# IN OVO INJECTION OF VITAMIN D3 TO PROMOTE POST-HATCH PERFORMANCE, INTESTINE HISTOMORPHOLOGY, BONE CHARACTERISTICS, AND BLOOD CONSTITUENTS OF BROILER CHICKENS.

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## SUMMARY

This study was conducted to investigate the role of vitamin D3 [25 (OH) D3] on embryonic development, hatchability %, bone growth, and intestine histomorphology in broiler chickens. Forty hundred broiler breeder eggs (Ross-308; average egg weight: 63.2 g) were divided into four groups (100 eggs in 4 replicates of 25 eggs each) as follows: Control (eggs were not- injected); Vehicle: eggs were injected with 100 µl sterile distilled water (diluent); Vit-50 and Vit-100: eggs were injected with 50 and 100 µl of Vit. D3, respectively. The eggs were incubated in an automatic incubator at 99.8°F and 60% RH. On the 18th day of incubation, in ovo injection was applied to eggs with live embryos within the amnion sac. At hatch, hatchability %, chick weights, and plasma constituents were recorded. Afterward, at 35 days of age, performance and intestinal development were recorded. Results revealed that chick weight at hatch and hatchability (%) were not influenced by the treatments. Also, the results showed that plasma total protein, albumin, and globulin values of day-old chicks were significantly higher in chicks hatched from eggs that were injected with 100 µl of Vit. D3 compared with the other groups and this trend was also observed at 35 days of age. Furthermore, body weight, body weight gain, and feed conversion ratio were significantly improved in response to in ovo injection with vitamin D3. Moreover, plasma calcium and phosphorus were significantly increased in chickens that were injected with Vit. D3, either at one or 35 days old. Parathyroid hormone (PTH) concentration was significantly higher in day-old chicks that hatched from the control and vehicle groups, but the opposite trend was recorded at 35-day-old, where the Vit. D3 injected groups showed high levels. Weights and lengths of tibia, femur, and keel bones were significantly heavier and longer for broilers that hatched from eggs injected with Vit. D3 than the other treatments. Jejunum histomorphometry revealed significant changes in response to in ovo Vit. D3 injection, including villus length, width, villus length to crypt depth ratio, and villus surface area. It is concluded that in ovo injection on the 18th day of incubation had a significant improvement in productive performance, plasma protein, Ca, P, and PTH levels and villi characteristics were developed. However, there was no significant effect on chick weight or hatchability percentage.

***Key words**: Broiler chickens,* in ovo *injection, vitamin D3, jejunum histomorphometry, parathyroid hormone, hatchability, bone characteristics.*

## INTRODUCTION

Vitamin D compounds are best known for their role in stimulating intestinal Ca absorption for optimal bone mineralization and improving skeletal system quality. Vitamin D3 (Cholecalciferol, Vit D3) is known to be involved in the promotion of calcium absorption in the small intestine (Bronner, 2003), maintenance of calcium homeostasis (DeLuca, 2004; Fleet, 2017), the proliferation of osteoblasts and affects bone growth and remodeling (Saunders-Blades and Korver, 2014). In addition, using 25-dihydroxycholecalciferol [25 (OH) D3], a metabolite of Vit D3, which is considered an intermediate hormone form of the Vit D3 family, has been reported to impose multifunctional mechanisms in relation to immunity, metabolism, proliferation, and differentiation of various cell types, and regulating tissue growth. Since dietary supplementation of Vit D3 is becoming critical in poultry nutrition, the use of

25(OH)D3 form is popular and better in terms of efficacy and cost than Vit D3 (Fatemi *et al.*, 2020b). Moreover, dietary 25 (OH)D3 was reported to improve the development of satellite cell activity and growth of skeletal muscles (Hutton *et al.*, 2014).

Bello *et al.* (2014a) reported that when in ovo 25 (OH)D3 was dissolved in vaccine diluent buffer and injected into broiler eggs, hatchability was improved without adverse effects on the chick weight at hatch. Moreover, in ovo injection of vitamin D3 (0.2 mg) into the amnion at 12 d of incubation has increased both Ca and P concentrations in yolk and embryonic tissue at 17 days of embryogenesis. In comparison to non-injected controls, the in ovo injection of 0.6 mg of 25(OH)D3 increased hatchability and bone quality in broilers (Bello *et al.*, 2014b).

