 in the maternal diet increased. Body weight gain and tibia ash increased and Ca rickets incidence decreased as the D_3 level in the chick diet increased. An evaluation of the study indicates that chicks hatched from eggs laid by hens fed 2,000 or 4,000 IU of D_3/kg as the maximum level of vitamin D_3 had the highest body weight gain and

chicks fed 3,200 IU had the highest body weight and tibia ash and the lowest TD and Ca rickets incidence.

Key Words: broiler breeders, progeny, vitamin D_3

INTRODUCTION

No studies have been reported on the effect of supplementing vitamin D_3 in the diet of broiler breeder hens on the performance and leg abnormalities of the progeny. Leg abnormalities in rapid growing chicks are still a worldwide problem in the poultry industry. The most common bone diseases of economical importance in the poultry industry are tibial dyschondroplasia (TD) and Ca and P rickets (Cook, 2000). The role of vitamin D_3 in the development of Ca rickets (sequential changes of the tibial growth plate) has been reported by Long et al. (1984) in a study conducted with broiler chickens fed a diet deficient in vitamin D_3. The NRC (1994) recommends 200 IU of vitamin D_3/kg of diet for 21-d old broiler and 300 IU/kg for egg-type white egg breeders with feed intakes of 100 g of feed/hen/day. However, studies conducted in environments excluding UV-light indicate that the vitamin D_3 requirement in young broiler chickens (Mitchell et al., 1997; Elliot et al., 1997; Kasim and Edwards, 2000) is higher than the NRC (1994) recommendation. Fritts and Waldroup (2003) observed a decrease in TD incidence and, specially, severity by supplementing vitamin D_3 up to 4,000 IU/kg in the diet of broiler chicks. More recently, McCormack et al. (2004) have reported that 10,000 IU of vitamin D_3/kg of diet can prevent TD almost completely. Several factors such as the presence of animal byproduct in the basal diet (Mattila, 1995; Atencio et al., 2003), UV-light (Edwards et al., 1992, 1994; Edwards, 2003), amount of cholecalciferol present in the chick at hatching (Murphy et al., 1936; Bethke et al., 1936; Griminger, 1966; Edwards, 1995) and level of other nutrients (Aburto and Britton, 1998; Aburto et al., 1998; Baker et al., 1998; Waldroup et al., 1965) may be influencing the growing chicks' quantitative requirement for vitamin D_3 for the maximum performance, maximum bone ash and minimum leg abnormalities. The broiler requirement for vitamin D_3 is usually based on bone ash or

incidence of rickets which have been found to be more sensitive indicators than growth rate.

Part of the vitamin D_3 requirement of chickens can be supplied by the birds being exposed to UV-light irradiation that enables them to synthesize vitamin D_3 (Tian et al., 1994). However, the use of windowless houses with incandescent lights in commercial settings (broiler breeder and broiler chicks) does no allow the skin to produce endogenous vitamin D, therefore, most of the vitamin must come from the feed or from the yolk sac in the case of young chicks. Edwards et al. (1992, 1994), Elliot and Edwards (1997), Edwards (2000 and 2003), and Ledwaba and Roberson (2003) have shown the beneficial effect of UV-light irradiation on reducing bone diseases in chickens. Murphy et al. (1936), Bethke et al. (1936), and Edwards (1995) showed that the amount of vitamin D_3 or light given to hens had a great effect on body weight gain and bone ash of the progeny during the first 2 or 3 weeks of their development. Stevens et al. (1984) observed an increase in body weight and tibia ash in poults hatched from turkey breeders fed 300, 900 and 2,700 IU /kg of vitamin D_3. Most of the work on leg abnormalities of young chicks has focused on the manipulation of their diet or environment. No investigation has been conducted on the manipulation of the maternal diet of the modern broiler chicks; therefore, the objective of this study was to determine the effect of maternal D_3 level in the diet on the performance and leg abnormalities of the progeny.

MATERIALS AND METHODS

Six experiments were conducted for a period of 16 days each using mixed-sex 1-d-old Ross x Ross[1] chicks hatched from eggs laid by hens fed various levels of

[1] Ross Breeders, Inc. Huntsville, Alabama 35805

vitamin D_3. The levels of D_3 used in the maternal diet and the conditions in which the hens were raised are reported by Atencio et al. (2004 a,b). Six levels of D_3 (0, 125, 250, 500, 1,000 and 2,000 IU/kg of diets) were fed to hens from 25 to 66 wk of age, however, the hens that were receiving the 0 level of D_3 were switched to a diet containing 4,000 IU/kg at 36 wk of age.

Chicks hatched from eggs laid when the hens were 27, 41, 29, 36, 45 and 52 weeks of age were used in Experiments 1, 2, 3, 4, 5 and 6, respectively. The basal diet was based on corn and soybean meal (Table 1). No D_3 source was used in the vitamin premix and no animal byproduct was used in the basal diet to guarantee that no unintentional vitamin D activity was present (Mattila, 1995; Atencio et al. 2003).

Chicks in Experiments 1 and 2 were fed the vitamin D_3 deficient basal diet while chicks in Experiments 3 and 4 were fed the basal diet supplemented with four levels of vitamin D_3 (0, 100, 200 and 400 IU/kg of diet) and chicks in Experiment 5 and 6 were fed with the same basal diet supplemented with six levels of vitamin D_3 (0, 200, 400, 800, 1,600 and 3,200 IU/kg of D_3). Experimental diets were mixed at the beginning of each experiment.

Chicks hatched were kept separate according to the maternal diet; therefore Experiments 1 and 2 had one independent variable (level of D_3 in the maternal diet), and Experiments 3, 4, 5 and 6 had two independent variables (level of D_3 in the chick diets and levels of D_3 in the maternal diet). A completely randomized design was used in Experiments 1 and 2 and a split plot design with vitamin D_3 in the chick diets as the whole plot and vitamin D_3 in the maternal diets as the subplot was used in Experiments 3, 4, 5 and 6.

Experiments 1 and 2 had 6 treatments with 3 and 4 replicates, respectively. Ten chicks per replicate were used in Experiments 1 and 2. Experiments 3 and 4 had

24 treatments (6 levels of maternal D_3 x 4 levels of chick D_3) and Experiments 5 and 6 had 30 treatments (5 levels of maternal D_3 x 6 levels of chick D_3). Four chicks from each maternal group were randomly selected, banded, weighed and randomly allocated to each of the 12 pens in Experiments 3 and 4 and each of the 24 pens in Experiments 5 and 6. No chicks were used from hens receiving no supplemental vitamin D_3 in Experiments 2, 4, 5 and 6 and no chicks were used from hens receiving 125 IU/kg of diet in Experiments 5 and 6. The reason for not using chicks from hens fed 0 or 125 IU/kg of D_3 was because of the absence of the 0 level (treatment) after 36 weeks of age and low hatchability, respectively. The numbers of chicks used in experiments 1, 2, 3, 4, 5 and 6 were 180, 240, 288, 288, 480 and 480, respectively.

All experiments were conducted in electrically heated wire mesh-floored battery brooders[2]. Ultraviolet irradiation was eliminated from the chick room and batteries pens by fitting Arm-a-Lite[3] sleeves to all the fluorescent fixtures in the room and battery brooders (Edwards et al., 1994). The fluorescent lights were on 24 h each day. The temperature of the room was maintained at 22 ºC. All experiments were conducted using the same environmental conditions. Chicks received water and feed *ad libitum*.

At the last day of each experiment, chicks in Experiments 2 and 6 were randomly selected for plasma samples that were analyzed for Ca[4]. Blood samples were obtained by heart puncture. Four chicks were bled from each of the six treatments in Experiment 2 (one from each replicate) while two chicks were bled from each of the 30 treatments in Experiment 6. At the same day all chicks were also weight and killed by carbon dioxide asphyxiation and examined at random for

[2] Petersime Incubator Co., Gettysburg, OH 54328.
[3] Arm-a-Lite® Thrermoplastic Processes, Sterling, NJ 07980.
[4] Section N-31 Technicon Autoanalyzer Methodology, Technicon Corp., Tarrytown, NY 10591.

TD and Ca rickets. A longitudinal cut was made across the right tibia and diagnosis for TD and Ca rickets were made based on the width of the proximal growth plate band and its appearance according to the methodology described by Edwards and Veltmann (1983) and Long et al. (1984). The left tibia was removed to determine the bone ash content on a dry fat-free basis (Association of Official Analytical Chemists, 1995). Feed intake and feed efficiency were calculated for every treatment in Experiments 1 and 2 while only the main effect of the D_3 levels in the chick diets for all other experiments. The reason for not calculating the feed intake and feed efficiency for the main effect of the vitamin D_3 levels on the maternal diet was that chicks hatched from every maternal group were present in every pen.

Statistical Analysis

The analyses were conducted using the mean of each replicate as an observation. Regression analyses (multiple and simple) were performed to fit a reduced quadratic regression equation using the General Linear Models procedure of SAS (SAS Institute, 1998). In Experiments 3, 4, 5 and 6 the probability for the regression coefficients, chick D_3 levels (CD_3) and CD_3^2, were found using an F value calculated by dividing their mean square by the mean square of the pen within vitamin D_3 treatment. All others probabilities were calculated using an F value found using the residual. Interactions were considered present when the regression coefficient CD_3 by maternal D_3 (MD_3) had a probability lower than 0.10 in the multiple factor regression. Linear or quadratic effects were considered present for the main effect (CD_3 or MD_3) when the probability was lower or equal to 0.05. All coefficients of determination (r^2) were calculated using the sum of squares of the regression divided by the total sum squares.

RESULTS

Experiment 1

No significant effects of the maternal diet were observed for any of the criteria measured in Experiment 1. However, body weight increased ($P < 0.11$) as the D_3 level in the maternal diet increased (Table 2). Calcium rickets was present in 100% of the chicks.

Experiment 2

Body weight gain, feed intake, and bone ash % increased as the vitamin D_3 level in the maternal diet increased (Table 3). Ca rickets incidence was observed in 100% of the chicks; however, a slight decrease in the score was observed as the vitamin D_3 in the maternal diet increased ($P<0.06$). No effect of the maternal diet was observed on plasma Ca.

Experiment 3

Significant interaction in the regression coefficient for CD_3 by MD_3 was observed on incidence of Ca rickets; however, the predicted inflection was a saddle point and not a true maximum (Table 4). Linear increases in body weight gain, tibia ash and TD incidence, and a linear decrease in Ca rickets incidence were observed as the D_3 levels in the chick diets increased. Linear decrease in Ca rickets incidence was observed as the D_3 level in the maternal diet increased; however no significant effects of the maternal diet were observed on body weight gain, bone ash and TD incidence.

Linear increases in feed intake and feed efficiency were observed as the D_3 level in the chicks' diets increased ($P<0.05$). The average feed intake values were

315±14, 333±3, 342±17 and 383±15 and the average feed efficiency values were 0.584±0.018, 0.712±21, 0.738±0.003 and 0.810±0.022 for the 0, 100, 200 and 400 IU/kg of D_3 in the chicks' diets, respectively.

Experiment 4

No significant interactions in the regression coefficient CD_3 by MD_3 was observed on any of the criteria studied (Table 5). When the simple factor regression was applied on the main effect of CD_3, an increase in body weight gain, tibia ash and TD incidence and a decrease in Ca rickets incidence were observed as the D_3 level in the chicks diet increased. By using the same technique on the main effect of MD_3, only body weight gain linearly increases as D_3 level in the maternal diet increased.

Feed intake and feed efficiency increased as the D_3 in the chicks diets increased ($P<0.05$); in addition, feed efficiency also responded in a non-linear manner (significant quadratic effect, $P<0.05$). The averages feed intake values were 301±13, 343±13, 364±12 and 449±19 and the average feed efficiency values were 0.627±0.026, 0.721±12, 0.737±0.023 and 0.704±0.13 for the 0, 100, 200 and 400 IU/kg of D_3, respectively.

Experiment 5

No interaction in the regression coefficient, CD_3 by BD_3 with a probability lower than 0.22 was observed on any of the criteria studied (Table 6). When the simple factor regression was used on the main effect of CD_3, a positive linear effect in body weight gain and tibia ash and a negative linear effect in incidence of TD and incidence of Ca rickets were observed as the D_3 in the chicks' diets increased. Body weight gain was the only characteristic that positively increased as the D_3 in the maternal diet increased.

Linear and quadratic effects of feed intake and linear effect of feed efficiency were observed as the vitamin D_3 levels in the chicks' diets increased ($P<0.05$). The averages feed intake values were 346±18, 400±7, 412±8, 462±13, 478±12 and 511±16 and the average feed efficiency values were 0.699±0.011, 0.797±0.016, 0.797±0.012, 0.809±0.017, 0.811±0.021 and 0.794±0.012 for the 0, 200, 400, 800, 1,600 and 3,200 IU/kg of D_3, respectively.

Experiment 6

In general, chicks hatched from hens fed the highest level of vitamin D_3 had higher body weights (Figure 1). A significant interaction in the regression coefficient CD_3 by MD_3 was observed for body weight gain (Table 7). This interaction indicated that at different levels of vitamin D_3 in the chicks' diets or maternal diets will result in different responses in body weigh gain. The combination of D_3 levels that resulted in the predicted maximum body weight gain (502 g) by using multiple regression were 2,125 IU/kg in the chicks' diets and 2,907 IU/kg in the maternal diet (Figure 2). Tibia ash followed the same general pattern (Figure 3); however, the interaction CD_3 *x* MD_3 was not significant ($P=0.22$).

Body weight gain, bone ash and plasma Ca increased and incidence of TD and incidence of Ca rickets decreased as the vitamin D_3 level in the chicks' diet increased. Body weight gain and bone ash ($P=0.08$) increased and incidence of TD ($P=0.09$) and Ca rickets ($P=0.13$) decreased as the D_3 in the maternal diet increased.

Linear and quadratic effects on feed intake and feed efficiency were observed as the vitamin D_3 level in the chicks' diets increased ($P<0.05$). The averages feed intake values were 390±12, 478±4, 521±6, 543±8, 569±8 and 585±10 and the average feed efficiency values were 0.732±0.006, 0.773±0.004, 0.785±0.002, 0.780±0.006, 0.780±0.005 and 0.776±0.014 for the 0, 200, 400, 800, 1,600 and 3,200 IU/kg of D_3, respectively.

Comparative results from all six experiments

Chicks in Experiment 2 had higher body weight gain and feed intake than chicks in Experiment 1 (Table 8). Lower bone ash and higher Ca rickets incidences were observed in Experiment 4 than Experiment 3. Higher body weight and bone ash and lower TD and Ca rickets incidences were observed in Experiment 6 than in Experiment 5. In general, chicks hatched from eggs laid by hens at older ages had higher body weight gains than chicks hatched from eggs laid by younger hens when comparing experiments within the same design.

In general chicks hatched from eggs laid by broiler breeder hens fed higher D_3 levels had higher body weight gains than chicks hatched from hens fed lower D_3 levels. However, significant effect of the maternal diet was observed for body weight gain in Experiments 2, 4, 5 and 6 and tibia ash in Experiments 2 and 6 (Table 9). No significant effects of the maternal diet were observed in Experiments 1 and 3 (chick hatched from eggs laid by hens at 27 and 29 weeks of age, respectively) for any of the criteria studied. When the same two designs (Experiments 1 and 3) were repeated with chicks hatched from eggs laid by hens at 41 and 36 week of age (Experiments 2 and 4) effects of the maternal diet was observed on body weight gain of the progeny.

In Experiments 5 and 6, TD incidence was reduced as the vitamin D_3 levels in the chicks' diet increased while in Experiments 3 and 4 the opposite occurred, TD increased. TD and Ca rickets incidence were present even when the level of vitamin D_3 in the chick' diets was the highest (3,200 IU/kg). In Experiments 5 and 6 TD incidences at the 3,200 IU/kg level were 7.5% and 6.7%, respectively. The corresponding values for Ca rickets were 21 and 13% (Tables 6 and 7).

DISCUSSION

The results of this work indicate that the level of vitamin D_3 in the maternal diet was effective in improving body weight of the progeny. These results are in agreement with the finding of Bethke et al. (1936), Griminger (1966) and Edwards et al. (1995) in experiments conducted with the progeny of laying hens. In their experiments the authors also observed improvement in body weight of chicks hatched from hens fed high levels of vitamin D_3.

Increasing the level of D_3 in the chicks' diet increased body weight gain linearly. Linear increase without plateau in body weight gain have also been observed by Edwards et al. (2002), and Kasim and Edwards (2000) using lower levels of vitamin D_3 (1,600 and 1,200 IU of D_3/kg of diet, respectively as the maximum level) and by McCormack et al., (2004) using levels much higher (0 to 10,000 IU/kg of diet). However, different from the current study a plateau in body weight gain was observed by several others investigator using lower levels of vitamin D_3 in the chicks' diet and a UV-light free environmental (Edwards et al., 1994; Elliot and Edwards, 1997; Aburto et al., 1998; Kasim and Edwards, 2000). The major difference between the chicks used in these studies was the level of vitamin D_3 reserved in the yolk sac of the progeny. In the current study, the data indicate that not all chicks had the same level of vitamin D_3 content at hatching. Mattila et al. (1999) showed a positive relationship between the D_3 level in the diet of laying hens and the level in their eggs' yolks. Chicks hatched from eggs laid by hens fed low levels of vitamin D_3 responded differently to the various levels of vitamin D_3 in the chicks' diet than chicks hatched from eggs laid by hens fed higher levels of vitamin D_3 (Figure 1).

The lack of effect of the maternal diet in Experiments 1 and 3 is an indication that all chicks had the same vitamin D reserved at hatching. In the study conducted by Atencio et al. (2004a, 2004b) with the broiler breeder hens that produced these chicks, no effect of vitamin D_3 levels used in the hens diet was observed on the performance of the hens before 29 weeks of age. It is thought that hens at 29 weeks of age were still using their body D_3 reserves and transferring some of these reserves to the eggs. As egg production continued, a D_3 effect was observed because the D_3 was being transferred from the body of the hens into the eggs at a rate that eventually depleted the body reserve of D_3. These results could be interpreted to mean that the chicks used in Experiments 1 and 3 (eggs laid at 27 and 29 weeks) had in general greater vitamin D_3 reserves in the yolk sac at 1 day than the chicks used in Experiments 2 and 4.

The higher performance of chicks in Experiment 4 (eggs laid at 52 weeks) in comparison to chicks from Experiment 3 (eggs laid at 42 weeks) might be a consequence of the lower egg production at 52 weeks. At lower egg production there is probably higher amounts of D_3 transferred to the eggs; therefore chicks used in Experiment 4 may have had higher D_3 reserves than chicks used in Experiment 3.

In agreement with several studies reported (Edwards et al., 1992; Edwards et al., 1994; Mitchell et al., 1997; Elliot and Edwards, 1997; Aburto et al., 1998; Kasim and Edwards, 2000; Edwards et al., 2002; McCormack et al., 2004) tibia ash in Experiments 3, 4, 5 and 6 increased without reaching a maximum as the vitamin D_3 in the chicks' diet increased. Tibia ash is one of the most sensitive criteria studied to the increasing level of vitamin D_3 in the chicks' diet. Similar to previous studies conducted by Murphy et al. (1936), Bethke et al. (1936), Griminger (1966) and Edwards (1995) increasing the level of vitamin D_3 in the maternal diet in the current work also had a positive effect on increasing bone ash of the progeny.

The occurrence of Ca rickets incidence in a 100% of the chicks (Experiments 1 and 2) suggests that no vitamin D metabolites were present in the basal diet and that the environment was free of UV-light. The role of vitamin D_3 on the development of rickets has been reported by Long et al. (1984). The effect of the maternal diet on tibia ash and Ca rickets incidence of the progeny throughout the different experiments in the current work was not consistent.

Similar to result of the current work, high incidence of TD has also been reported by several investigators. Edwards et al. (1992) observed 52% and 40% of TD in two experiments conducted in a UV-light free environment with chicks fed 0.95% of Ca and 2,000 IU/kg of D_3. Elliot and Edwards (1994) observed high incidence of TD in three strains of commercial broilers (58, 49 and 43%) fed a TD inducing diet. However, the lesion was very low in Athens Canadian Randombred (8%) and absent in Single Comb White Leghorn (0%). Mitchell et al. (1997) in an experiment conducted with chickens selected for high and low incidence of TD and fed various levels of vitamin D_3 (0 to 1,600 IU/kg) observed a high incidence of TD. The chicks fed the 1,600 IU/kg by Mitchell et al. (1997) had 53% and 33% of TD incidence and 24% and 13% of Ca rickets for the chicks selected for high and low incidence of TD, respectively. Elliot and Edwards (1997) also observed a high incidence of TD and Ca rickets in chickens raised in a UV-light free environment and fed various levels of vitamin D_3 (0 to 2,000 IU/kg of diet). The chicks fed the highest level of D_3 (2,000 IU/kg) had the lowest incidence of TD (50%) and Ca rickets (7%). Fritts and Waldroup (2003) observed a decrease in TD incidence and severity in broiler chicks fed a diet with the NRC (1994) recommendation of Ca and P, supplementing with 4,000 IU of vitamin D_3/kg. McCormack et al. (2004) reported that dietary supplementation with vitamin D_3 up to 10,000 IU/kg

completely prevented TD in 14-d-old chicks fed a diet containing 0.80% of Ca and 0.35% of available P or 1.00% of Ca and 0.50% of available P but not with a diet low in Ca and normal P which is known to increase the occurrence of TD (Edwards and Veltmann, 1983). Baker et al. (1998) in an experiment conducted in a UV-light free environment observed that chicks were able to tolerate high level of vitamin D_3 in diet (50,000 IU/kg).

The low incidence of TD in the chicks fed the lowest levels of vitamin D_3 in the current study (Experiments 3 and 4) may have been related to the poor growth rate. Several researchers have observed lower incidence of TD in chicks with poor growth rate than in chicks with rapid growth rate (Mitchell et al., 1997; Fritts and Waldroup, 2003; McCormack et al., 2004).

None of the previous studies reported the level of D_3 fed to the hens that produced their chicks. The NRC (1994) recommends 300 IU/kg for egg-type white egg breeders with feed intakes of 100 g of feed/hen/day. Suggested levels for broiler breeder in books and commercial guidelines vary from 1,000 (Scott et al., 1982) to 3,500 IU of vitamin D_3/kg (Ross Feeding Program Summary, 2000). In the current work, it is believed that not all chicks had the same vitamin D_3 in the yolk sac because they were hatched from hens fed various levels of vitamin D_3. This new variable in the current study (maternal D_3) limits the comparison between the effects of vitamin D_3 levels in the chick diet between studies.

An evaluation of the current study shows that increasing the level of vitamin D_3 in the maternal diet increased body weight gain of the progeny. The results from these studies, although not always statistically significant for tibia ash, TD and Ca rickets incidence throughout the different experiments with the progeny, suggest that the requirement of broiler breeder hens to produce the

heaviest and healthiest chicks is considerably higher than the requirement for maximum egg production of the hens (Atencio et al. 2004a,b) and higher than the NRC (1994) or Scott et al. (1982) recommendations, especially when birds are raised in environments having almost no measurable UV light.

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Table 1 – Composition of the basal diet

Ingredient	%
Corn, ground	52.87
Soybean meal (48.5 % CP)	38.00
Limestone	1.28
Dicalcium phosphate	1.86
Vegetable oil	5.00
Salt (NaCl)	0.450
Vitamin Premix[1]	0.250
Mineral Premix[2]	0.075
Dl-Methionine	0.220
Total	100.00
Calculated Composition[3]	
Crude Protein, %	23.2
ME, kcal/kg	3150
Ca, %	1.01
Total Phosphorus, %	0.73
Non-phytate phosphorus, %	0.49

[1] Vitamin premix provides the following (per kilogram of diet): Vitamin A (trans-retinyl acetate), 5500 IU; vitamin E(all-rac-tocopherol acetate), 11 IU; riboflavin (B_2), 4.4 mg; Ca pantothenate, 12 mg; pyridoxine HCl (B_6), 4.0 mg; nicotinic acid 44 mg; folic acid 3 mg; biotin 0.3 mg, thiamine mononitrate (B_1) 2.2 mg; vitamin B_{12} (cobalamin), 0.01 mg; menadione sodium bisulfate complex, 1.1 mg; choline chloride added to meet the requirement 220 mg; ethoxyquin, 125 mg.
[2] Mineral premix provides the following (per kilogram of diet): Manganese ($MnSO_4H_2O$), 60 mg; Iron ($FeSO_47H_2O$), 30 mg; Zinc (ZnO), 50 mg; copper ($CuSO_45H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide, 1.5 mg; Selenium, 0.3 mg.
[3] NRC (1994).

Table 2 – Body weight gain (BWG), gain:feed, feed intake, tibia ash, Ca rickets incidence and Ca rickets score of 16-d-old chicks fed a vitamin D_3 deficient diet and hatched from eggs laid by 27 week-old hens fed various levels of vitamin D_3 (Experiment 1).

D_3 level in the maternal diet	n[1]	BWG	Gain:feed	Feed intake	Tibia Ash	Ca rickets incidence	Ca rickets score[3]
(IU/kg)		(g)	(g/g)	g	%	%	
0	3	136±16[2]	0.582±0.066	233±07	22.87±.20	100	2.92±.04
125	3	166±13	0.624±0.013	266±16	22.20±.20	100	2.93±.07
250	3	154±10	0.699±0.056	221±08	22.70±.38	100	3.00±.00
500	3	137±07	0.604±0.048	229±15	20.83±.61	100	2.93±.07
1000	3	158±14	0.609±0.056	260±08	22.47±.20	100	2.89±.11
2000	3	179±06	0.675±0.021	265±02	22.93±.38	100	3.00±.00
Regression analyses							
	df	----------Probabilities----------					
Linear	1	0.1147	0.4329	0.1425	0.8149	-	0.7217
Quadratic	1	0.4684	0.7195	0.1358	0.0103	-	0.9166

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM
[3] Average score from chicks having Ca rickets lesions score 1 to 3.

Table 3 –Body weight gain (BWG), gain:feed, feed intake, tibia ash, Ca rickets incidence and Ca rickets score of 16-d-old chicks fed a vitamin D_3 deficient diet and hatched from eggs laid by 41 week-old hens fed various levels of vitamin D_3 (Experiment 2).

Maternal diet D_3 level	n[1]	BWG	Gain:feed	Feed intake	Tibia ash	Ca rickets incidence	Ca rickets score[3]	Plasma Ca
(IU/kg)		*(g)*	*(g/g)*	*(g)*	*(%)*	*(%)*		*(mg/100 mL)*
125	4	138±20[2]	0.600±.127	124±80	21.68±.94	100	3.00±.00	6.00±.00
250	4	180±18	0.588±.030	308±35	22.05±.27	100	2.94±.06	6.25±.46
500	4	205±23	0.691±.041	298±33	21.22±.45	100	2.95±.05	6.50±.70
1000	4	248±18	0.698±.070	359±15	21.47±.42	100	2.81±.09	5.94±.34
2000	4	217±15	0.638±.039	341±21	22.31±.24	100	2.86±.09	5.83±.25
4000	4	270±27	0.732±.015	369±36	23.38±.21	100	2.79±.08	6.23±.27
Regression analyses								
	df	----------Probabilities----------						
Linear	1	0.0009	0.0979	0.0011	0.0149	-	0.0647	0.8705
Quadratic	1	0.4431	0.8097	0.0522	0.0156	-	0.8514	0.8816

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM
[3] Average score from chicks having Ca rickets lesions score 1 to 3.

Table 4 – Regression analysis and main effect means of body weight gain (BWG), tibia ash, TD incidence and Ca rickets incidence of 16-d-old chicks fed various levels of D_3 in the diet (CD_3) and hatched from eggs laid by 29 week-old hens fed various levels of vitamin D_3 (MD_3) (Experiment 3)

D_3 level in chicks diet	D_3 level in maternal diet	n[1]	BWG	Tibia ash	TD incidence	Ca rickets incidence
(IU/kg)	*(IU/kg)*		*(g)*	*(%)*	*(%)*	*(%)*
0		3	180±5[2]	23.79±0.48	5.6±3.0	98.6±1.4
100		3	236±7	27.85±0.44	9.7±3.4	94.4±3.2
200		3	252±8	30.93±0.47	12.9±3.6	88.4±4.5
400		3	311±6	35.60±0.49	31.5±5.7	78.7±5.8
	0	12	243±15	29.23±1.36	10.4±4.5	97.2±2.8
	125	12	228±18	29.79±1.49	21.5±8.5	95.2±3.3
	250	12	241±14	28.74±1.35	15.9±5.2	89.6±4.8
	500	12	248±14	29.93±1.27	15.9±5.2	91.7±3.6
	1,000	12	251±16	30.10±1.41	16.6±6.0	86.8±6.7
	2,000	12	258±19	30.20±1.80	9.0±3.9	79.9±8.2
Multiple regression analysis		df	----------Coefficient (probabilities)----------			
Intercept		1	175.870715 (0.0001)	23.478826 (0.0001)	2.964920252 (0.5797)	100.22452836 (0.0001)
CD_3		1	0.440130 (0.0001)	0.042182 (0.0013	0.030287563 (0.5505)	-0.024011 (0.6163)
CD_3*CD_3		1	-0.000330 (0.1007)	-0.00003238 (0.1422)	0.000122045 (0.2850)	-0.000003 (0.9779)
MD_3		1	0.019497 (0.3488)	0.00148137 (0.2858)	0.011860992 (0.3182)	-0.0045429 (0.6848)
MD_3*MD_3		1	-0.000004 (0.6157)	-0.00000069 (0.2882)	-0.00000526 (0.3332)	0.0000017 (0.7356)
CD_3*MD_3		1	0.000008 (0.8151)	0.00000027 (0.9127)	-0.00002422 (0.2249)	-0.0000391 (0.0404)
R^2			0.73	0.84	0.30	0.30
CD_3 at maximum response			699	656	SP[3]	SP
MD_3 at maximum response			2,640	1,206	SP	SP
Predicted maximum			355	38.2	SP	SP
Root MSE			29.84	1.98	17.03	16.09
Simple regression analysis			----------Probabilities ----------			
CD_3 main effect						
Linear		1	0.0001	0.0001	0.0001	0.0006
Quadratic		1	0.8400	0.5145	0.0813	0.4994
MD_3 main effect						
Linear		1	0.2526	0.5715	0.6550	0.0154
Quadratic		1	0.6471	0.8484	0.1946	0.6571

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM
[3] SP (Saddle point) = Optimal not found

Table 5 – Regression analysis and observed main effect means of body weight gain (BWG), tibia ash, TD incidence and Ca rickets incidence of 16-d-old chicks fed various levels of D_3 in the diet (CD_3) and hatched from eggs laid by 36 week-old hens fed various levels of vitamin D_3 (MD_3) (Experiment 4)

D_3 level in chicks diet	D_3 level in maternal diet	n[1]	BWG	Tibia ash	TD incidence	Ca rickets incidence
(IU/kg)	*(IU/kg)*		*(g)*	*(%)*	*(%)*	*(%)*
0		3	180±9[2]	22.01±0.35	9.4±4.4	96.7±3.3
100		3	241±10	25.49±0.33	26.1±6.2	100.0±0.0
200		3	264±12	29.21±0.52	33.9±6.3	98.3±1.7
400		3	312±10	34.83±0.57	40.0±7.2	73.3±6.9
	125	12	227±14	28.33±1.28	22.2±8.0	95.8±4.2
	250	12	233±22	28.78±1.62	23.6±6.6	89.6±5.7
	500	12	247±20	26.81±1.46	28.5±5.8	95.8±2.8
	1,000	12	275±15	27.60±1.73	34.7±7.7	85.4±6.8
	2,000	12	264±16	27.89±1.41	27.8±9.5	93.8±6.3
Multiple regression analysis		df	----------Coefficient (probabilities)----------			
Intercept		1	143.0023968 (0.0001)	22.96832356 (0.0001)	7.142442810 (0.4521)	101.827406 (0.0001)
CD_3		1	0.5739828 (0.0001)	0.03812770 (0.0001)	0.137794444 (0.0765)	0.0709893 (0.1609)
CD_3*CD_3		1	-0.0005808 (0.0301)	-0.00001687 (0.1878)	-0.00024866 (0.1463)	-0.0003258 (0.0047)
MD_3		1	0.0975172 (0.0028)	-0.00290992 (0.0591)	0.018930426 (0.3513)	-0.0171672 (0.1981)
MD_3*MD_3		1	-0.0000334 (0.0173)	0.00000115 (0.0860)	-0.00001117 (0.2092)	0.0000075 (0.1994)
CD_3*MD_3		1	-0.0000286 (0..5358)	0.00000115 (0.6058)	0.000047113 (0.1180)	0.0000021 (0.9156)
R^2			0.69	0.89	0.26	0.38
CD_3 at maximum response			463	SP[4]	446	SP
MD_3 at maximum response			1,263	SP	1,788	SP
Predicted maximum			337	SP	54.8	SP
Root MSE			35.61	1.74	23.48	15.98
Simple regression analysis			----------Probabilities----------			
CD_3 main effect						
Linear		1	0.0001	0.0001	0.0006	0.0001
Quadratic		1	0.5346	0.0222	0.3923	0.00006
MD_3 main effect						
Linear		1	0.0398	0.6684	0.3594	0.6244
Quadratic		1	0.7548	0.6677	0.5935	0.5353

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM
[3] SP (Saddle point) = Optimal not found

Table 6 – Regression analysis and observed main effect means of body weight gain (BWG), tibia ash, TD incidence and Ca rickets incidence in 16-d-old chicks fed various levels of D_3 in the diet (CD_3) and hatched from eggs laid by 45 week-old hens fed various levels of vitamin D_3 (MD_3) (Experiment 5)

D_3 in the chicks diet	D_3 level in maternal diet	n[1]	BWG	Tibia ash	TD incidence	Ca rickets incidence
(IU/kg)	*(IU/kg)*		*(g)*	*%*	*%*	*%*
0		4	241±10[2]	23.4±0.3	26.3±4.9	95.8±2.3
200		4	318±8	30.7±0.5	39.2±4.7	97.5±2.5
400		4	328±9	33.0±0.5	45.4±6.0	85.4±6.0
800		4	373±7	37.1±0.6	47.9±6.9	64.2±6.5
1,600		4	387±6	38.4±0.4	23.3±4.8	34.6±5.8
3,200		4	406±8	39.5±0.3	7.5±3.2	21.3±4.9
	250	24	304±15	32.8±1.1	25.7±5.9	65.6±7.7
	500	24	328±14	33.4±1.3	34.0±5.7	71.9±7.3
	1,000	24	358±11	34.1±1.2	32.3±5.7	63.2±7.5
	2,000	24	350±12	33.7±1.2	39.2±5.2	72.2±7.2
	4,000	24	372±11	34.1±1.3	26.7±4.8	59.4±8.1
Multiple regression analysis		df	----------Coefficient (probabilities)----------			
Intercept		1	230.0952873 (0.0001)	25.72108447 (0.0001)	27.20889518 (0.0001)	99.97157718 (0.0001)
CD_3		1	0.1340155 (0.0001)	0.01367742 (0.0001)	0.00859195 (0.2883)	-0.0545427 (0.0001)
CD_3*CD_3		1	-0.0000278 (0.0001)	-0.00000308 (0.0001)	-0.00000518 (0.0271)	0.00000944 (0.0001)
MD^3		1	0.0432541 (0.0001)	0.00086544 (0.2939)	0.01472324 (0.0465)	0.00667564 (0.3238)
MD_3*MD_3		1	-0.0000061 (0.0109)	-0.00000017 (0.3582)	-0.00000326 (0.0459)	-0.00000178 (0.2348)
CD_3*MD_3		1	-0.0000026 (0.2212)	0.00000009 (0.5917)	-0.00000103 (0.4859)	-0.00000087 (0.5227)
R^2			0.73	0.80	0.23	0.65
CD_3 at maximum response			2,264	2,265	SP[3]	SP
MD_3 at maximum response			3,063	3,181	SP	SP
Predicted maximum			448	42.6	-	-
Root MSE			35.06	2.67	24.10	22.19
Simple regression analysis			----------Probabilities----------			
CD_3 main effect						
Linear		1	0.0001	0.0001	0.0018	0.0001
Quadratic		1	0.0004	0.0001	0.0001	0.0018
MD_3 main effect						
Linear		1	0.0001	0.4547	0.6764	0.6124
Quadratic		1	0.3835	0.7358	0.1091	0.4706