More recently, the in ovo injection of 2.4 mg of 25(OH)D3 has been observed to result in an improvement in broiler hatch quality (Fatemi *et al.*, 2020b) as well as a decrease in feed conversion ratio (FCR) in broilers from hatch to 14 d of age (Fatemi *et al*., 2020a). Additionally, in comparison to the in ovo injection of diluent with or without Vit. D3, an improvement in the inflammatory response of 39-day-old broilers was observed when they received 2.4 mg of 25(OH) D3 by in ovo administration (Fatemi *et al.*, 2021). Moreover, there is evidence that in young embryos and early hatched chicks, the conversion of D3 to 25(OH) D3 is low due to the immaturity of their livers, which was reported to limit the increase in serum 25(OH) D3 when D3 alone is supplemented in broilers diet during early post-hatch growth (Saunders-Blades and Korver, 2014).

Therefore, the purpose of the present work was to study the effect of in ovo injection of Vit D3 on post-hatch chick weights, bone growth, jejunum histomorphology and some blood parameters in broiler chickens.

## MATERIALS AND METHODS

The present study was carried out at the department of poultry production, faculty of agriculture, Ain Shams university.

***Experimental design:***

A total number of 400 eggs with an average weight of 63.2 ± 2.1 g were obtained from a commercial broiler breeder flock (Ross) at 44 weeks of age. Eggs were divided into four groups of 100 eggs to four replicates, 25 eggs each as follows: control: (Eggs were not injected); vehicle: eggs were injected with the 100 µl sterile distilled water, Vit- 50 and Vit- 100 groups: eggs were injected with 50 and 100 of µl of Vit. D3, respectively. All eggs were stored and incubated under standard conditions (37.6°C and 60% RH) in an automatic incubator. Egg positional effects in the incubator were prevented by randomizing all treatment replicates between incubator try levels. All eggs were candled at 11 and 18 days of incubation to remove infertile eggs and early dead embryos. The in ovo injection treatments were applied by hand injection to eggs with live embryos on the 18th day of incubation following the procedures described by El-Shazly (2012).

***In ovo injection Protocol:***

The applied treatments included a control non-injected and a diluent-injected control groups and two 25- hydroxycholecalciferol vitamin D3 [25(OH)D3] levels injected groups in which 50 µl and 100 µl of 25 (OH)D3 were in ovo administered targeting the amnion of each egg. The in ovo injection of each treatment was completed within 20 minutes of taking out from the incubator, where the temperature of the chamber was maintained at 35°C. The injections were done through a pinhole made at the board end of the egg. Immediately after the injection, the site was sealed with sterile melted paraffin wax and eggs were returned to the incubator.

On the day of hatch chicks were weighed, wing banded and transferred to the battery brooders for growth performance study. The relative chick weight (as a percentage of egg weight before incubation) and hatching percentage were calculated based on the number of fertile eggs. Chicks from the four groups were fed ad libitum a commercial basal diet that was formulated to cover the recommended requirements of the broiler chickens according to the performance guide of nutrition of Ross-308.

***Bird management and the collected data:***

The management conditions were similar for all groups throughout the experimental period. The light was provided 24h during 1-4 days of age, followed by 23h during 5–35 DOA. Chicks were individually

weighed at hatch, at 21 and 35 days of age, while feed consumption (FC) was recorded for the entire experimental period. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated.

***Blood constituents:***

On the first day post-hatching, six chicks per treatment group were killed by decapitation and the total blood was collected in dry heparinized tubes, then centrifuged at 4000rpm for 15 minutes, whereas another six chicks per treatment group were slaughtered at 5 weeks of age and blood samples were also collected, then centrifuged as described previously. Plasma samples were stored at -20°C until analysis. Plasma samples were assigned for the determination of total protein, albumin, calcium, phosphorus, and alkaline phosphatase (ALK) using available commercial kits (Spectrum). Parathyroid gland hormone (PTH) was determined by RIA technique as reported by Woodhead (1990).