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM
[3] SP (Saddle point) = Optimal not found

Table 7 – Regression analysis and observed main effect means of body weight gain (BWG), tibia ash, TD incidence, Ca rickets incidence and plasma Ca of 16-d-old chicks fed various levels of D_3 in the diet (CD_3) and hatched from eggs laid by 52 week-old hens fed various levels of D_3 (MD_3) (Experiment 6)

D_3 in the chick diet	D_3 level in the maternal diet	n[1]	BWG	Tibia ash	Incidence of TD	Incidence of Ca rickets	Plasma Ca
(IU/kg)	*(IU/kg)*		*(g)*	*(%)*	*(%)*	*(%)*	*(mg/100 mL)*
0		4	285±10.3[2]	26.2±0.3	38.8±6.3	97.5±2.5	7.71±0.36
200		4	369±8.7	32.3±0.6	41.7±7.3	93.8±3.1	9.46±0.45
400		4	409±6.8	36.5±0.5	32.5±6.3	70.0±5.6	10.08±0.50
800		4	423±9.4	39.1±0.4	33.8±5.4	53.8±6.5	11.43±0.18
1,600		4	443±8.3	40.9±0.2	2.5±2.5	17.5±4.8	11.51±0.10
3,200		4	455±8.7	41.4±0.2	6.7±2.7	12.9±3.9	11.22±0.17
	250	24	384±15.6	34.7±1.1	28.1±6.1	63.5±8.2	9.91±0.50
	500	24	392±13.7	34.7±1.3	37.5±5.5	67.0±7.1	10.34±0.49
	1,000	24	406±12.6	36.0±1.2	20.5±5.1	52.1±8.4	10.04±0.59
	2,000	24	407±12.1	36.7±1.2	23.9±6.1	54.2±8.2	10.57±0.43
	4,000	24	426±12.3	37.3±1.1	19.8±5.7	51.1±8.1	10.31±0.48
Multiple regression analysis							
		df	----------Coefficient (probabilities)----------				
Intercept		1	280.5880	27.02029902	54.13426557	111.8433896	8.073377145
			(0.0001)	(0.0001)	(0.0001)	(0.0001)	(0.0001)
CD_3		1	0.1458	0.01417735	-0.03244855	-0.0750926	0.003806685
			(0.0001)	(0.0001)	(0.0001)	(0.0001)	(0.0001)
CD_3*CD_3		1	-0.00003	-0.00000315	0.00000568	0.0000147	-0.000000937
			(0.0001)	(0.0001)	(0.0166)	(0.0001)	(0.0001)
MD_3		1	0.0461	0.00197688	-0.00949749	-0.0120521	0.000467429
			(0.0003)	(0.0090)	(0.2018)	(0.0525)	(0.3553)
MD_3*MD_3		1	-0.000006	-0.00000027	0.00000119	0.0000019	-0.000000095
			(0.0208)	(0.0991)	(0.4677)	(0.1556)	(0.3841)
CD_3*MD_3		1	-0.000004	-0.00000017	0.00000131	0.0000002	0.000000024

		(0.1011)	(0.2656)	(0.3801)	(0.8690)	(0.8096)
R^2		0.66	0.83	0.29	0.74	0.58
CD_3 at maximum response		2,125	2,175	2,559	2,536	2,066
MD_3 at maximum response		2,907	2,934	2,579	2,968	2,714
Predicted maximum		502	45.3	0.36	-1.23	12.64
Root MSE		40.88	24.37	20.27	2.44	1.1439
Simple regression analysis		---------- Probabilities ----------				
CD_3 main effect						
Linear	1	0.0001	0.0001	0.0001	0.0001	0.0001
Quadratic	1	0.0001	0.0001	0.0994	0.2033	0.0001
MD_3 main effect						
Linear	1	0.0003	0.0623	0.0970	0.1378	0.5167
Quadratic	1	0.3008	0.9099	0.7573	0.8988	0.7728

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM

Table 8 – Overall experiments means of body weight gain (BWG), tibia ash, TD incidence, Ca rickets incidence and plasma Ca of 16 days old chicks hatched from eggs laid by hens at 27, 41, 29, 36, 45 and 52 weeks of age (Experiments 1, 2, 3, 4, 5 and 6, respectively).

Hens age	Experiment	Number of chicks	BWG	Feed intake	Feed efficiency	Tibia ash	Incidence of TD	Incidence of Ca rickets	Plasma Ca
(wk)			*(g)*	*(g)*		*(%)*	*(%)*	*(%)*	*(mg/100 mL)*
27	1	180	159±6.0	248±7.0	0.642±0.019	22.23±0.25	-	100	-
41	2	240	204±11	286±26	0.648±0.024	21.77±0.19	-	100	6.07±0.18
29	3	288	245±7.3	344±9.0	0.711±0.026	29.65±0.57	15.83±2.62	88.62±2.53	-
36	4	240	249±7.9	364±17	0.697±0.015	27.88±0.66	27.36±3.33	92.08±2.37	-
45	5	480	342±5.99	434±12	0.785±0.010	33.59±0.54	31.59±2.45	66.46±3.36	-
52	6	480	397±6.38	514±14	0.770±0.005	36.02±0.53	25.97±2.58	57.57±3.57	10.23±0.22

Experiments 1 and 2 do not include the 0 and 4,000 maternal D_3 levels.
Experiments 3 and 4 do not include the 0 maternal D_3 level.

Table 9 – Summary of main effects of the vitamin D_3 levels in the chick diet and in the maternal diet of 16-d-old chicks hatched from eggs laid by hens at 27, 41, 29, 36, 45 and 52 weeks of age (Experiments 1, 2, 3, 4, 5 and 6, respectively).

	Main effect simple regression (chick D_3)				Main effect simple regression (maternal D_3)					
Hens age (week)	29	36	45	52	27	41	29	36	45	52
Experiment	3	4	5	6	1	2	3	4	5	6
Criteria										
Body weight gain	L	L	L,Q	L,Q	NS	L	NS	L	L	L[3]
Feed intake	L	L	L	L,Q	NS	L	-	-	-	-
Feed efficiency	L	L,Q	L,Q	L,Q	NS	L	-	-	-	-
Tibia ash	L	L,Q	L,Q	L,Q	Q	L,Q	NS	NS	NS	L
TD incidence	L	L	L[1],Q	L[1],Q	-	-	NS	NS	NS	L[1 2]
Ca rickets incidence	L[1]	L[1]	L[1],Q	L[1]	NS	NS	L	NS	NS	L[1 3]
Ca Plasma	-	-	-	L,Q	-	NS	-	-	-	NS

L = Linear increase (P<0.05)
Q = Quadratic effect (P<0.05)
[1] Linear decree se
[2] (P<0.10)
[3] (P<0.13)

Figure 1 – Body weight gain of 16-d-old chicks fed increasing level of D_3 and hatched from eggs laid by hens fed various levels of vitamin D_3 (Experiment 6).

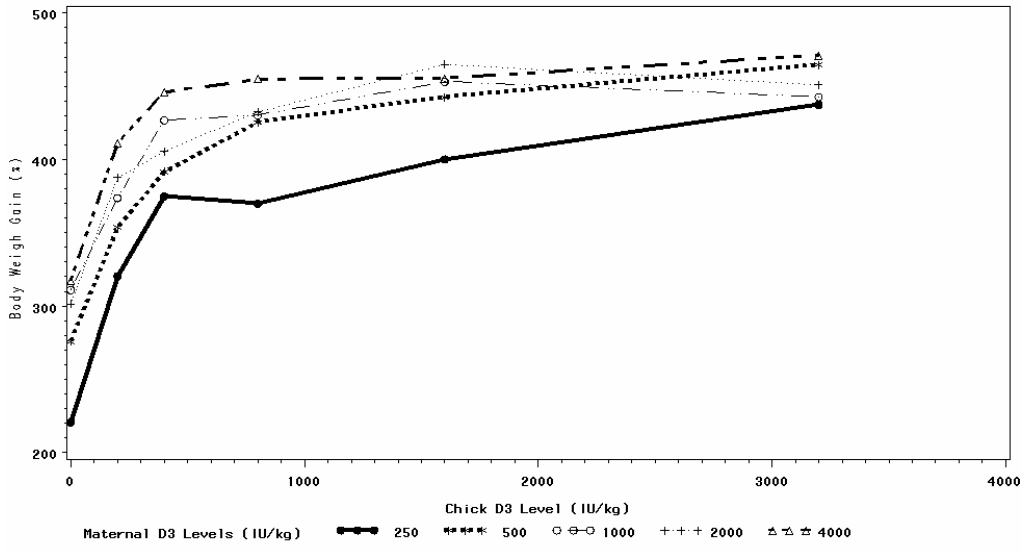


Figure 2 – Surface response representation of data from Experiment 6, predicting 16-d body weight gain against the levels of vitamin D_3 in the chicks' diet and in the maternal diet. A) Contour plot; B) response surface

A B

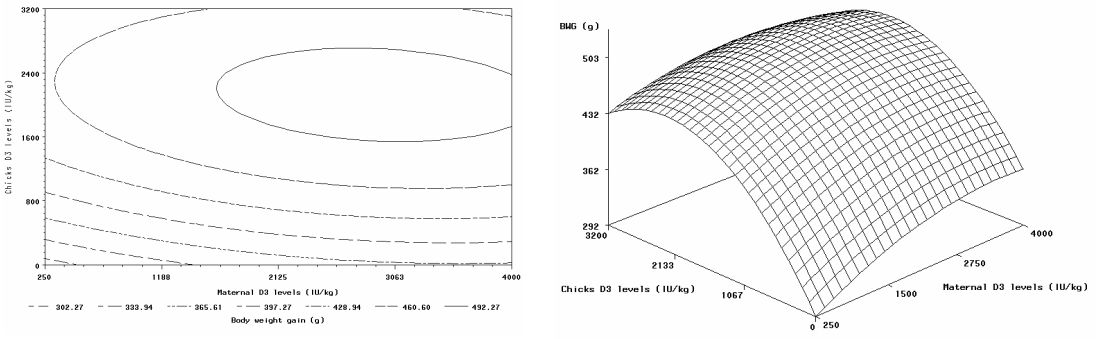
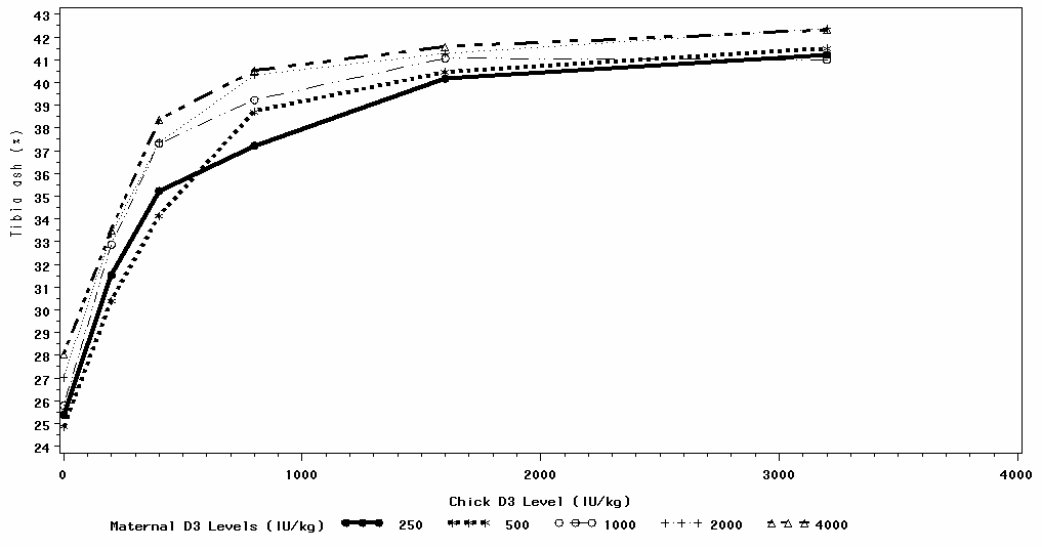


Figure 3 – Tibia ash of 16-d-old chicks fed increasing level of D_3 and hatched from eggs laid by hens fed various levels of vitamin D_3 (Experiment 6).



CHAPTER 5

EFFECT OF DIETARY VITAMIN D_3 LEVEL ON THE PERFORMANCE AND BONE ABNORMALITIES OF BROILER BREEDER HENS PROGENY FED VARIOUS LEVELS OF CALCIUM OR 25-HYDROXYCHOLECALCIFEROL[1]

[1] Aencio, A., G.M. Pesti and H.M. Edwards Jr. To be Submitted to Poultry Science.

ABSTRACT

Four experiments were conducted using Ross x Ross chicks hatched from broiler breeder hens fed various levels of vitamin D_3 to determine the effect of the maternal diet on the performance and leg abnormalities of the progeny. Six levels of D_3 (0, 125, 250, 500, 1,000 and 2,000 IU/kg of diets) were fed to the hens from 25 to 66 weeks of age, however at 36 weeks of age hens that were receiving the 0 level of D_3 were switched to a diet containing 4,000 IU/kg. Chicks hatched from eggs laid when the hens were 30, 37, 49 and 55 weeks of age were used in Experiments 1, 2, 3 and 4, respectively. The studies were conducted in an ultraviolet light free environment as split plot designs, with Ca levels and 25-OHD_3 in the chicks' diet as the whole plot and D_3 in the maternal diet as a subplot. Chicks in Experiments 1 and 2 were fed two levels of Ca (0.63% and 0.90%) and chicks in Experiments 3 and 4 were fed six levels of 25-OHD_3 (0, 2.5, 5, 10, 20 and 40 µg/kg of diet). Interactions between Ca levels and maternal D_3 were observed in Experiment 2 and between 25-OHD_3 and maternal D_3 in Experiment 4 for body weight gain. Linear positive effects in body weight gain at both levels of Ca were observed as the vitamin D_3 level in the maternal diet increased (Experiment 2). In Experiment 4, the predicted maximum body weight gain was reached at 25 µg of 25-OHD_3 per kg of diet and 1,202 IU of maternal D_3 per kg. Significant increases in body weight gain of the progeny were observed in Experiments 1, 2 and 4 as the vitamin D_3 level in the maternal diet increased. Chicks hatched from eggs laid by hens fed the highest levels of D_3 generally had the highest tibia ash. Significant reductions in Ca rickets incidence (Experiments 1 and 2) and tibial dyschondroplasia (TD) incidence (Experiment 1) were observed as the level of vitamin D_3 in the maternal diet increased. Chicks fed lower levels of Ca had lower body weight gains and tibia ash and higher incidences of

TD and Ca rickets than chicks fed higher levels of Ca (Experiment 1 and 2). Increasing the level of 25-OHD_3 in the chicks' diet significantly improved body weight gain, tibia ash and plasma Ca and reduced TD and Ca rickets incidence in both Experiments, 3 and 4. An overall evaluation of the study indicates that chicks hatched from eggs laid by hens fed the highest levels of vitamin D_3 and fed high levels of Ca or 25-OHD_3 had the highest body weight gains, tibia ash and plasma Ca, and the lowest incidences of TD and Ca rickets.

Key Words: broiler breeders, progeny, Ca, 25- hydroxycholecalciferol

INTRODUCTION

The effect of cholecalciferol (vitamin D_3) in the maternal diet on body weight, tibia ash and bone abnormalities of progeny has been shown in laying type hens by Murphy et al. (1936), Bethke et al. (1936a,b), Griminger, (1966) and Edwards (1995) and in turkey poults by Robertson et al (1941), Stadelman et al, (1950) and Stevens et al (1984). However, no reports have been made using the progeny of broiler breeders even though bone abnormalities in rapidly growing broilers is still a problem in the poultry industry. Many experiments have been conducted manipulating the diet of young broiler chicks with the objective of reducing bone disease; however, no relation has been made with the level of vitamin D_3 fed to the hens that produced those chicks.

Edwards and Veltmann (1983) showed the effect of feeding different levels of Ca and P on the incidence of tybial dyschondroplasia (TD) and also showed that chicks fed low levels of calcium (Ca) and normal levels of phosphorus (P) develop TD. A high incidence of TD has also been observed by Edwards et al. (1984) in chicks fed low level of Ca (0.44%) with low level total phosphorus (0.52%). Long et al. (1984a, 1984b and 1984c) in experiments conducted with broiler chickens fed a P rickets-inducing diet (low level of P and normal Ca or high level of Ca and normal P) and a Ca rickets-inducing diet (vitamin D or Ca deficient) described the histological changes in the proximal tibia growth plate. Edwards et al. (2004) observed a high incidence of Ca rickets in chicks fed 0.65% of Ca, 0.49% of available P and various levels of 1-alpha hydroxycholecalciferol [1-αOHD_3 (0 to 20 µg/kg of diet)]. Many studies have shown the beneficial effects of high levels of vitamin D_3, 25-hydroxycholecalciferol (25-OHD_3), 1-αOHD_3, 1,25-dihdroxycholecalciferol [$1,25(OH)_2D_3$] and ultraviolet (UV) light irradiation on the increase of tibia ash and

reductions of TD and Ca rickets incidences (Boris et al., 1977; Edwards et al., 2002 Fritts and Waldroup, 2003; Ledwaba and Roberson, 2003, Edwards, 2003). Boris et al. (1977) and Edwards et al. (2002) reported higher potencies of 1-αOHD$_3$ than 25-OHD$_3$ and higher potencies of 25-OHD$_3$ than D$_3$; however the potency between these D metabolites depends on the levels used in the diet. Potency is indirectly proportional to dietary level. Boris et al. (1977), Fritts and Waldroup (2003) and Atencio et al. (2004a) observed higher potencies of 25-OHD$_3$ than D$_3$ at low levels of supplementation compared to higher levels. The D metabolite, 25-OHD$_3$ has been shown to improve tibia ash and lessen the incidence and severity of TD and Ca rickets in broiler chicks; however, other works have shown no reduction in TD incidence in broiler chicks selected for high incidence of TD by supplementing 25-OHD$_3$ to the diet (Mitchell et al., 1997; Elliot and Edwards (1997); Zhang et al., 1997). Edwards et al. (1992, 1994), Edwards (2000 and 2003), and Ledwaba and Roberson (2003) have shown the beneficial effect of UV-light irradiation on reducing bone disease in chickens. In recent experiments conducted in our laboratory, Driver et al. (2004) observed higher body weight gain and tibia ash and lower bone abnormalities in chicks hatched from eggs laid by broiler breeder hens fed 2,000 IU of vitamin D$_3$ than in chicks from hens fed 250 IU of vitamin D$_3$ per kg of diet. The objective of this study was to test the effects of various levels of vitamin D$_3$ fed to broiler breeder hens on the performance and leg abnormalities of their progeny fed various levels of Ca and 25-OHD$_3$.