***Bone collection:***

Bone characteristics were tested using some bones related to the productive performance of broiler chicks. Since bones were taken from birds that were slaughtered for blood samples collection. The tibia, femur, and keel were dissected, de-fleshed, weighed and their length was recorded. The breaking strength of the tibia was measured by using an Instron Universal testing machine (Model 1011, Instron Corp. Canton MA, 02021). The machine was set at a maximum load of 50 kg and a cross-head speed of mm/minute.

***Tibia bone characteristics and jejunum histomorphometry:***

At autopsy, representative tissue specimens from the upper jejunum were dissected during the slaughtering time. Samples were fixed in a 10% formalin-Saline solution, before applying the paraffin method technique. All sections were dehydrated in ascending grades of ethyl alcohol; cleared in xylol and then embedded in paraffin wax. Transverse sections (4-5 microns, thick) were taken, mounted on glass slides, and stained with Hematoxylin and Eosin stains. All sections were examined under an electric microscope provided with a computerized camera and then subjected to morphometric analysis by using an Olympus inverted microscope cell Sens Entry. The villus length was measured from the villus tip to the villus-crypt junction, while crypt depth was defined as the depth of the invagination between two villi.

***Statistical analyses:***

Data were subjected to analysis of variance using general linear model described in SAS User's Guide (SAS Institute, 2003). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSIONS

***Egg weight, hatchability, and embryonic mortality:***

The effects of in ovo injection with vitamin D3 on hatchability percentage, chick weight at hatching and late embryonic mortality are presented in Table (1). It is clear from the results that the differences between treatments in chick weight at hatch, relative chick weight and hatchability (%) were not significant. These results indicate that the time of injection (day 18 of incubation) was no longer enough to exert the expected effect of vitamin D3 on chick weight at the hatch. It is worth noting that chicks from eggs in ovo injected with 50 µl of Vit. D3 were heavier numerically than those receiving the other treatments. Both Vit. D3 injected groups have recorded the lowest mortality rate. Moreover, during the late incubation period, the dead embryos (%) were significantly higher in the control and the diluent-injected groups compared to the vitamin-injected groups. These results support the previous findings which claimed that vitamin D3 injection could improve embryonic viability which in turn decreases the percentage of dead embryos (Ohta *et al.*, 1999; Bhanja *et al.*, 2006). Numerous studies have stated that the weight of newly hatched chickens is a major predictor of marketing weight in chickens. Wilson (1991) reported that each one gram of increase in weight of a newly- hatched chick leads to an 8 to 13 g increase in final body weight at marketing age. It is worse noting that various factors influencing hatch traits, embryonic development, and post-hatch growth, such as genetic make-up of parent stock, breeder hen age, egg size and quality characteristics, incubation conditions, and the time of in ovo injection (Egbeyale *et al.*, 2011). On the other hand, some authors, using different in ovo injection times, reported that the addition

of fat-soluble vitamins, glutamine, amino acids, or carbohydrates to chick embryos at different incubation periods enhanced intestinal development and enzyme expression at hatch, thereby allowing more efficient post-hatch development (Uni and Tako, 2004 and Lopes *et al.*, 2006).

**Table (1): Effect of *in ovo* injection with vitamin D3 on hatchability, chick weight at hatch and embryonic mortality rate in broiler chickens.**

| Traits | Treatments | | | | |
|---|---|---|---|---|---|
| | **Control** | **Vehicle** | **Vit-50** | **Vit-100** | **Sig.** |
| **Egg weight (g)** | 62.18± .02 | 62.06± .73 | 61.80± .18 | 61.63± .38 | NS |
| **Chick weight at hatch (g)** | 42.32± .21 | 42.77± .13 | 43.27± .72 | 42.51± .65 | NS |
| **Chick weight (%)** | 68.16± .82 | 69.23± .51 | 70.13±1.3 | 69.34± .64 | NS |
| **Hatchability (%)** | 86.75 | 87.68 | 87.24 | 88.04 | NS |
| **LEM (%)** | $2.71^{a}$ | $2.80^{a}$ | $2.22^{b}$ | $2.31^{b}$ | * |

*$^{a-b}$...Means with different superscripts within the same row differ significantly (P <0.05). LEM = late embryonic mortality, NS= not significant.*

***Growth performance data:***

Table (2) shows the chick weights from day old tell five weeks of age (WOA) as affected by in ovo injection with vitamin D3. It is observed that there was no significant difference in the live body weight (LBW) of day-old chicks among vitamin-injected and either non-injected or diluent-injected groups. However, at three and five WOA chicks that hatched from in ovo Vit. D3-injected eggs (50 or 100 µl) had significantly heavier LBW compared to the other groups. A similar trend was observed for body weight gain of broiler chicks as influenced by in ovo injection with 25(OH)D3 at day 18 of incubation (Table 3). . On the other hand, during the period from 3 to 5 WOA, chicks that hatched from non-injected, vehicle and 50 µl of 25 (OH)D3 - injected eggs have nearly similar BWG compared with those from 100 µl - injected eggs which recorded the lowest significantly value. However, during the whole experiment period (1d - 5 weeks of age), both Vit. D3 injected groups achieved the best significant gain in weight compared with the other groups.

**Table (2): Effect of in ovo injection with Vit. D3 on live body weight and body weight gain in broiler chickens at 35 days.**

| Age | Treatments | | | | |
|---|---|---|---|---|---|
| | **Control** | **Vehicle** | **Vit-50** | **Vit-100** | **Sig.** |
| | | | **Live body weight (g)** | | |
| **One-day-old** | 42.32± 2.21 | 43.27± 1.72 | 42.77± 1.13 | 42.51± 1.65 | NS |
| **3 weeks** | $989.62^{b}$±22.30 | $1016.52^{b}$± 43.4 | $1091.06^{a}$± 32.8 | $1126.65^{a}$± 2.21 | * |
| **5 weeks** | $2089.1^{b}$± 31.26 | $2116.8^{b}$± 35.10 | $2198.5^{a}$± 40.61 | $2203.4^{a}$± 28.19 | ** |
| | | | **Body weight gain (g)** | | |
| **0 -3 weeks** | $947.34^{b}$± 18.35 | $973.23^{b}$± 21.4 | $1048.23^{a}$± 27.1 | $1084.1^{a}$± 25.29 | ** |
| **3-5 weeks** | 1099.40± 38.7 | 1100.38± 29.3 | 1107.54± 33.55 | 1076.82± 44.21 | NS |
| **0- 5 weeks** | $2046.7^{b}$± 21.86 | $2073.5^{b}$± 28.73 | $2155.7^{a}$± 35.08 | $2160.5^{a}$± 33.14 | **** |

*$^{a-b}$...Means with different superscripts within the same row differ significantly (*=P <0.05, **= P <0.01). NS= not significant.*

The reason for this incremental increase in LBW and BWG in response to in ovo injection of 25(OH)D3 may be related to a synergism effect between in ovo vitamin D3 injection and subsequent growth of broiler. In this concern, Fatemi *et al.* (2020 a) attribute this result to the expected increase in the circulating levels of 25(OH)D3 which may have the potential to enhance the growth performance of broilers, this confirms the results of the current study. There is gross evidence for the stimulation effect of in ovo injection with Vit. D3 on satellite cells activity, growth of skeletal muscles, osteoblasts proliferation and bone calcification (Fatemi *et al.*, 2021). The role of vitamin D3 in the improvement in body weight might be due to an increase in calcium and phosphorus utilization by embryos and enhancement of bone development which in turn influence hatching and post-hatch weight as proposed by Hutton *et al.* (2014), which support our results. It is well known that the addition of fat-soluble vitamins to chick embryos at