MATERIALS AND METHODS

Four experiments were conducted for periods of 16 days each using mixed-sex 1-d-old Ross x Ross[2] chicks hatched from eggs laid by hens fed various levels of vitamin D_3. The levels of vitamin D_3 used in the maternal diet and the conditions in which the hens were raised are reported by Atencio et al. (2004a, 2004b). Six levels of D_3 (0, 125, 250, 500, 1,000 and 2,000 IU/kg of diets) were fed to the hens from 25 to 66 wk of age, however, the hens that were receiving the 0 level of D_3 were switched to a diet containing 4,000 IU/kg at 36 wk of age.

Chicks hatched from eggs laid when the hens were 30, 37, 49 and 55 weeks of age were used in Experiments 1, 2, 3 and 4, respectively. The basal diet fed to the chicks was based on corn and soybean meal (Table 1). No animal byproduct was used in the basal diets to guarantee that no unintentional vitamin D activity was present (Mattila, 1995 and Atencio et al., 2003). Diets in Experiments 1 and 2 had 1,100 IU/kg of vitamin D_3, while diets in Experiments 3 and 4 were formulated with no D_3 supplementation.

Chicks in Experiments 1 and 2 were fed two levels of Ca (0.63 and 0.90%) and normal nonphytate phosphorus (0.49%). The low Ca and normal phosphorus diet is known to induce TD in broiler chicks (Edwards and Veltmann, 1983; Edwards et al., 1984). Chicks in Experiments 3 and 4 were fed the basal diet supplemented with six levels of 25-OHD_3 (0, 2.5, 5, 10, 20, 40 µg/kg). Diets were mixed at the beginning of each experiment.

Chicks were identified by maternal diet; therefore, every experiment had two independent variables. No chicks were used from hens receiving no D_3 in

[2] Ross Breeders, Inc. Huntsville, Alabama 35805

Experiments 2, 3 and 4 and no chicks were used from hens fed 125 or 4,000 IU of vitamin D_3 per kg of diet in Experiments 1 and 4, respectively. The reasons for not using chicks from hens fed 0 or 125 IU/kg of vitamin D_3 was because of the absence of the 0 level (treatment) after 36 weeks of age, and low hatchability, respectively.

After hatching, three, four or five chicks from each maternal group according to hatchability, were randomly selected, banded and weighed and randomly allocated to each of the 12 pens in Experiments 1 and 2 and each of the 24 pens in Experiments 3 and 4; thus all experiments consisted of a split plot design. The number of chicks used in Experiments 1, 2, 3 and 4 were 288, 288, 432 and 528, respectively.

All experiments were conducted in electrically heated wire mesh-floored battery brooders[3] located in a room that excluded UV-light. Ultraviolet irradiation was eliminated from the chick room and battery pens by fitting Arm-a-Lite[4] sleeves to all fluorescent fixtures in the room and battery brooders (Edwards et al., 1994). The fluorescent lights were on 24 h each day. The temperature of the room was maintained at 22 ºC. All experiments were conducted using the same environmental conditions. Chicks received water and feed *ad libitum*.

On the last day of each experiment, two chicks in Experiments 3 and 4 were randomly selected for plasma samples that were analyzed for Ca[5]. Blood samples were obtained by heart puncture. On the same day all chicks were weighed and then killed by carbon dioxide asphyxiation and examined at random for TD and Ca rickets. A longitudinal cut was made across the right tibia and diagnoses for TD and Ca rickets were made based on the width of the proximal growth plate band and its appearance according to the methodology described by Edwards and Veltmann (1983)

[3] Petersime Incubator Co., Gettysburg, OH 54328.
[4] Arm-a-Lite® Thrermoplastic Processes, Sterling, NJ 07980.
[5] Section N-31 Technicon Autoanalyzer Methodology, Technicon Corp., Tarrytown, NY 10591.

and Long et al. (1984). The left tibia was removed to determine bone ash content on a dry fat-free basis (Association of Official Analytical Chemists, 1995). Feed intake and feed efficiency were calculated only for the main effect of the Ca level in Experiments 1 and 2.

Statistical Analysis

Analyses were conducted using the mean of each replicate as an observation. Analysis of variance and regression analyses were completed in Experiments 1 and 2. Linear and quadratic effects for level of vitamin D_3 in the maternal diet were performed by Ca level when interactions were present ($P<0.05$) or without Ca level when the interaction was not significant. Multiple factor and simple factor regression analysis were used in Experiments 3 and 4. The simple factor regression was used when the regression coefficient for the interaction, 25-OHD_3 by maternal D_3, in the multiple factor regression was not significant at a probability value lower than 0.10. In all experiments, linear or quadratic effects were considered present at a probability value lower or equal to 0.05. All analyses were performed using the General Linear Models procedure of SAS (SAS Institute, 1998). The probabilities for the regression coefficients of 25-OHD_3 and 25-OHD_3^2 were found using the F value calculated by dividing the mean square by the mean square of the pen within vitamin D_3 treatment. All others probabilities were calculated using an F-value found using the residual. All coefficients of determination (r^2) were calculated using the sum of squares of the regression divided by the total sum of squares.

RESULTS

Experiment 1

No interactions between Ca and the maternal diet were observed for any of the criteria measured (Table 2). In general, chicks hatched from eggs laid by hens fed the highest levels of vitamin D_3 had the highest body weights, tibia ash and the lowest TD incidence, TD score, Ca rickets incidence and Ca rickets score. Body weight gain increased and TD incidence, TD score, Ca rickets incidence and Ca rickets score decreased as the level of D_3 in the maternal diet increased ($P<0.01$).

Ca levels significantly affected all criteria measured, except body weight gain ($P<0.17$). Chicks fed 0.90% Ca had higher tibia ash and lower TD incidence and score and lower Ca rickets. Significant differences were observed between the low and the high levels of Ca on feed efficiency ($P<0.06$). The average values were 0.747±0.013 vs 0.713±0.009 for the low and high level Ca, respectively. Chicks fed 0.63% of Ca consumed significantly ($P<0.05$) less feed (390.9±9) than those fed 0.90% of Ca (432±9).

Experiment 2

Chicks hatched from eggs laid by hens fed 4,000 IU of vitamin D_3/kg had intermediate body weight gain, tibia ash, TD and Ca rickets incidence when compared with the chicks from the other treatments. The reason for these intermediate values might have been due to the hens having received only one week of the 4,000 IU of vitamin D_3; therefore, chicks hatched from eggs laid by these hens were not included in the statistical analysis. An interaction between Ca and maternal diet ($P<0.001$) was observed for body weight gain (Table 3). Body weight gain increased as the levels of vitamin D_3 increased in the maternal diet at

both levels of Ca ($P<0.01$); however, weight gain was higher in the chicks fed 0.90% of Ca (Figure 1), except at the highest maternal D_3 level. Similar to Experiment 1, chicks hatched from eggs laid by hens fed the highest levels of D_3 had the highest tibia ash ($P<0.007$) and the lowest TD ($P<0.002$) and Ca rickets ($P<0.21$) incidences and TD score ($P<0.001$) regardless of the Ca level.

The main effect of Ca level was significant ($P<0.03$) for all criteria measured. Chicks fed 0.90% Ca had higher tibia ash and lower incidence of TD, incidence of Ca rickets, TD score and Ca rickets score.

Significant differences were observed between the two Ca levels for feed intake ($P<0.01$). Chicks fed 0.63% of Ca consumed less feed (430±7) than the one fed 0.90% of Ca (478±3). No differences were observed between the two levels of Ca on feed efficiency. The average values were 0.750±0.013 vs 0.738±0.015 for the 0.63% and 0.90% of Ca, respectively.

Experiment 3

No significant effects in the regression coefficient for 25-OHD_3 by maternal D_3 were observed for any of the criteria studied (Table 4). Linear and quadratic effects were observed for all the criteria as 25-OHD_3 increased in the chicks' diet, except Ca rickets which responded only in a negative linear manner. No significant effects of the maternal diet were observed for any of the criteria; however, chicks hatched from eggs laid by hens fed high level of vitamin D_3 had generally higher body weight ($P<0.1030$) and tibia ash ($P<0.6850$) and lower TD ($P<0.1131$) and Ca rickets ($P<0.08$) incidences.

Experiment 4

A significant interaction in the regression coefficient for 25-OHD_3 by maternal D_3 was observed for body weight gain (Table 5). This interaction indicates that at

different levels of 25-OHD_3 in the chick diets, or D_3 in the maternal diets, will result in different responses in body weight gain of the progeny. The combination of 25-OHD_3 and maternal D_3 that resulted in the predicted maximum body weight gain (490 g) using a multiple regression equation was 25 µg and 1,282 IU/kg, respectively (Figure 2).

No effect of the maternal diet was observed on TD incidence ($P<0.51$), Ca rickets incidence ($P<0.61$) and plasma Ca ($P<0.89$). Similar to Experiment 3, linear and quadratic effects were observed for all criteria measured as 25-OHD_3 increased in the chicks' diet, except Ca rickets incidence which had only a linear increase, but not quadratic.

Comparative results from all four experiments

In general chicks fed diets high in Ca or 25-OHD_3 and hatched from eggs laid by hens fed higher levels of vitamin D_3 had higher body weight gains and tibia ash and lower incidences of TD and Ca rickets than chicks fed low levels of Ca or 25-OHD_3 and hatched from eggs laid by hens fed low levels of vitamin D_3. Significant effects of the maternal diet were observed on body weight gains and TD incidence (Experiments 1 and 2), tibia ash (Experiment 2) and Ca rickets incidence (Experiment 1). No significant effects of the maternal diet were observed in any of the criteria studied in Experiment 3 and 4, except body weight gain in Experiment 4. Even though body weight gain numerically increased in Experiment 3 as the D_3 in the maternal diet increased, no significance difference was observed at a probability value lower than 0.10 (Table 6).

No differences were observed for the different criteria between Experiments 1 and 2; however large differences in TD and Ca rickets incidence were observed between Experiments 3 and 4 (Table 7). The average TD incidence in Experiment 3 was 61% while in Experiment 4 was 19%. The opposite was observed for Ca rickets incidence, Experiment 3 had higher incidence (23%) than Experiment 4 (50%).

DISCUSSION

The positive effect of the maternal diet on body weight gains, tibia ash and leg disease of the progeny in the current work are similar to the result reported by Driver et al. (2004) using chicks hatched from eggs laid by the same hens. Murphy et al. (1936), Griminger, (1966), Bethke et al. (1936) and Edwards et al. (1995) in experiments conducted with the progeny of laying hens and Robertson et al (1941), Stadelman et al, (1950) and Stevens et al. (1984) in experiment conducted with the progeny of turkey breeders observed higher performance and tibia ash in chicks and poults hatched from eggs laid by hens fed higher level of vitamin D_3.

In previous studies conducted in our laboratory, chicks hatched from eggs laid by the same hens, increases in body weight were observed as the vitamin D_3 level in the maternal diet increased. However, in the current work body weight reached a plateau when the maternal D_3 levels in the diet were high (Tables 4 and 5). The main differences between the two studies were that in the previous study chicks were fed various levels of D_3 and in the current work chicks were fed various levels of 25-OHD_3. In agreement with the results of Experiment 3, no effect of the maternal diet on body weight, tibia ash and Ca rickets was observed by Edwards et al. (2004) in an study conducted with chicks fed various levels of Ca and 1-αOHD_3 and hatched from

eggs laid by hens fed various levels of vitamin D_3 (1,000, 2,000, and 4,000 IU/kg of diet). These results suggest that when 25-OHD_3 (Experiments 3 and 4) or 1-αOHD_3 (Edwards et al. 2004) gets high in the chicks' diet, the effect of the maternal diet was reduced and may have been eliminated due to the high potency of 25-OHD_3 and 1-αOHD_3. Differences between Experiment 3 and 4 and those of Edwards et al. (2004) may have been due to the levels of vitamin D sources added. Studies have shown different potencies for D_3, 25-OHD_3 and 1-αOHD_3. Boris et al. (1977) reported the potency of these D metabolites for tibia ash as follows: 1-αOHD_3 > 25-OHD_3 > D_3. A higher potency of 1- αOHD_3 in comparison to D_3 has also been reported by Edwards et al. (2002).

The consistent low body weight gain, tibia ash and higher TD and Ca rickets in chicks hatched from eggs laid by hens fed low levels of D_3 in the current work suggest that chicks might have lower vitamin D contents in the yolk sac as a consequence of the low D_3 transfer from the dam to the egg. Mattila et al. (1999) showed a positive relationship between the vitamin D_3 level in the diet of laying hens and the level of D_3 and 25-OHD_3 in the egg yolk.

Similar to the experiment conducted by Edwards and Veltmann (1983) the TD inducing diet of the current study (0.63% Ca and 0.49% nonphytate P) had lower body weight gain, low tibia ash and high TD incidence and Ca rickets than the chicks fed the higher level of Ca (0.90%). Edwards (1984) also observed a high incidence of TD (74%), low tibia ash and body weight in chicks of different strains fed a diet low in Ca (0.44%) and low level of total phosphorus (0.52%). The results of the current studies suggest that the diet low in Ca (0.63%) and normal in nonphytate phosphorus (0.49%) was effective in inducing TD. Ca rickets and TD incidence were also observed in chicks fed the 0.90% Ca, 0.49% nonphytate P and 1,100 IU of vitamin D_3

per kg of diet. The NRC (1994) recommendation for Ca and nonphytate phosphorus for young broilers (0 to 3 weeks of age) is 1.00% and 0.45%, respectively. Recently, McCormack et al. (2004) reported that dietary supplementation of vitamin D_3 up to 10,000 IU per kg completely prevented TD in diets containing 0.80% Ca/0.35% of nonphytate and 1.00% Ca and 0.50% nonphytate P. A high incidence of TD was also observed by Driver et al. (2004) in chicks hatched from eggs laid by hens fed 2,000 IU of vitamin D_3/kg and fed a diet with 1.00% Ca, 0.45% nonphytate P and 1,100 IU vitamin D_3 per kg of diet. A high incidence and severity of Ca rickets and low tibia ash were also observed by Edwards et al. (2004) by feeding chicks a low level of Ca. The results from the current work demonstrated that the amount of vitamin D_3 transfer from the dam to the egg was not enough to prevent TD and Ca rickets under all circumstances.

Mitchell et al. (1997), Zhang et al. (1997), Fritts and Waldroup (2003) and Ledwaba and Roberson (2003) reported that as the levels of 25-OHD_3 increased in the diet body weight and tibia ash increased and TD incidence and severity decreased; however no reduction in TD was observed in chickens genetically selected to develop TD. In the current study 25-OHD_3 in the chicks diet was effective in improving body weight gain, tibia ash, plasma Ca and reducing TD and Ca rickets; however TD incidence and Ca rickets were still present regardless of the levels of 25-OHD_3 supplemented to the diet (Tables 4 and 5).

The effect of 25-OHD_3 on the different criteria measured in the current work is in agreement with the study conducted by Boris et al. (1977), Mitchell et al. (1997) and Ledwaba and Roberson (2003) even though no report is made on the level of D_3 fed to the hens that produced their chicks. Although no work has been reported on the vitamin D_3 requirement of broiler breeder hens the NRC (1994) refers the reader to

the requirement for egg-type white egg breeders which is 300 IU of vitamin D_3/kg diet for a hen with a 100 g of feed intake per day. Suggested levels for broiler breeders in books and commercial guidelines vary from 1,000 (Scott et al., 1982) to 3,500 (Ross Feeding Program Summary, 2000)[6] IU of vitamin D_3/kg. In the current work it is believed that not all chicks had the same vitamin D content in the yolk sac, because they were hatched from eggs laid by hens fed various levels of vitamin D_3. This new variable in the current study (maternal D_3) limits the comparison between studies. An evaluation of the current work shows that increasing the level of vitamin D_3 in the maternal diet was effective in improving performance, tibia ash and in reducing leg abnormalities of the progeny fed the different levels of Ca. Although no significant effects were observed for the maternal diet on most of the criteria measured when 25-OHD_3 was supplemented to the chicks' diet, increasing the level of vitamin D_3 in the maternal diet improved body weight gain, tibia ash and the general health of the progeny. This study suggests that the vitamin D_3 requirement of broiler breeders hens to produce good chicks is higher than the NRC (1994) or Scott et al. (1982) recommendations.

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Table 1 – Composition of the basal diets

	Experiments 1 and 2		Experiments 3 and 4
	Low Ca (0.63%)	Marginal Ca (0.90%)	D_3 deficient diet
Ingredient	%	%	%
Corn, ground	54.86	54.15	52.87
Soybean meal (48.5% CP)	37.00	37.00	38.00
Vegetable oil	5.00	5.00	5.00
Dicalcium phosphate	1.86	1.86	1.86
Limestone	0.28	0.99	1.28
Salt (NaCl)	0.45	0.45	0.450
Vitamin Premix[1]	0.250	0.250	0.250
Mineral Premix[2]	0.100	0.100	0.075
Dl-Methionine	0.200	0.200	0.220
Total	100	100	100.00
Calculated Composition[3]			
Crude Protein, %	22.9	22.83	23.2
ME, cal/kg	3.158	3.134	3.150
Ca, %	0.63	0.90	1.01
Total Phosphorus, %	0.72	0.72	0.73
Non-phytate phosphorus, %	0.49	0.49	0.49

[1] Vitamin premix provides the following (per kilogram of diet): Vitamin A (trans-retinyl acetate), 5500 IU; vitamin E(all-rac-tocopherol acetate), 11 IU; riboflavin (B_2), 4.4 mg; Ca pantothenate, 12 mg; pyridoxine HCl (B_6), 4.0 mg; nicotinic acid 44 mg; folic acid 3 mg; biotin 0.3 mg, thiamine mononitrate (B_1) 2.2 mg; vitamin B_{12} (cobalamin), 0.01 mg; menadione sodium bisulfate complex, 1.1 mg; choline chloride added to meet the requirement 220 mg; ethoxyquin, 125 mg.