different incubation periods enhanced intestinal development and enzyme expression at hatch, thereby allowing more efficient post-hatch development (Lops *et al.*, 2006). Similar findings were also reported by El-Shazly (2012) who used different levels of Vit. D3 in ovo injections and Vignale *et al*. (2015) who observed an improvement in LBW and breast meat yield by feeding a diet with 25(OH)D3 (5520 IU/kg feed) to broiler chickens for 42 day of age. They attributed their results to the increase of the fractional rate of protein synthesis by 3-fold compared with the control diet. In contrast, our results disagree with those obtained by Michalczuk *et al.* (2010), who reported that at the end of the rearing period (6WOA), statistically significant differences were no longer found in the body weight of broilers. However, Tako and Uni (2004) reported that in ovo feeding treatment increased body gain by 3% over control during 0-7 days of age. On the other hand, Ohta *et al.* (1999) recorded that the injection of an amino acid mixture into growing embryos in broiler breeder eggs resulted in a higher body weight at hatch and at 56 d of age compared with chick from control embryos.

***Feed consumption (FC) and Feed conversion ratio (FCR):***

Feed consumption and FCR of broiler chicks as influenced by prehatch in ovo injection of 25(OH)D3 are presented in Table (3). It is clear from the results that during the first 3WOA, FC was significantly increased for chicks from Vit. D3, 50µl and Vit. D3,100 µl treatment groups compared with those from control and vehicle groups. Moreover, during the 2nd growing phase (3-5 WOA), data showed that birds from the control and vehicle treatments consumed significantly more feed than the other chick groups, while the lowest value was recorded for chicks of Vit-100 µl treatment. This finding may be due to chicks of G1 group gained more weight during this period, which needs more nutrients to support this growth demand. For the whole period (1d -5 WOA), birds from the vehicle are still consuming more feed, followed by Vit-50 µl, both were significantly different compared with the G4 and G1. On the other hand, the feed conversion ratio (FCR) was significantly better for the Vit-100 µl chicks compared to the other treatments during the first three weeks of age. This trend was also observed during the second period (3 -5 WOA) and for the whole experiment period, with chicks from both Vit-D injected groups having the best significant FCR values. It seems likely that the best FCR of chicks from the it-D injected groups may reflect their heavier LBW, and hence BWG. It appears also that FCR, as a function of feed consumption to weight gain, may be influenced by nutrient utilization and gut health in terms of nutrient digestion, absorption, and villus surface area.

In agreement with the current results, Saunders-Blades and Korver (2014) similarly reported that the in ovo injection of 25 (OH)D3 increases its concentration in broiler embryo blood, which in turn improved post-hatch performance of chicks.

**Table (3): Effect of in ovo injection with Vit.D3 on feed consumption and feed conversion ratio in broiler chickens at 35 days.**

| Traits | Treatments | | | | |
|---|---|---|---|---|---|
| | **Control** | **Vehicle** | **Vit-50** | **Vit-100** | **Sig.** |
| | | | **Feed consumption (g)** | | |
| **0 -3 wk** | 1212.3$^{b}$± 22.81 | 1233.8$^{b}$± 30.72 | 1302.8$^{a}$± 38.13 | 1294.5$^{a}$±29.65 | * |
| **3 -5 wk** | 2146.6± 35.30 | 2213.5± 38.46 | 2095.6±42.81 | 2046.5± 40.21 | NS |
| **0 -5 wk** | 3359.1± 51.26 | 3446.8± 65.10 | 3398.5± 55.61 | 3342.4± 58.19 | NS |
| | | | **Feed conversion ratio** | | |
| **0 -3 wk** | 1.24$^{a}$± 0.15 | 1.26$^{a}$± 0.11 | 1.23$^{a}$± 0.10 | 1.19$^{b}$± 0.13 | * |
| **3-5 wk** | 1.95$^{a}$± 0.12 | 2.01$^{a}$± 0.10 | 1.89$^{b}$± 0.09 | 1.90$^{b}$± 0.08 | ***** |
| **0 - 5 wk** | 1.64$^{a}$± 0.06 | 1.65$^{a}$± 0.10 | 1.56$^{b}$± 0.08 | 1.54$^{b}$± 0.04 | ***** |

*$^{a-b}$...Means with different superscripts within the same row differ significantly (* = P <0.01). NS= not significant.*