[2] Mineral premix provides the following (per kilogram of diet): Manganese ($MnSO_4H_2O$), 60 mg; Iron ($FeSO_47H_2O$), 30 mg; Zinc (ZnO), 50 mg; copper ($CuSO_45H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide, 1.5 mg; Selenium, 0.3 mg.

[3] NRC (1994).

Table 2 – Body weight gain (BWG), tibia ash, TD incidence, TD score, Ca rickets incidence, Ca score and plasma Ca of 16-d-old chicks fed two levels of Ca and hatched from eggs laid by 30 weeks old hens fed various levels of vitamin D_3 (MD_3) (Experiment 1)

Progeny Dietary Ca	Maternal D_3	n[1]	BWG	Tibia ash	Incidence of TD	TD score	Incidence of Ca rickets	Ca rickets score
(%)	(IU/kg of diet)		(g)	(%)	(%)		(%)	
0.63	0	6	265±15[2]	34.03±0.82	91.7±5.3	2.83±0.12	70.8±10.0	1.06±0.15
0.63	125	6	283±8	35.67±0.35	95.8±4.2	2.96±0.04	54.2±14.3	0.74±0.20
0.63	250	6	269±13	35.45±1.06	54.2±13.6	1.97±0.37	68.2±3.9	0.95±0.08
0.63	500	6	313±10	35.02±0.45	58.3±11.4	2.08±0.23	73.7±13	1.00±0.19
0.63	1000	6	289±17	33.98±0.44	61.2±8.3	2.07±0.28	51.3±5.5	0.72±0.17
0.63	2000	6	333±5	36.45±0.58	65.0±10.0	2.35±0.24	38.8±8.3	0.49±0.08
0.90	0	6	303±14	36.63±1.32	41.7±8.3	1.71±0.18	37.5±5.6	0.58±0.14
0.90	125	6	293±15	37.87±0.36	61.2±10.5	2.24±0.22	62.5±10.7	0.83±0.08
0.90	250	6	301±16	38.14±1.01	25.0±0.0	1.11±0.15	37.5±10.7	0.63±0.24
0.90	500	6	326±12	38.82±0.65	31.3±6.3	1.04±0.22	29.2±10.0	0.33±0.11
0.90	1000	6	321±9	37.77±0.88	31.3±6.3	1.04±0.22	20.8±7.7	0.25±0.11
0.90	2000	6	312±12	38.38±0.96	25.0±0.0	1.00±0.20	34.7±5.0	0.40±0.12
Main effect means								
	0	12	284±11	35.59±0.92	66.7±8.9	2.27±0.20	54.2±7.4	0.82±0.12
	125	12	288±8	36.77±0.41	78.5±7.5	2.60±0.15	58.3±5.6	0.78±0.10
	250	12	285±11	36.67±0.82	42.5±9.2	1.54±0.23	52.8±7.1	0.79±0.13
	500	12	320±8	36.92±0.69	47.5±8.3	1.56±0.22	51.4±10.3	0.67±0.14
	1000	12	305±10	35.88±0.74	49.2±7.2	1.56±0.23	36.1±6.4	0.49±0.12
	2000	12	322±7	37.33±0.59	45.0±8.2	1.67±0.25	36.8±4.6	0.45±0.07
0.63		36	292±6	35.16±0.29	71.2±4.5	2.38±2.38	59.5±4.3	0.82±0.07
0.90		36	309±5	37..91±0.36	37.7±3.7	1.36±1.36	37.0±3.9	0.51±0.07
ANOVA		df	----------Probabilities ----------					
Whole Plot								
Ca level		1	0.1712	0.0001	0.0001	0.0001	0.0005	0.0064

Pen (Ca level)	10	0.0013	0.3927	0.7165	0.9970	0.7762	0.3661
Split-plot							
MD_3 Level	5	0.0004	0.1589	0.0004	0.0001	0.1013	0.0708
Ca`*x*`MD_3 level	5	0.0719	0.7034	0.7448	0.8353	0.0683	0.1300
Regression analysis							
MD_3 main effect							
Linear	1	0.0001	0.3008	0.0007	0.0001	0.0177	0.0068
Quadratic	1	0.7797	0.7144	0.2348	0.0450	0.4192	0.5142

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM

Table 3 – Body weight gain (BWG), tibia ash, TD incidence, TD score, Ca rickets incidence, Ca score and plasma Ca of 16-d-old chicks fed two levels of Ca and hatched from eggs laid by 37 week-old hens fed various levels of vitamin D_3 (MD_3) (Experiment 2)

Progeny dietary Ca	Maternal D_3	n[1]	BWG	Tibia ash	Incidence of TD	TD score	Incidence of Ca rickets	Ca rickets score
(%)	(IU/kg of diet)		(g)	(%)	(%)		(%)	
0.63	125	6	244±19[2]	34.05±1.27	83.3±10.5	2.50±0.32	83.3±10.5	2.33±0.44
0.63	250	6	297±16	34.44±0.55	75.0±6.5	2.25±0.19	75.0±11.2	2.25±0.34
0.63	500	6	341±09	35.43±0.56	86.2±6.3	2.50±0.23	86.2±6.3	2.50±0.23
0.63	1000	6	303±16	34.00±0.30	75.0±7.9	2.15±0.24	75.0±7.9	2.30±0.18
0.63	2000	6	374±08	36.97±0.35	45.8±6.1	1.25±0.20	51.3±10.7	1.36±0.29
0.63	4000	6	332±14	34.63±1.40	65.3±12.3	1.74±0.35	75.0±13	1.92±0.31
0.90	125	6	323±26	37.17±0.75	41.7±20.1	1.25±0.60	41.7±20.1	1.25±0.60
0.90	250	6	336±13	36.70±1.25	52.8±11.5	1.58±0.35	61.2±8.3	1.83±0.25
0.90	500	6	350±05	37.75±0.43	45.8±12.7	1.29±0.35	50.0±10.1	1.42±0.27
0.90	1000	6	366±07	38.69±0.56	13.8±06.3	0.42±0.19	38.8±13.9	1.04±0.44
0.90	2000	6	357±13	38.54±0.75	31.6±04.8	0.95±0.15	41.6±12.4	1.10±0.30
0.90	4000[3]	6	358±05	38.36±0.51	22.2±07.9	0.67±0.24	30.5±7.6	0.88±0.24
Main effect means								
	125	12	284±19	35.61±0.84	62.5±12.5	1.88±0.38	62.5±12.5	1.79±0.39
	250	12	317±12	35.57±0.74	63.9±07.1	1.92±0.21	68.1±6.9	2.04±0.21
	500	12	345±05	36.59±0.49	66.0±09.1	1.90±0.27	68.1±7.9	1.96±0.24
	1000	12	337±13	36.56±0.80	41.6±10.7	1.20±0.31	55.3±9.8	1.61±0.32
	2000	12	366±07	37.68±0.44	39.4±04.4	1.11±0.13	46.9±7.8	1.24±0.20
	4000[3]	12	345±08	36.49±0.91	43.7±09.5	1.20±0.26	50.7±9.6	1.35±0.25
0.63		36	315±09	34.94±0.38	71.7±4.0	2.06±0.13	74.3±4.4	2.10±0.14
0.90		36	348±06	37.85±0.31	34.7±5.1	1.03±0.15	44.0±5.1	1.26±0.15
ANOVA		df	----------Probabilities ----------					

Whole Plot							
Ca level	1	0.0312	0.0001	0.0027	0.0038	0.0224	0.0306
Pen (Ca level)	10	0.0030	0.9001	0.0201	0.0165	0.0189	0.0132
Split-plot							
MD_3 level	4	0.0001	0.0781	0.0122	0.0086	0.2349	0.1161
Ca level *x* MD_3 level	4	0.0015	0.3791	0.0716	0.0604	0.3482	0.3133
Regression analysis							
Low Ca (0.63%)							
Linear	1	0.0001		0.0112			
Quadratic	1	0.3212		0.0624			
High Ca (0.90%)							
Linear	1	0.0176		0.0676			
Quadratic	1	0.3584		0.8660			
MD_3 main effect							
Linear	1	0.0001	0.0075	0.0026	0.0011	0.2167	0.1544
Quadratic	1	0.3423	0.5536	0.1479	0.1823	0.1779	0.1095

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM
[3] Chicks hatched from eggs laid by hens fed the 4,000 IU of vitamin D_3/kg of diet was not included in the analyses.

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Table 4 – Main effect mean of body weight gain (BWG), tibia ash, TD incidence, Ca rickets incidence and plasma Ca of 16-d-old chicks fed various levels of 25-OHD_3 in the diet and hatched from eggs laid by 49 week-old hens fed various levels of vitamin D_3 (MD_3) (Experiment 3)

25-OHD_3 in chick diet	D_3 level in maternal diet	n[1]	BWG	Tibia ash	Incidence of TD	Incidence of Ca rickets	Plasma Ca
(µg/kg)	*(IU/kg)*		*(g)*	*(%)*	*(%)*	*(%)*	*(mg/100 mL)*
0		3	253±15[2]	25.63±0.78	10.6±4.9	100±0	8.57±0.50
2.5		3	341±16	34.99±0.54	47.9±8.4	89.4±4	11.42±0.40
5		3	384±8	38.77±0.70	36.7±6.5	56.7±7	12.22±0.16
10		3	422±6	40.54±0.39	21.1±4.8	48.9±6	12.42±0.16
20		3	419±7	40.06±0.43	7.8±3.5	34.4±5	12.25±0.12
40		3	441±12	42.08±0.43	1.7±1.7	18.9±4	12.39±0.12
	250	18	347±20	36.86±1.52	25.9±7.4	63.0±7	11.54±0.36
	500	18	388±17	37.28±1.61	24.1±7.0	64.8±8	11.81±0.33
	1,000	18	370±16	37.69±1.40	22.7±5.7	61.1±8	11.34±0.64
	2,000	18	380±21	36.76±1.58	19.4±6.3	55.6±9	11.55±0.39
	4,000	18	398±16	38.08±1.33	12.5±4.2	45.8±8	11.50±0.48
Multiple regression analysis							
		df	----------Coefficient [standard error] (probabilities) ----------				
Intercept		1	272.521856	30.119300	41.106418	102.841005	10.274966
			[15.984374]	[1.288790]	[7.391105]	[5.870502]	[0.458617]
			(0.0001)	(0.0001)	(0.0001)	(0.0001)	(0.0001)
25-OHD_3		1	12.882100	1.043590	-1.190287	-5.012035	0.244208
			[1.583652]	[0.126681]	[0.732273]	[0.581620]	[0.046416]
			(0.0001)	(0.0001)	(0.1078)	(0.0001)	(0.0001)
25-OHD_3^2		1	-0.223734	-0.019011	0.001868	0.0770056	-0.005053
			[0.036349]	[0.002842]	[0.0168079]	[0.0133500]	[0.001067]
			(0.0001)	(0.0001)	(0.9118)	(0.0001)	(0.0001)
MD_3		1	0.019249	0.000266	-0.006430	-0.005118	-0.000218

		[0.017981]	[0.001367]	[0.008314]	[0.006604]	[0.000538]
		(0.2875)	(0.8462)	(0.4414)	(0.4405)	(0.6867)
MD_3^2	1	-0.000001	0.00000001	0.00000005	-0.0000001	0.00000002
		[0.000003]	[0.0000003]	[0.000001]	[0.000001]	[0.0000001]
		(0.7194)	(0.9815)	(0.9779)	(0.9320)	(0.8546)
25-OHD_3 *x* MD_3	1	-0.000310	-0.000006	0.000213	0.000053	0.000007
		[0.000288]	[0.000021]	[0.000133]	[0.000106]	[0.000007]
		(0.2855)	(0.7783)	(0.1134)	(0.6174)	(0.3371)
R^2		0.59	0.62	0.23	0.71	0.40
25-OHD_3 at Maximum response		26	SP[3]	SP	SP	SP
MD_3 at maximum response		3986	SP	SP	SP	SP
Predicted maximum		477	-	-	-	-
Root MSE		51.46	3.69	23.73	18.84	1.35
Simple regression analysis		----------Probabilities----------				
25-OHD_3 main effect	1	0.0001	0.0001	0.0001	0.0001	0.0001
Linear	1	0.0001	0.0001	0.0001	0.2690	0.0001
Quadratic						
MD_3 main effect						
Linear	1	0.1030	0.6850	0.1131	0.0891	0.7969
Quadratic	1	0.7783	0.9341	0.6061	0.4059	0.9757

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM
[3] SP (Saddle point) = Optimal not found

Table 5 – main effect mean of body weight gain (BWG), tibia ash, TD incidence, Ca rickets incidence and plasma Ca of 16-d-old chicks fed various levels of 25-OHD_3 in the diet and hatched from eggs laid by 55 weeks old hens fed various levels of vitamin D_3 (MD_3) (Experiment 4).

25-OHD_3 in chick diet	D_3 level in maternal diet	n[1]	BWG	TD incidence	Ca rickets incidence	Plasma Ca
(µg/kg)	*(IU/kg)*		*(g)*	*%*	*%*	*(mg/100 mL)*
0		4	226±12[2]	8.0±3.6	97.7±1.6	8.17±0.45
2.5		4	293±11	28.1±5.8	99.0±1.0	7.51±0.31
5		4	378±8	43.9±5.3	74.0±4.8	10.79±0.32
10		4	404±9	17.7±4.5	19.8±5.5	11.45±0.14
20		4	427±6	8.7±3.1	7.8±3.1	11.27±0.21
40		4	420±12	9.0±5.3	4.0±1.8	11.38±0.19
	125	24	341±20	18.5±5.9	50.0±9.5	10.33±0.54
	250	24	322±18	21.3±5.2	55.6±8.3	9.77±0.55
	500	24	373±16	22.9±5.5	50.8±9.1	10.35±0.49
	1,000	24	375±15	17.2±4.1	49.6±9.1	9.84±0.54
	2,000	24	379±15	15.2±4.2	45.8±9.2	10.18±0.63

Multiple regression analysis

	df	Coefficient [standard error] (and probabilities)			
Intercept	1	213.842260	25.332444	108.189320	8.383214
		[12.704960]	[6.555781]	[5.014438]	[0.494696]
		(0.0001)	(0.0002)	(0.0001)	(0.0001)
25-OHD_3	1	16.717966	-0.304227	-8.132852	0.307908
		[1.258744]	[0.641904]	[0.496805]	[0.049012]
		(0.0001)	(0.6365)	(0.0001)	(0.0001)
25-OHD_3^2	1	-0.303439	-0.002329	0.139512	-0.005925
		[0.028892]	[0.014730]	[0.011403]	[0.001124]
		(0.0001)	(0.8746)	(0.0001)	(0.0001)
MD_3	1	0.098227	0.000563	-0.002723	-0.000683
		[0.028585]	[0.014680]	[0.011282]	[0.001113]
		(0.0008)	(0.9695)	(0.8097)	(0.5415)
MD_3^2	1	-0.000029	-0.000001	-0.0000008	0.0000002
		[0.000012]	[0.000006]	[0.000004]	[0.0000004]
		(0.0230)	(0.8212)	0.8760	(0.5948)
25-OHD_3 *x* MD_3	1	-0.000919	-0.000036	0.000062	0.000008
		[0.000459]	[0.000234]	[0.000181]	[0.000017]
		(0.0478)	(0.8774)	0.7330	(0.6245)
R^2		0.71	0.07	0.83	0.55
CD_3 at maximum response		25	SP[3]	SP	SP
MD_3 at maximum response		1282	SP	SP	SP
Predicted maximum		490	-	-	-
Root MSE		47.09	24.01	18.58	1.30

Simple regression analysis	----------Probabilities----------			
25-OHD_3 main effect				
Linear	0.0001	0.0462	0.0001	0.0001
Quadratic	0.0001	0.0001	0.3903	0.0004

MD_3 main effect				
Linear	0.0002	0.5105	0.6171	0.8924
Quadratic	0.9845	0.3681	0.6548	0.7262

[1] n = number of replicates per mean value
[2] Observed mean and observed SEM
[3] SP (Saddle point) = Optimal not found

Table 6 – Summary of main effect of Ca and 25-OHD_3 levels in the chick diet and main effect of D_3 in the maternal diet of 16-d-old chicks hatched from eggs laid by hens at 30, 37, 49 and 55 weeks of age (Experiments 1, 2, 3 and 4, respectively).

	Chick diet main effect (simple regression)				Maternal diet main effect (simple regression)			
	Ca		25-OHD_3		Maternal D_3			
Hens age	30	37	49	55	30	37	49	55
Experiment	1	2	3	4	1	2	3	4
	---------- Probabilities----------							
Criteria								
Body weight gain	0.1712[1]	0.0312						
Linear			0.0001	0.0001	0.0001	0.0001	0.1030	0.0002
Quadratic			0.0001	0.0001	0.7797	0.3423	0.7783	0.9845
Bone ash	0.0001	0.0001						
Linear			0.0001	-	0.3008	0.0075	0.6850	-
Quadratic			0.0001	-	0.7144	0.5536	0.9341	-
TD incidence	0.0001	0.0027						
Linear			0.0001	0.0462	0.0007	0.0026	0.1131	0.5105
Quadratic			0.0001	0.0001	0.2348	0.1479	0.6061	0.3681
Ca rickets incidence	0.005	0.0224						
Linear			0.0001	0.0001	0.0177	0.2167	0.0891	0.6171
Quadratic			0.2690	0.3903	0.4192	0.1779	0.4059	0.6548
Ca Plasma	-	-						
Linear			0.0001	0.0001	-	-	0.7969	0.8924
Quadratc			0.0001	0.0004	-	-	0.9757	0.7262

[1] Probability value taken from ANOVA, Ca effect.

Table 7 – Overall experiments mean of body weight gain (BWG), tibia ash, TD incidence, Ca rickets incidence and plasma Ca of 16 days old chicks hatched from eggs laid by hens at 29, 37, 49 and 55 weeks of age (Experiments 1, 2, 3 and 4, respectively).

Hens age (weeks)	Experiment	Number of chicks	BWG	Tibia ash	Incidence of TD (TD score)	Incidence of Ca rickets (Ca rickets score)	Plasma Ca
			g	*(%)*	*(%)*	*(%)*	*(mg/100 mL)*
30	1	288	304±4	36.70±0.29	53.54±.97 (1.79±0.11)	47.08±3.51 (0.64±0.05)	-
37	2	288	329±6	36.38±0.31	55.17±4.29 (1.62±0.13)	59.79±4.14 (1.72±0.13)	-
49	3	324	371±9	37.15±0.75	61.11±3.92	23.03±3.26	11.56±0.21
55	4	528	362±8	-	19.18±2.39	50.47±4.42	10.03±0.27

Experiment 1 and 2 does not include the 0 and 4,000 IU/kg of D_3 levels in the maternal diet.
Experiment 3 and 4 does not include 125 and 4,000 IU/kg of D_3 levels in the maternal diet.