***Blood biochemical constituents:***

Results in Table (4) showed the effect of in ovo injection of 25(OH)D3 at day 18 of incubation on blood plasma proteins in day-old chicks and at the marketing age (35 DOA). It is clear to observe that plasma total protein, albumin and globulin values of day-old chicks were significantly higher in chicks hatched from eggs that injected with 100 µl of 25(OH)D3 compared with the other groups. However, the group of G4 had the lowest A / G ratio. Similarly, at 35 DOA, all plasma protein fractions were significantly higher for chick from the group of G4 compared with the other treatments. However, data revealed that the plasma total protein value of the control chicks at 35 DOA was lower, but albumin and globulin levels

were nearly comparable to those of the other treatment, except the values recorded for chicks from the Vit.100 group, which has significantly the higher levels. It is worth noting that plasma protein levels were dramatically increased with age, this coincident with the fast growth of long bones, along with the process of bone calcification related to an improvement in calcium and phosphorus metabolism associated with bone formation. This process is accompanied by enhancement of protein utilization as it is the sole source for the organic matrix bone. This view was documented in an earlier study by Itoh and Hatano (1964). It is also possible that 25 (OH)D3 may facilitate bone formation by inducing biosynthesis of osteocalcin (VD-bind protein) which is a specific product of the osteoblasts during bone formation and or remodeling. These results are in close agreement with the previous studies by Dake (2000); Hassan *et al.* (2006) and Salim *et al.* (2019).

**Table (4): Effect in ovo injection with Vit.D3 on blood plasma protein fractions in broiler chickens at one and 35 days.**

| Traits | Treatments | | | | |
|---|---|---|---|---|---|
| | **Control** | **Vehicle** | **Vit-50** | **Vit-100** | **Sig.** |
| | | | **One-day-old** | | |
| **TP (g/dl)** | $3.88^{b}\pm 0.34$ | $3.69^{b}\pm 0.41$ | $3.96^{b}\pm 0.53$ | $4.35^{a}\pm 0.26$ | * |
| **Alb (g/dl)** | $2.24^{b}\pm 0.26$ | $2.18^{b}\pm 0.32$ | $2.34^{a}\pm 0.36$ | $2.42^{a}\pm 0.35$ | * |
| **Glob (g/dl)** | $1.64^{b}\pm 0.12$ | $1.51^{b}\pm 0.26$ | $1.62^{b}\pm 0.23$ | $1.93^{a}\pm 0.19$ | ** |
| **A/G ratio** | $1.37^{ab}\pm 0.16$ | $1.44^{a}\pm 0.12$ | $1.43^{a}\pm 0.09$ | $1.25^{b}\pm 0.11$ | * |
| | | | **At 35 DOA** | | |
| **TP (g/dl)** | $4.66^{b}\pm 0.50$ | $4.83^{ab}\pm 0.39$ | $4.75^{ab}\pm 0.44$ | $5.14^{a}\pm 0.36$ | * |
| **Alb (g/dl)** | $2.39^{b}\pm 0.34$ | $2.58^{a}\pm 0.23$ | $2.40^{b}\pm 0.25$ | $2.68^{a}\pm 0.28$ | * |
| **Glob (g/dl)** | $2.27^{b}\pm 0.15$ | $2.25^{b}\pm 0.17$ | $2.35^{ab}\pm 0.21$ | $2.46^{a}\pm 0.12$ | * |
| **A/G ratio** | $1.05^{b}\pm 0.13$ | $1.15^{a}\pm 0.19$ | $1.02^{b}\pm 0.10$ | $1.09^{ab}\pm 0.09$ | ** |

*$^{a-b}$…Means with different superscripts within the same row differ significantly (*=P <0.05, ** P <0.01). TP=total protein, Alb=albumin, Glob =globulin and A/G=albumin to globulin*

***Plasms calcium regulating parameters:***

Concerning the effect of in ovo injection of 25 (OH)D3 on plasma calcium, phosphorus and parathyroid hormone aspects, the present results showed considerable changes (Table 5). On the first day post-hatching, results clearly showed that parathyroid hormone (PTH) concentration was significantly higher in the plasma of chicks that hatched from the control and diluent-injected eggs while the Vit-D3 injected groups recorded the lowest values. The highest values for plasma calcium levels were observed for the in ovo injected treatments with Vit. D3 compared with the diluent injected and the control groups. This trend was also observed for the plasma phosphorus concentration and alkaline phosphatase (ALP) activity. In contrast, PTH concentration was significantly lower in the plasma chicks that hatched from eggs which enriched with 25 (OH)D3 via in ovo injection at d 18 of embryonic development compared with the control and diluent injected groups.

At 35 DOA, the results also showed that plasma Ca, P, and ALP activity exhibited the same trend where the 25 (OH)D3 in ovo injected groups recorded higher levels than the other groups. This means that there were significant differences between the injected treatments and the control and diluent injected groups. Concerning PTH hormone concentration, the highest value was recorded for chicks from Vit.100, followed by Vit-50 group, while the other groups showed the lowest values. It appears that the main physiological response of post-hatched chicks to in ovo injection of 25 (OH)D3, is marked increases in the criteria of calcium regulation mechanisms during bone formation.

This mechanism includes PTH-VD3 - Ca interrelationships during pre- and post-hatching growth. In this concern, there is strong evidence that both PTH and the active form of VD3 (1, 25 (OH)2 D3) increased the concentration of calcium in the blood, which promotes bone calcification (Bilal *et al.*, 2010 and Salim *et al.*, 2019).

The role of ALP in calcium metabolism and bone mineralization is well documented as it is considered a reliable marker for the maturation of the bone-forming cells (osteoblasts). Since the function of these cells is regulated by PTH, thyroid gland hormones, and IGFs (El-Ansary *et al.*, 2007; Kim *et al.*, 2011). In the case of osteopenia and (or) poor bone mineralization, the osteoblasts promote ALP activity, then its level in serum increases. There is also another source for ALP from chondrocytes either during late

embryonic development or during the fast growth period after hatching, where it is important for bone calcification (Roberson and Edward, 1994).

**Table (5): Effect of in ovo injection with Vit. D3 on plasma concentration levels of calcium, phosphorus, alkaline phosphatase and parathyroid hormone in broilers chickens at one and 35 days of age.**

| Traits | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | Vehicle | Vit-50 | Vit-100 | Sig. |
| | | One-day-old | | | |
| Ca (mg/dl) | $9.88^{b}$± 1.62 | $10.04^{b}$± 2.51 | $10.96^{a}$± 2.43 | $10.75^{a}$± 2.27 | * |
| P (mg/dl) | $4.17^{b}$± 0.73 | $4.28^{b}$± 0.32 | $5.07^{a}$± 0.36 | $4.82^{a}$± 0.35 | * |
| ALP (IU/dl ) | $120.6^{b}$ ± 8.42 | $112.5^{b}$± 0.15 | $139.6^{a}$± 13.37 | $155.9^{a}$± 17.25 | ** |
| PTH (ng/dl) | $2.98^{a}$± 0.31 | $2.74^{a}$± 0.60 | $2.45^{b}$± 0.45 | $2.38^{b}$± 0.58 | * |
| | | At 35 DOA | | | |
| Ca (mg/dl) | $10.52^{c}$± 2.44 | $10.46^{c}$± 1.89 | $11.83^{a}$± 2.34 | $10.95^{b}$± 2.55 | * |
| P (mg/dl) | $5.48^{b}$± 0.87 | $4.88^{b}$± 0.92 | $5.75^{a}$± 1.04 | $5.97^{a}$± 1.16 | * |
| ALP (IU/dl ) | $144.36^{b}$± 13.65 | $166.65^{b}$± 18.34 | $192.43^{a}$± 21.20 | $212.56^{a}$± 15.89 | * |
| PTH (ng/dl) | $4.35^{b}$± 0.72 | $4.62^{b}$± 0.58 | $5.59^{a}$± 0.40 | $5.84^{a}$± 0.49 | * |

$^{a-c}$…Means with different superscripts within the same row differ significantly (*=P <0.05, ** P = <0.01). Ca=calcium, Pi=phosphorus, ALP =alkaline phosphatase and PTH=parathyroid hormone.