Figure 1 – Body weight gain of 16-d old chicks fed two levels of Ca and hatched from eggs laid by hens fed various levels of vitamin D_3 (Experiment 2)

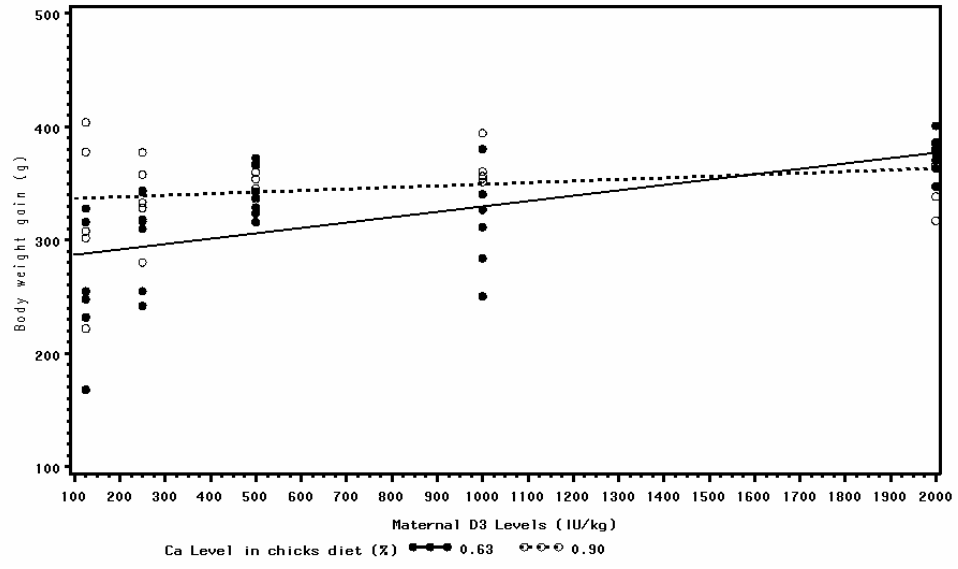
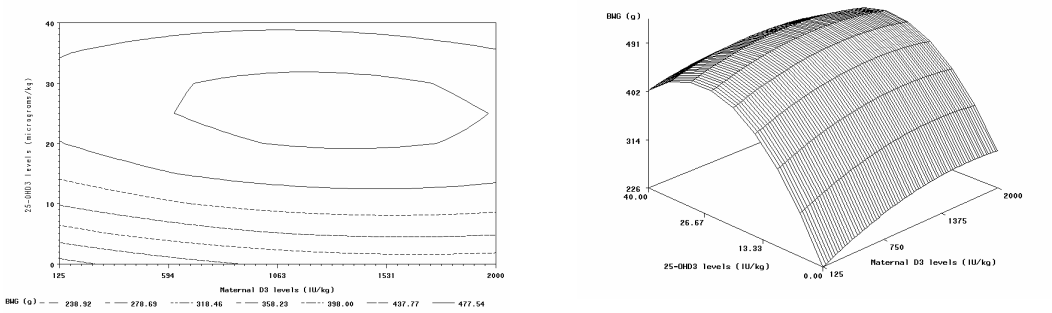


Figure 2 – Surface response representations of data from Experiment 4, predicted 16-d body weight gain (BWG) against the levels of 25-OHD_3 in the chicks diet and vitamin D_3 in the maternal diet. A) Contour plot; B) response surface

A B



CHAPTER 6

25-HYDROXYCHOLECALCIFEROL AS A CHOLECALCIFEROL SUBSTITUTE IN BROILER BREEDER HENS DIETS AND ITS EFFECT ON THE PERFORMANCE AND GENERAL HEALTH OF THE PROGENY[1]

[1] Atencio, A., G.M. Pesti and H.M. Edwards Jr. To be submitted to Poultry Science.

ABSTRACT

An experiment was conducted with broiler breeder hens to determine the relative biological value (RBV) of 25-hydroxycholecalciferol (25-OHD_3) compared to cholecalciferol (D_3) for hen-day egg production (HDEP), hatchability (HAT), early embryo mortality [1-to-10-d of incubation (EEM)], late embryo mortality [11-to-21-d (LEM)] and body ash of the progeny (BAP). The study was conducted with 73-to-90 wk old molted Ross broiler breeder hens in an environment excluding UV light. A basal D_3 deficient diet supplemented with four levels of D_3 [0, 3,125, 12,500, and 50,000 nanogram (ng)/kg of diet] and two level of 25-OHD_3 (3,125 and 12,500 ng/kg of diet) was fed. Three repetitions of seventeen hens were used for each treatment group. No D_3 source was used in the vitamin premix and no animal byproduct was used in the corn, soybean meal and wheat middlings basal diet to assure that no unintentional vitamin D activity was present. Slope ratio analyses with the data from 81 to 88 wk of age were performed using the logarithm of D_3 level + 1 for hens fed 0, 3,125 and 12,500 ng/kg. The RBVs of 25-OHD_3 statistically different from the standard (D_3), using slope ratio methodology were 138, 133, 128 and 111 % for HDEP, HAT, LEM and BAP, respectively (average 128%). When comparing 25-OHD_3 against D_3 at the 3,125 ng/kg level, the RBVs were 209, 167, 400 and 108% for the same criteria, respectively (average=221%). However, at the 12,500 ng/kg level no statistical differences between 25-OHD_3 and D_3 were observed (average=108%). With the progeny of these hens, four experiments were conducted for 16-d each with chicks hatched at 80, 87, 89 and 90 wk of age (Experiments 1, 2, 3 and 4, respectively) to determine the effect of the maternal diet on the performance and general health of the progeny. Chicks were fed corn-soybean meal basal diets. In Experiment 1 no vitamin D source was added to the chicks diets and in Experiments

2, 3 and 4 the basal diet was supplemented with 27.5 µg of D_3/kg of diet. Linear increases in body weight and tibia ash were observed in the progeny as the vitamin D_3 level in the diet increased (Experiment 1). Chicks hatched from hens fed 3,125 ng/kg of 25-OHD_3 had higher body weight (Experiments 1, 2 and 3) and tibia ash (Experiment 1) than chicks hatched from hens fed the same level of vitamin D_3; however at the 12,500 ng level no differences were observed between the two D activity sources. In the progeny study, the average RBV of 25-OHD_3 at the 3,125 and 12,500 ng levels were 115 and 101%, respectively. The potency of 25-OHD_3 in relation to D_3 depends on the level tested. When comparing vitamin D sources, 25-OHD_3 has greater potency than D_3 only at very low levels of supplementation.

Key Words: Broiler breeders, Vitamin D_3 and 25-hydroxycholecalciferol

INTRODUCTION

No studies have been found in the literature testing the potency of 25-hydroxycholecalciferol (25-OHD_3) in comparison to D_3 on the performance of broiler breeder hens. Both 25-OHD_3 and D_3 have been reported to be absorbed in the gut (Bar et al. 1980). However, the authors observed a higher absorption of 25-OHD_3 (74.9%) than D_3 (66.5%) in 14 day-old chicks.

In experiments conducted with chicks, 25-OHD_3 has shown to be more potent than D_3. McNutt et al. (1973) reported that 25-OHD_3 is twice as active as D_3 in supporting growth and 1.3 to 1.5 times as active as D_3 in terms of increasing percentage of bone ash in chickens. Boris et al. (1977) observed that high levels of 25-OHD_3 and D_3 in the diet of chicks resulted in approximately the same tibia ash; however when the tibia ash from chicks fed low levels of D_3 or 25-OHD_3 were compared, 25-OHD_3 was twice as potent as D_3. Fritts and Waldroup (2003) also observed higher potency of 25-OHD_3 than vitamin D_3 in supporting body weight gain, tibia ash, and in reducing the incidence and severity of TD in broilers; however the difference were observed primarily at lower levels of vitamin D_3.

Several papers have been published comparing the effects of 25-OHD_3 and D_3 on commercial laying hens (Charles and Ernest, 1974; Charles et al., 1978; Marret et al. 1975; Roland and Harms, 1976; Abdulrahim et al. 1979; Keshavarz, 1996, 2003). Some researchers have observed no differences between the D-sources while others have observed significant differences. A mixture of factors such as levels of D metabolites used in the experimental diets, duration of the studies, exposure to UV light and presence of animal byproducts containing D_3 activities may have contributed to these inconsistent results by different investigators.

Charles and Ernest (1974) in an experiment conducted for 28 days reported an increase in egg specific gravity in old laying hens (144 wk of age) fed 25-OHD_3 as compared to vitamin D_3; however, such improvement was not observed with young laying hens (28 wk of age). The same authors also observed a lower egg production by hens fed 25-OHD_3 when compared with hens fed D_3. The authors did not specify the levels of D sources used in their experiments. In a subsequent study Charles et al. (1978) observed better egg shell quality from laying hens fed 200 IU/kg of 25-OHD_3 than hens fed 200 IU/kg of D_3. No beneficial effects of supplementing 25-OHD_3 as compared to D_3 were found to effect performance, shell quality and bone ash in three different experiments conducted for 12, 20 and 16 wk with laying hens (Keshavaraz, 1996, 2003). In the 1996 experiment the level of both D sources was 12.5 µg/kg diet and in the two experiments published in 2003 the level was 69 µg/kg of diet.

Abdulrahim et al. (1979), in an experiment conducted for 13 wk with 21 wk old leghorn hens, did not observe differences between treatments in egg production and hatchability by supplementing the diet with D_3 or 25-OHD_3 at 120 and 360 IU/kg of diet. No regression analysis was performed by the authors, but a numerical increase in egg production and hatchability was observed when the 25-OHD_3 and D_3 increased in the diet. When comparing the means between the two D sources at the 120 IU/kg level, hatchability increased 5%. However, no difference in percent production was observed at the 360 IU/kg level between the two D sources. Hart and DeLuca (1985) and Hart et al. (1986) reported that hens fed 25-OHD_3 support normal hatchability and embryo development in the same proportion as hens fed D_3. Fraser and Emtage (1976) and Mattila et al. (1999) reported higher D_3 transfer to eggs than 25-OHD_3 when the hens were fed diets supplemented with D_3. Mattila et al. (1999) also showed a positive correlation between the D_3 content of the hens' feed and the D_3 and 25-

OHD_3 contents of egg yolk. Ameenuddin et al. (1983) reported normal embryo development by injecting 25-OHD_3 in fertile eggs produced by hens fed 1,25-$(OH)_2D_3$ in the diet as the only source of dietary vitamin D_3. The more active form of vitamin D_3, 1,25-$(OH)_2D_3$, when fed to hens as the only source of vitamin D_3 failed to support normal embryo development.

Variations in results between experiments may have been also related to the duration of the experiments. Previous experiments conducted in our laboratory show that it takes approximately 5 to 6 wk for broiler breeder hens fed a previous diet with 27.5 µg/kg of vitamin D_3 from 0 to 24 wk of age to use their vitamin D_3 body reserved or become deficient in D_3. Another source of variation not taken in consideration in some of the experiments conducted is the present of animal byproducts. Mattila (1995) found vitamin D_3 in fish, wild mushrooms, meat and milk. Atencio et al. (2003) also observed vitamin D_3 in some samples of poultry fat. Several studies have shown the effect of UV light in de novo vitamin D synthesis (Tian et al., 1994) and in reducing bone diseases in chickens (Edwards et al., 1994, 2000 and 2003; Elliot and Edwards, 1997; Ledwaba and Roberson, 2003). Murphy et al. (1936) and Bethke et al. (1936) reported that D_3 and sunlight given to hens affected bone calcification of the progeny and the mineral composition of the embryo. The effect of vitamin D_3 levels in the maternal diets on the performance and general health of the progeny has been also reported by Griminger (1966) and Edwards (1995).

The potency of 25-OHD_3 in comparison to D_3 in laying hens appears to be related to the levels used in the diet. In general, differences between the two D sources have been observed at lower levels than at high levels. Therefore, the objectives of this experiment were to determine the relative biological value of 25-

OHD_3 in comparison to vitamin D_3 for broiler breeders hens and relate their levels in the maternal feed with the performance and general health of the progeny.

MATERIALS AND METHODS

Three hundred and six, 73 wk old, molted female broiler breeders (Ross - 508) [2] and thirty six 26 wk old males (Ross 344) were randomly allocated to 18 floor pens (2.4 m wide x 3.6 m length). The experiment was conducted in a house where incandescent light bulbs that emit no UV light were used and the outside light was excluded by baffles on the ventilation system. In this house the day length throughout the experiment was 16 hours. Room temperature was maintained at approximately 22 ºC.

A basal D_3 deficient diet (Table 1) supplemented with four levels of D_3[3] (0, 3,125, 12,500, and 50,000 ng/kg of diets) and two levels of 25-OHD_3[4] (3,125 and 12,500 ng/kg of diet) were fed to hens from 73 to 90 wk of age. Three repetitions of seventeen hens and two males per pen were used for each treatment group.

No D_3 source was used in the vitamin premix and no animal byproduct was used in the corn, soybean meal and wheat middlings based diet to guarantee that no unintentional vitamin D activity was present in the experimental diets. The same basal diet was used throughout the experiment. Feed was mixed at approximately monthly intervals for all treatments.

Hens were fed every day the experimental diets using the amount of energy and protein recommended by the breeders' guidelines[2] for peak production and post-peak production. Because males received the same experimental diet as hens, they

[2] Ross Breeders, Inc. Huntsville, Alabama 35805
[3] Pure crystalline cholecalciferol (D_3) obtained from Sigma Chemical Co., St. Louis, MO 63178-9916.
[4] Hy-D, (Concentration 0.1835 mg/g) Roche Vitamins, Inc., Parsippany, NJ.

were given 18,380 IU D_3/bird by oral administration once per month. Male an females were fed in separate feeder according to the requirement for energy of each sex.

Eggs collected every day were stored in a cooler at 17 ºC. Eggs collected during 7-d were grouped and set in the incubator by treatment and pen. Total incubation time was 21.5-d. Temperature and relative humidity settings from 0-18 and 19-21 day of incubation were 37.8 °C and 37.2°C, respectively. All incubated eggs were candled after 10 days of incubation, and clear eggs were removed, opened and classified as early embryo mortalities or infertile eggs. Clear eggs were classified as early dead embryos if the opaca and pellucida areas could be differentiated or if positive development was observed. At 18 days of incubation the eggs were transferred to the hatcher. All eggs cracked prior to incubation or at transfer were removed from the data set. Those eggs that failed to hatch were broken out to determine percentages of late embryo mortality (11-21 d) and pips. Pips were any embryos that were completely covered with feathers and had the shell of the egg broken. There were 14 hatches in this study. No eggs were incubated during the first 3 first week of the experiment because no enough eggs were laid. Hatchability, early embryo mortality and late embryo mortality were calculated based on the number of fertile eggs. Egg weight and specific gravity were measured every two wk on all eggs collected during one particular day. Two newly hatched chicks per pen, hatched every other week were killed placed in individual crucibles, dried and ashed (600 ºC) in a muffle furnace overnight for the determination of body ash.

Four experiments were conducted for a period of 16 days using mixed-sex 1-d-old chicks hatched from these hens. Chicks hatched from eggs laid by hens at 80, 87, 89 and 90 wk of age were used in experiments 1, 2, 3 and 4, respectively. The corn-

soybean meal basal diet was adequate in all nutrients, except vitamin D (Table 1). Chicks in Experiment 1 were fed the basal diet and chicks in Experiments 2, 3 and 4 were fed the same basal diet supplemented with 27.5 μg/kg of D_3. Diets were mixed at the beginning of each experiment. Chicks were kept separate according to the maternal diet and all experiments used the maternal diet as the treatment groups (5 treatments per experiment). No chicks were used from hens receiving no supplementation of vitamin D source, because of low hatchability.

All experiments were conducted in electrically heated wire mesh-floor battery brooders[5] located in the same room. Ultraviolet irradiation was eliminated from the chick room by fitting Arm-a-Lite[6] sleeves to all the fluorescent fixtures in the room and battery brooders (Edwards et al., 1994). The photoperiod was 24L:0D. The temperature of the room was maintained at 22 ºC. Chicks received water and feed *ad libitum*.

At the termination of each experiment, chicks were weighed and killed by carbon dioxide asphyxiation and examined at random for tibial dyschondroplasia (TD) and Ca rickets. A longitudinal cut was made across the right tibia and diagnosis for TD and Ca rickets were made based on the width of the growth plate band and its appearance according to the methodology describe by Edwards and Veltmann (1983) and Long et al. (1984). The left tibias were removed to determine bone ash content on a dry fat-free basis (Association of Official Analytical Chemists, 1995). In Experiment 1, three birds from each pen were randomly selected for plasma Ca[7] analyses.

[5] Petersime Incubator Co., Gettysburg, OH 54328.
[6] Arm-a-Lite® Thrermoplastic Processes, Sterling, NJ 07980.
[7] Section N-31 Technicon Autoanalyzer Methodology, Technicon Corp., Tarrytown, NY 10591.

Statistical Analysis

The experiment with the hens had a split plot design with two vitamin D sources, 25-OHD_3 and D_3 as the whole plot and subplot measurements taken over time at different ages. The analyses were conducted using the mean of each replicate per week as an observation. The analysis was performed with data collected from 81 to 88 wk of age. The relative biological values of 25-OHD_3 in comparison to that of the standard were calculated using the slope ratio techniques and comparing the means between the two D sources at the same level. The slope ratio analyses were performed using the logarithm ($\log_{10}$) of D_3 level plus 1 (0, 3,125 and 12,500 ng/kg level) on the data collected from 81 to 88 wk of age. The mean comparison was performed with data averaged over the 14 wk of collection of data (77 to 90 wk of age).

The progeny studies were conducted with a complete randomized design (Experiment 1) and incomplete block designs (Experiments 2, 3 and 4). Experiments 2, 3 and 4 used the same blocks and same environmental conditions at different times. In Experiment 1 an ANOVA and regression analyses were completed using the General Linear Model and Genmod procedures of SAS (SAS Institute, 1996). Means were compared using the Student-Newman-Keuls (SNK) test. In Experiments 2, 3 and 4, means between the two D sources at the same levels (3,125 and 12,500 ng/kg) were compared using orthogonal contrasts at $\alpha = 0.05$. All coefficients of determination (r^2) were calculated using the sum of squares of the regression divided by the sum of squares of the total.

RESULTS

No effects of 25-OHD_3 and D_3 in the hens' diet were observed for fertility, body weight of the progeny at 1 day of age, egg specific gravity, egg weight or pipped eggs (data no presented). In general hens fed the highest and the lowest levels of 25-OHD_3 and D_3 had the highest and the lowest hen day egg production, hatchability and body ash of the progeny and the lowest and highest embryo mortality, respectively (Table 2; Figures 1, 2, 3, 4, 5). No 25-OHD_3 or D_3 effects were observed in any of the criteria studied before 79 wk of age. Hens fed the 50,000 ng/kg of diet had the highest egg production throughout the experiment after 79 wk of age (Figure 1). Large differences in hen day egg production responses between 25-OHD_3 and D_3 were observed at the 3,125 ng/kg level, but almost no differences were observed at the 12,500 ng/kg level. Hatchability, late embryo mortality and body ash of the progeny followed the same pattern.

The relative biological values of 25-OHD_3 in comparison to D_3 for hen day egg production, hatchability, early embryo mortality, late embryo mortality and body ash of the progeny were 138, 133, 122, 128 and 111% respectively, using the logarithm of the level plus 1 and the slope ratio analysis. The relative biological value of 122% for early embryo mortality was not statistically different from that of the D_3.

Because significant quadratic effects were observed as the D_3 level in the diet increased (Table 2) a comparison between the means of the 25-OHD_3 and D_3 at the same levels were performed. Hen day egg production was 2.09 times higher for 25-OHD_3 when compared with D_3 at the 3,125 ng/kg level. However, at the 12,500 ng/kg level the difference was only 1.05 higher for 25-OHD_3. Hatchability was also 1.67 times higher for 25-OHD_3 than D_3 at 3,125 ng/kg level; however, no difference was

observed between the 25-OHD_3 and D_3 at 12,500 ng/kg. Late embryo mortality was reduced 4 times by 25-OHD_3 when compared with D_3 at 3,125 ng/kg level and only 1.26 times at 12,500 ng/kg level. Body ash of the progeny was 1.08 and 1.02 times higher for 25-OHD_3 than D_3 at 3,125 ng/kg and 12,500 ng/kg, respectively. In order to determine if the two means were statistically different, comparisons by orthogonal contrast were performed. Statical differences were observed between 25-OHD_3 and D_3 at the 3,125 ng/kg level; however no differences were observed at the 12,500 ng/kg level for hen day egg production, hatchability, late embryo mortality or body ash of the progeny.

Chick studies with progeny

Experiment 1

Body weight, feed efficiency, feed intake and tibia ash increased lineally as the D_3 level in the maternal diet increased; however no slope ratio analyses was performed because no significant differences between the slopes (source by level) was observed (Table 3). Higher body weight, feed intake and bone ash, and lower mortality was observed in chicks hatched from hens fed 50,000 ng of D_3 per kg of diet. In general chicks hatched from hens fed 3,125 ng/kg of 25-OHD_3 had higher body weight, gain:feed, feed intake and tibia ash (mg/tibia) and lower mortality than chicks hatched from hens fed the same level of D_3. However, body weight and tibia ash (mg/tibia) were the only characteristics that were statistically different when the two D sources at the 3,125 ng/kg level were compared by orthogonal contrasts. Body weight and tibia ash were 1.23 and 1.20 times higher for 25-OHD_3 when compared to D_3 at the 3,125 ng/kg of diet. However, at the 12,500 ng/kg level the difference was only 1.01 (body weight) and 1.04 (mg of tibia ash) times higher for 25-OHD_3.

No significant effects of the maternal diet were observed on TD, Ca rickets incidence and plasma Ca. Tibial dyschondroplasia and Ca rickets incidence (data not presented) were present in chicks from all treatments. Tibial dyschondroplasia and Ca rickets were observed in 100% of the treatments because no D metabolites were added to the experimental diet and no UV light was present in the room in which the experiment was conducted. The same inference can be made for the low level of plasma Ca and low % tibia ash.

Experiments 2, 3 and 4

In Experiments 2 and 3 the lowest body weight was observed in chicks hatched from hens fed 3,125 ng D_3/kg of diet (Table 4). However, in Experiment 4 this response was not observed, the highest body weight was observed in chicks hatched from hens fed 3,125 ng of D_3/kg of diet. These results indicate that 25-OHD_3 was 1.06 and 1.11 times more potent than D_3 at 3,125 ng/kg diet for Experiments 2 and 3, respectively. However, no differences were observed between 25-OHD_3 and D_3 at 12,500 ng/kg level for Experiments 2 and 3. Higher TD and Ca rickets incidences were observed in chicks hatched from hens fed 3,125 ng/kg of D_3 (when comparing the overall means of Experiments 2, 3 and 4); however the opposite was observed at the 12,500 ng/kg level, the highest incidence was observed from chicks hatched from hens fed 25-OHD_3 (Tables 5 and 6). There is no obvious explanation for these results. No significant effect of the maternal diet on mortality of the progeny was observed in any of the treatments.

The same pattern observed with the performance of the hens was observed with the progeny; a higher improvement by 25-OHD_3 was observed at the lower level than at the higher level. The overall mean relative bioavailability values of 25-OHD_3

found in the experiment conducted with broiler breeder were 128%, 221% and 108% using slope ratio techniques and mean comparison at the 3,125 and 12,500 ng/kg levels, respectively (Table 7). In the progeny experiments the average relative bioavailability value found were 115% and 101% for the 3,125 and 12,500 ng/kg levels, respectively.

DISCUSSION

The reason for using the data collected from 81 to 88 wk of age in the regression analyses was to reduce the presence of interactions between D sources by age observed when all 14 wk were included in the analysis. The absence of any interaction allows the calculation of the relative biological value for the 8 wk period instead of each week. Before 81 wk the responses changed each wk as the hens became depleted, so the relative biological values would have been different for the different age. This indicates that it took about 6 to 7 wk after the hens were fed the experimental diets for them to use their body D reserves and become dependent on the dietary sources. It is important to emphasize that the hens were fed a diet that contained 27.5 µg/kg of vitamin D_3 per kg of diet after they were molted (during four weeks preceding the beginning of the experiment). In a previous experiment conducted in our laboratory, also in an environment that excluded UV light, it was observed that it takes five to six wk for broiler breeder hens to become deficient in vitamin D when grower diet contained 27.5 µg/kg of vitamin D_3. Vitamin D_3 absorbed can be stored in adipose tissue from which it is slowly mobilized (Norman and DeLuca, 1963; Mawer et al., 1972; Combs, 1992).

No linear differences or quadratic effects were observed for fertility, early embryo mortality, body weight of the progeny, egg specific gravity, egg weight and

pipped eggs due to vitamin D source. In a previous experiment conducted in our laboratory with younger broiler breeder hens increases in egg specific gravity and egg weight were observed as the D_3 level in the diet increased. The lack of effect of D source on egg specific gravity and egg weight in this experiment could be a consequence of low egg production (Figure 1) or the advancing layers age. The lower the egg production, the lower the amount of D_3 taken from the hens body D_3 reserve. Charles and Ernest (1974) reported an increase in specific gravity in old laying hens (144 wk of age), but not in young laying hens (28 wk of age) fed 25-OHD_3 as compared to vitamin D_3. In a subsequent study Charles et al. (1978) observed improved shell quality in leghorn laying type hens fed 25-OHD_3 when compared to D_3 at 200 IU/kg. Roland and Harms (1976) supplemented young and old leghorn laying type hens with 2,200 µg of D_3/kg diet and 1.1 mg of 25-OHD_3/ kg diet during 6 wk and observed no effect on egg production, serum calcium, egg weight and egg specific gravity. No beneficial effects of supplementing 25-OHD_3 as compared to D_3 were found for egg production, egg weight, specific gravity, shell quality or bone mineralization in laying hens by Keshavaraz (1996, 2003). The levels of 25-OHD_3 and D_3 were 12.5 µg/kg (Keshavaraz, 1996) and 69 µg/kg (Keshavaraz, 2003) and the duration of the experiment of 1996 was 16 weeks and the two experiments of 2003 were 20 and 16 weeks.

The highest and the lowest egg production, hatchability, embryo mortality and body ash of the progeny throughout the experiment were observed in the hens that received the highest and the lowest supplements of D_3, respectively (Figures 1 to 5). These results are in agreement with a previous experiment in which hens fed with the highest level of vitamin D_3 had the highest responses for the same criteria (Atencio, et al. 2004a,b). Abdulrahim, et al. (1979) in 13 wk experiment with leghorn laying type

hens observed an increase in egg production and hatchability by increasing the levels of 25-OHD_3 and D_3 from 0 to 360 IU/kg. Couch et al. (1947) also observed an increase in egg production, hatchability and egg quality and a reduction in embryo mortality by feeding hens with various levels of D_3 (190 to 760 IU/kg of diet).

The relative biological values of 25-OHD_3 were different for the different criteria and for the different methodologies used. Similar results to the present study were observed by Boris et al. (1977) in an experiment conducted with 1 to 21 day old chicks. Boris et al. (1977) observed higher potency of 25-OHD_3 when compared to D_3 in improving tibia ash at low levels than at a higher levels. Fritts and Waldroup (2003) also observed that 25-OHD_3 is more metabolically potent than vitamin D_3 for support of body weight gain, tibia ash, and reduction in incidence and severity of TD in broiler; however the difference were observed primarily at lower levels of vitamin D_3.

The average plasma Ca and tibia ash in percentage found in Experiment 1 were 7.1 mg/100 mL and 23.45%, respectively. Similar levels of plasma Ca (average: 7.0 mg/100 mL) and higher percentage of tibia ash (average: 28%) were observed by Edwards et al. (1994) in three different experiments conducted with 16 d old chicks in a UV-light free environment. The higher bone ash observed in Edwards et al. (1994) experiments may have been due to the presence of poultry fat in the vitamin D deficient diet. Vitamin D activity in some samples of poultry fat has been reported (Atencio et al. 2003).

Broiler breeder hens younger than the ones used in this experiment and with a higher egg production may result in different relative biological values than the one found for the 3,125 and 12,500 ng/kg level. Mattila, et al., (1999) showed that 25-

OHD_3 and D_3 are transferred from the hens to the eggs; therefore requirements could be expected to be directly proportional to egg production.

On average, higher potencies were observed in the study with the hens than with the progeny. A general evaluation of the data obtained indicates that the potency of 25-OHD_3 in relation to D_3 depends on the levels tested. When comparing vitamin D sources, 25-OHD_3 has greater potency than D_3 only at very low levels of supplementation. The D metabolite, 25-OHD_3 is at least as potent as D_3. It may be more potent for various criteria, but further research with more levels is needed.

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Table 1 – Composition of the experimental diets

Ingredient	Broiler Breeders (%)	Progeny (%)
Corn, ground	43.060	52.87
Wheat middling	30.000	-
Soybean meal (48.5 % CP)	16.788	38.00
Limestone	6.759	1.28
Tricalcium phosphate	1.376	-
Dicalcium phosphate	-	1.86
Vegetable oil	1.000	5.00
Salt (NaCl)	0.400	0.450
Vitamin Premix[1]	0.250	0.250
Mineral Premix[2]	0.075	0.075
Dl-Methionine	0.237	0.22
Coccidiostat[3]	0.050	-
Total	100.00	100.00
Calculated composition[4]		
Crude Protein, %	16.9	23.2
ME, kcal/kg	2,489	3,150
Ca, %	3.10	1.03
Total Phosphorus, %	0.74	0.73
Non-phytate phosphorus, %	0.40	0.49

[1] Vitamin premix provides the following (per kilogram of diet): Vitamin A (trans-retinyl acetate), 5500 IU; vitamin E(all-rac-tocopherol acetate), 11 IU; riboflavin (B_2), 4.4 mg; Ca pantothenate, 12 mg; pyridoxine HCl (B_6), 4.0 mg; nicotinic acid 44 mg; folic acid 3 mg; biotin 0.3 mg, thiamine mononitrate (B_1) 2.2 mg; vitamin B_{12} (cobalamin), 0.01 mg; menadione sodium bisulfate complex, 1.1 mg; choline chloride added to meet the requirement 220 mg; ethoxyquin, 125 mg.
[2] Mineral premix provides the following (per kilogram of diet): Manganese ($MnSO_4H_2O$), 60 mg; Iron ($FeSO_47H_2O$), 30 mg; Zinc (ZnO), 50 mg; copper ($CuSO_45H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide, 1.5 mg; Selenium, 0.3 mg.
[3] 66.15 mg of monensin sodium/kg
[4] NRC (1994).

Table 2 – Performance of broiler breeder hens fed cholecalciferol (D_3) or twenty five hydroxycholecalciferol (25-OHD_3) from 73 to 0 wks of age. Performance measures include hen day egg production (HDEP), hatchability (HAT), early embryo mortality (EEM), late embryo mortality (LEM) and body ash of the progeny (BAP)[1].

D_3	25-OHD_3	HDEP	HAT	EEM	LEM	BAP
(ng/kg)	*(ng /kg)*	*(%)*	*%*	*%*	*%*	*mg*
0	0	7.2±1.3[2]	28.3±6.7	16.3±4.1	70.2±6.9	0.549±0.00
3,125	0	13.1±1.5	52.8±5.6	12.8±3.6	40.3±5.2	0.690±14.65
0	3,125	27.3±1.8	88.2±1.6	4.2±0.6	10.0±1.6	0.751±11.36
12,500	0	30.8±1.5	95.9±0.6	2.5±0.6	2.9±0.5	0.788±10.73
0	12,500	32.5±1.8	95.9±0.7	4.1±0.8	2.3±0.6	0.804±10.15
50,000	0	43.8±1.9	96.7±0.5	3.7±0.5	2.6±0.4	0.805±8.27
Slope ratio analysis[3]						
Probability (Linear differences in slope)		0.0001	0.0001	0.0903	0.0001	0.0059
Probability (Quadratic differences in slope)		0.0001	0.0001	0.1048	0.0001	0.0106
Regression equation						
Intercept		1.1659±2.1174	1.0004±5.1551	22.6207±3.6569	98.13490336±4.5904	0.4934±0.03
Linear slope of D_3		5.7445±0.6824	17.3686±1.6074	-3.9600±1.1371	-18.3409±1.4314	0.1071±0.01
Linear slope of 25-OHD_3		7.9708±0.6824	23.1151±1.6028	-4.8325±1.1371	-23.4753±1.4272	0.1202±0.01
R_2		0.53	0.66	0.15	0.71	0.56
Relative biological value						
25-OHD_3		1.38	1.33	1.22	1.28	1.11
Standard Error		0.090	0.066	0.205	0.056	0.05
Lower 95% fiducial limit		1.210	1.20	0.814	1.17	1.01
Upper 95% fiducial limit		1.565	1.46	1.627	1.39	1.21

[1] Mean included data collected during 77 to 90 wk, except BAP which includes wk 82, 84, 86, 88 and 90.
[2] Observed mean and Observed SEM.
[3] Slope ratio analyses performed using data collected from 81 to 88 wk of age and the logarithm of D level (0, 3,125 and 12,500 only) plus 1.

Table 3 – Body weight (BW), gain:feed, feed intake (FI), bone ash, mortality and plasma calcium of chicks hatched from eggs laid by hens at 80 weeks of age and fed various levels of vitamin D_3 and twenty five hydroxycholecalciferol (25-OHD_3) (Experiment 1)

Maternal diet and levels									
D_3	25-OHD_3	n[1]	16-d BW	Gain:Feed	Feed intake	Tibia ash	Tibia ash	Mortality[2]	Plasma Ca
(ng/kg)	*(ng/kg)*		*(g)*	*(g:g)*	*(g)*	*(%)*	*(mg/tibia)*	*(%)*	*(mg/100 mL)*
3,125		2	156±2 ^{c}	0.584±0.03 ^{a}	188±09.1 ^{c}	22.8±0.3 ^{ab}	472±20 ^{d}	30	7.06±0.46
	3,125	3	192±3 ^{b}	0.679±0.08 ^{ab}	218±14.7 ^{bc}	21.3±0.5 ^{b}	564±24 ^{c}	16	7.09±0.41
12,500		3	217±3 ^{bc}	0.639±0.004 ^{ab}	261±08.6 ^{bc}	22.9±0.2 ^{ab}	646±15 ^{b}	33	6.40±0.12
	12,500	3	220±3 ^{b}	0.795±0.03 ^{b}	219±16.4 ^{bc}	22.6±0.3 ^{b}	671±21 ^{b}	16	7.76±0.48
50,000		3	285±3 ^{a}	0.709±0.005 ^{ab}	336±15.3 ^{b}	24.4±0.6 ^{a}	808±24 ^{a}	13	7.20±0.23
ANOVA									
Source of Variation		df	----------Probabilities----------						
Source		1	0.1743	0.0168	0.6752	0.0469	0.0296	0.5277	0.0995
Level		4	0.0003	0.0411	0.0003	0.0238	0.0001	0.1004	0.6058
Source *x* level		2	0.2594	0.5696	0.0322	0.1596	0.1686	0.6621	0.1132
Regression analysis for D_3 levels[3]			.0003	.0006	0.0005	.0478	.0001	-	-

[a-c] Means within a column with no common superscript differ significantly ($P \leq 0.05$). The Student-Newman-Keuls (SNK) test was applied when the one way ANOVA (considering all 5 treatments) was significant ($P \leq 0.05$).

[1] n = number of replicates used per treatment.

[2] Arc sign transformations were applied before the ANOVA was performed

[3] Linear effects were observed for the criteria studied. Linear regression equations: BW= 169.8444 + 0.0596±0.009 X_1 (r^2=0.86); Gain:feed = 0.5948 + 0.00005±0.000009 X_1 (r^2=0.80); Feed intake = 206.1888 + 0.0670±0.01 X_1 (r^2=0.83); Tibia ash (%) = 22.5733 + 0.0009±0.0002 X_1 (r^2=0.66); Tibia ash (mg/tibia) = 517.6767 + 0.1508±0.02 X_1 (r^2=0.84); where X_1 = vitamin D_3 level

Table 4 – Body weight of chicks hatched from eggs laid by hens fed different D sources and levels in the diet (Experiments (Ex) 2, 3 and 4).

Maternal diet		Ex 2	Ex 3	Ex 4	Ex 2	Ex 3	Ex 4	Ex 2,3,4
D_3	25-OHD_3	n[1]	n	n	16-d BW	16-d BW	16-d BW	Mean
ng/kg	*ng/kg*				*(g)*	*(g)*	*(g)*	*(g)*
3,125		14	24	10	438±30 [b]	394±10 [b]	470±13 [a]	423±12 [b]
	3,125	41	53	39	468±7 [a]	438±6 [a]	435±8 [b]	446±4 [a]
12,500		70	63	31	484±8	436±8	455±9	458±5 [a]
	12,500	80	72	45	487±6	435±6	443±8	456±5 [a]
50,000		126	111	74	474±4	418±5	427±6	442±3
	Mean				475±3	425±3	439±4	

[1] n = number of chicks used per treatment.
[a-b] Means within the levels with no common superscript differ significantly ($P \leq 0.05$); result of orthogonal contrast comparisons.

Table 5 – Tibial dyschondroplasia incidence in chicks hatched from eggs laid by hens fed different D sources and levels in the diet (Experiments (Ex) 2, 3 and 4)

Level in the diet		Ex 2	Ex 3	Ex 4	Ex 2	Ex 3	Ex 4	Ex 2,3,4
D_3	25-OHD_3	n[1]	n	N	TD incidence	TD incidence	TD incidence	Mean
ng/kg	*ng/kg*				*(%)*	*(%)*	*(%)*	*(%)*
3,125		14	24	10	21.4	20.8	10	18.75 [a]
	3,125	41	53	39	0.0	3.8	5.1	3.01 [b]
12,500		70	63	31	7.1	9.38	0.0	6.67 [b]
	12,500	80	72	45	8.7	12.5	13.3	12.63 [a]
50,000		126	111	74	5.4	7.1	2.7.	5.41
	Mean				6.61	9.51	6.53	

[1] n = number of chicks used per treatment

[a,b] Means between the same level with no common superscript differ significantly ($P \leq 0.05$); result of orthogonal contrast comparisons.

Table 6 – Rickets incidence in chicks hatched from eggs laid by hens fed different D sources and levels in the diet (Experiment (Ex) 2, 3 and 4)

Level in the diet		Ex 2	Ex 3	Ex 4	Ex 2	Ex 3	Ex 4	Ex 2,3,4
D_3	25-OHD_3	n[1]	n	n	Rickets incidence	Rickets incidence	Rickets incidence	Mean
ng/kg	*ng/kg*				*(%)*	*(%)*	*(%)*	*(%)*
3,125		14	24	10	14.29	4.17	10.00	8.33 [a]
	3,125	41	53	39	0.00	0.00	10.26	3.01 [b]
12,500		70	63	31	2.86	3.13	0.00	2.42 [b]
	12,500	80	72	45	6.25	5.48	22.22	9.60 [a]
50,000		126	111	74	5.47	5.36	13.51	7.32
	Mean				4.80	3.99	12.56	

[1] n = number of chicks used per treatment

[a,b] Means within the same level with no common superscript differ significantly ($P \leq 0.05$); result of orthogonal contrast comparisons.

Table 7 – Potency of twenty five hydroxycholecalciferol in comparison to D_3 for the different criteria

Criteria	Relative biological values: Slope ratio[1] analysis	25-OHD_3 and D_3 levels: 3,125 ng/kg	12,500 ng/kg
	----------Potency % ----------		
D_3 (standard)	100	100	100
25-OHD_3			
HDEP	138	209[2]	105
Hatchability	133	167[2]	100
Late embryo mortality	122	400[2]	126
Body ash of the progeny	111	108[2]	102
Average potency (25-OHD_3)	128	221	108
Progeny experiment 1			
Body weight		123[2]	101
Tibia ash		120[2]	104
Progeny Experiments 2,3,4			
Body weight (Ex 2)		106[2]	100
Body weight (Ex 3)		111[2]	100
Body weight (Ex 4)		92[2]	97[2]
Average potency of 25-OHD_3 [3]		115	101

[1] Relative biological values for criteria with significant differences.
[2] Significant differences between 25-OHD_3 and D_3 by comparing means at the same levels using orthogonal contrasts.
[3] Experiment 3 was not included.
[4] Ex = experiment.

FIGURES

Figure 1 – Effect of vitamin D source and level on hen day egg production (HDEP) of broiler breeders from 77 to 90 wk of age. Vitamin D source 1, 2, 4, and 6 correspond to D_3 levels 0, 3,125, 12,500 and 50,000 ng/kg and D source 3 and 5 correspond to 25-OHD_3 levels 3,125 and 12,500 ng/kg). Symbols shown are the treatment means per week

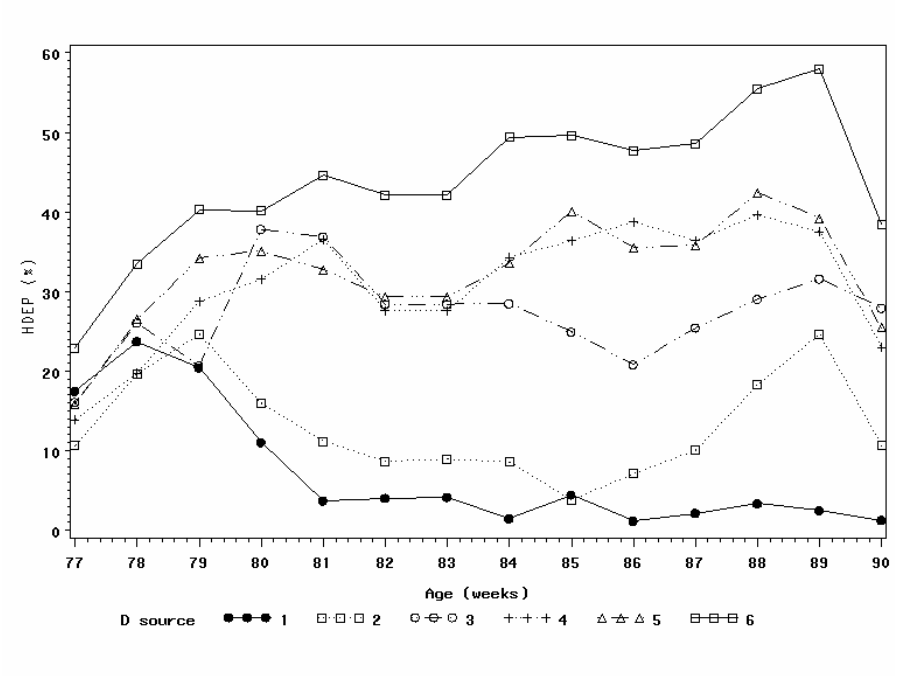


Figure 2 – Effect of vitamin D source and level on hatchability of broiler breeders from 77 to 90 wk of age. Vitamin D source 1, 2, 4, and 6 correspond to D_3 levels 0, 3,125, 12,500 and 50,000 ng/kg and D source 3 and 5 correspond to 25-OHD_3 levels 3,125 and 12,500 ng/kg). Symbols shown are the treatment means per week

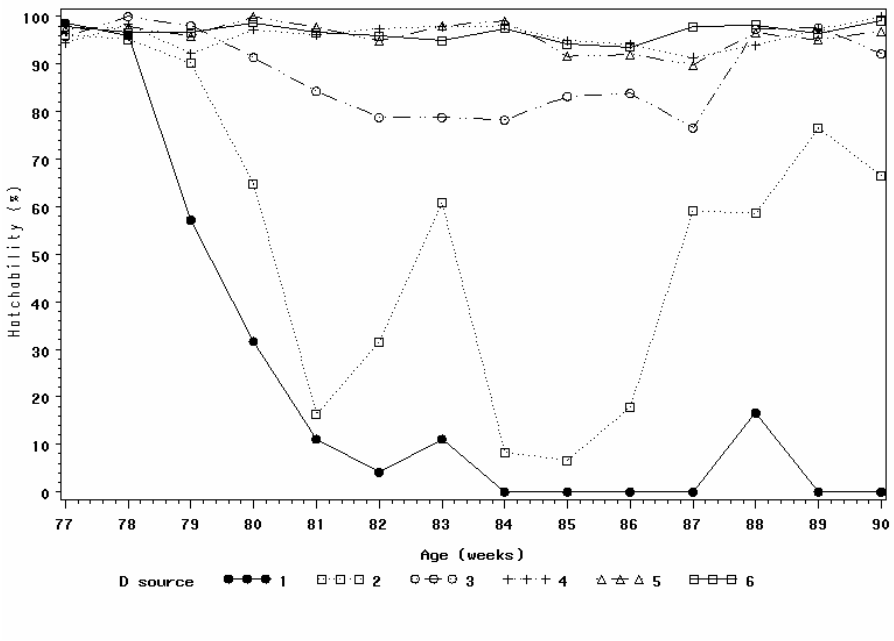


Figure 3 – Effect of vitamin D source and level on early embryo mortality (EEM %) of broiler breeders from 77 to 90 wk of age. Vitamin D source 1, 2, 4, and 6 correspond to D_3 levels 0, 3,125, 12,500 and 50,000 ng/kg and D source 3 and 5 correspond to 25-OHD_3 levels 3,125 and 12,500 ng/kg). Symbols shown are the treatment means per week

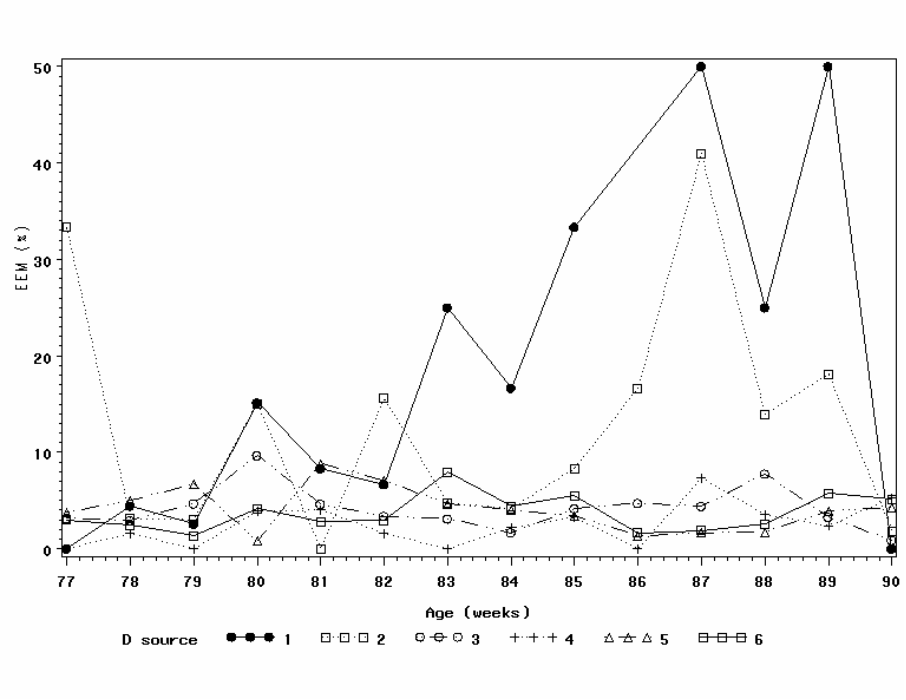


Figure 4– Effect of vitamin D source and level on early embryo mortality (LEM %) of broiler breeders from 77 to 90 wk of age. Vitamin D source 1, 2, 4, and 6 correspond to D_3 levels 0, 3,125, 12,500 and 50,000 ng/kg and D source 3 and 5 correspond to 25-OHD_3 levels 3,125 and 12,500 ng/kg). Symbols shown are the treatment means per week

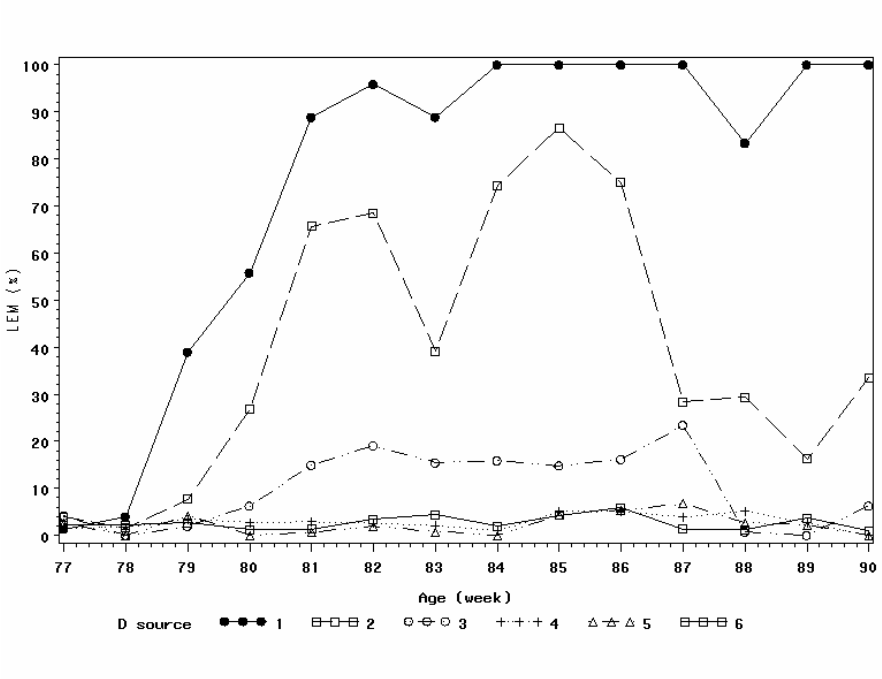
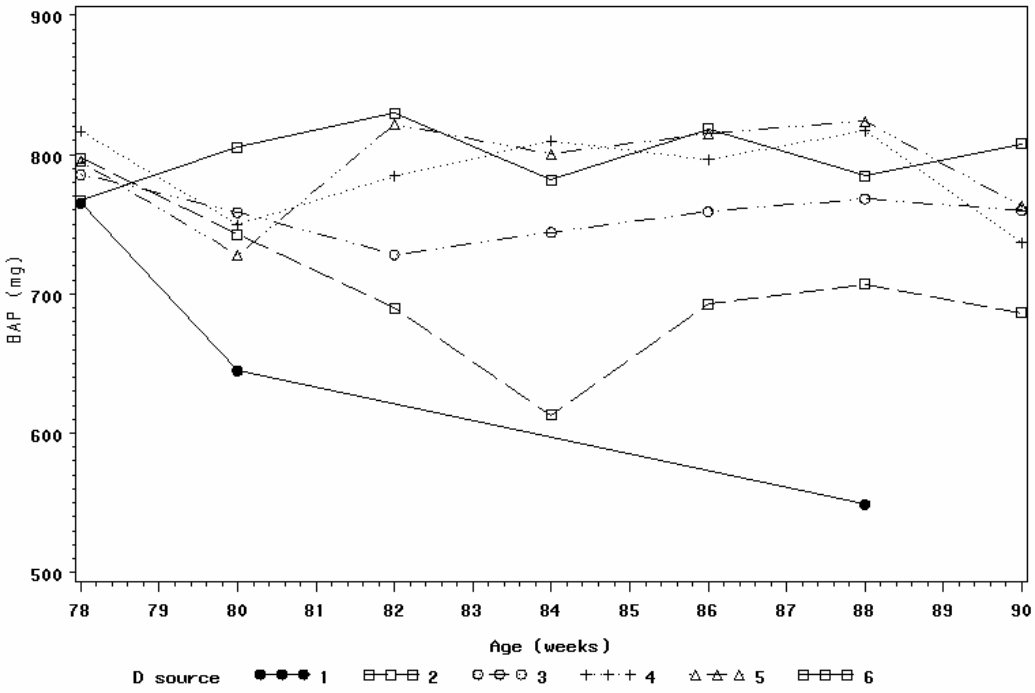


Figure 4– Effect of vitamin D source and level on body ash of the progeny (BAP) of broiler breeders from 77 to 90 wk of age. Vitamin D source 1, 2, 4, and 6 correspond to D_3 levels 0, 3,125, 12,500 and 50,000 ng/kg and D source 3 and 5 correspond to 25-OHD_3 levels 3,125 and 12,500 ng/kg). Symbols shown are the treatment means per week



CHAPTER 7

GENERAL CONCLUSIONS

Using data from the original design of the experiment (treatment 0, 125, 250, 500, 1,000 and 2,000 D_3 per kg for the 27-36-weeks of age) the vitamin D_3 requirement for maximum performance of broiler breeder hens is approximately 1,400 IU per kg of feed. When the data from the modified experiment 37-66 weeks of age include the conversion of the 0 IU of D_3/kg treatment to the 4,000 IU of vitamin D_3 per kg treatment, the requirement may be approximately 2,800 IU of D_3/kg.

Increasing the level of vitamin D_3 in the maternal diet was effective in improving body weight gain, feed intake, feed efficiency, tibia ash and in reducing tibial dyschondroplasia and Ca rickets incidence of the progeny fed either diets deficient in vitamin D_3 or diets with various levels of vitamin D_3, Ca or 25-hydroxycholecalciferol. Although not always statistically significant for tibia ash, tybial dyschondroplasia and Ca rickets incidence throughout the different experiments with the progeny, the results from these studies suggest that the requirement of broiler breeders to produce the heaviest and healthiest chicks is considerably higher than the requirement for maximum performance of the hens and higher than the NRC (1994) or Scott et al. (1982) recommendations.

Using vitamin D_3 as the standard, the average relative biological value of 25-OHD_3 for hen egg production, hatchability, embryo mortality and body ash of the progeny using slope ratio methodology was 128%. When comparing 25-OHD_3

against D_3 at the 3,125 ng/kg level, the average relative biological value was 221%. However, at the 12,500 ng/kg level the average relative biological value was 108%. In the study with progeny, the average relative biological value of 25-OHD_3 at the 3,125 and 12,500 ng levels were 115 and 101%, respectively. These results indicate that the potency of 25-OHD_3 in relation to vitamin D_3 depends on the level tested. When comparing vitamin D sources, 25-OHD_3 has greater potency than D_3 only at very low levels of supplementation.