***Bone characteristics:***

The bone traits of broiler chicks as influenced by in ovo injection of 25 (OH)D3 at the 18th day of incubation are presented in Table (6). The results showed that tibia, femur and keel lengths were longer (P<0.05) for broilers that hatched from eggs injected with VD3 (Vit.50 & Vit.100) than the other treatments. On the other hand, the tibia and femur weights were heavier (P<0.05) for both in ovo vitamin 25 (OH)D3 - injected groups compared to the diluent-injected and the control groups. It appears from the previous results that the in ovo injection with VD3, could improve long bones (tibia and femur) and keel measurements. This elongation may be due to the physiological role of VD3 for enhancing bone formation during the post-hatch growth period. This result was confirmed by several authors (Kim, *et al.*, 2011; El-Shazly, 2012; Zamani *et al.*, 2018; Salim *et al.*, 2019) who reported that VD3 enrichment of eggs by direct in ovo injection or by using higher levels of vitamins in the diet to increase their contents in the yolk, could be recommended for improving bone quality of fast-growing broiler. Similarly, tibia bone breaking strength was significantly higher for chicks from Vit.50 and Vit.100 treatment groups compared by other treatments. These results provide evidence for the influence of VD3 fortification of eggs on bone quality measurements. The rise in plasma Ca, P, and ALP activity may be accounted for by increasing the osteoblastic activity in medullary bone and then accelerating bone mineralization. This is in close agreement with the results obtained in growing chicks as observed by Hurwitz, 1992); Rath *et al.*, (2000), Applegate and Lilburn, (2002) and Mabelebele *et al.*, (2017).

**Table (6): Effect of in ovo injection with Vit. D3 on in broilers chickens at one and 35 days of age.**

| Traits | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | Vehicle | Vit-50 | Vit-100 | Sig. |
| Tibia Length-(cm) | $12.86^{b}$ ± 1.22 | $12.59^{b}$± 1.34 | $14.87^{a}$± 1.42 | $13.75^{a}$± 1.65 | * |
| Tibia Weight (g) | $15.65^{b}$ ± 1.45 | $14.73^{b}$ ± 1.46 | $16.53^{a}$ ± 1.92 | $16.98^{a}$ ± 2.18 | * |
| Femur Length-(cm) | $9.94^{b}$ ±0.74 | $9.86^{b}$ ± 0.69 | $10.19^{a}$ ± 0.78 | $10.38^{a}$ ± 1.82 | NS |
| Femur Weight (g) | $12.60^{b}$ ± 2.19 | $13.24^{b}$ ± 1.68 | $14.52^{a}$ ± 1.46 | $14.93^{a}$ ± 1.34 | ** |
| Keel Length (cm) | $12.45^{b}$ ± 1.56 | $12.57^{b}$ ± 1.44 | $13.29^{a}$ ± 1.13 | $12.90^{a}$ ± 1.21 | * |
| TBS Neuton ( $N/cm^2$ ) | $1.86^{b}$ ± 0.23 | $2.12^{b}$ ± 0.19 | $2.44^{a}$ ± 0.08 | $2.35^{a}$ ± 0.16 | ** |

*$^{a-b}$…Means with different superscripts within the same row differ significantly (*=P <0.05, ** = P <0.01). NS= not significant.*

***Histomorphological examination:***

The data presented in Table (8) revealed a highly significant increase in villi height, width, and surface area at 35 days of age due to the in ovo injection with Vitamin D3 on the 18th day of incubation. This may explain the improvement in the BWG and FCR of the injected groups with vitamin D3. The increase observed in villi height and surface area means an increase in the absorption area of the intestine and maximizes feed utilization.

**Table (7): Effect of in ovo injection with Vit. D3 on on jejunum histomorphometry in broilers chickens at 35 days of age.**

| Traits | Treatments | | | | |
|---|---|---|---|---|---|
| | **Control** | **Vehicle** | **Vit-50** | **Vit-100** | **Sig.** |
| **VL (µm)** | $780 \pm 48.4^{b}$ | $720 \pm 36.3^{c}$ | $860 \pm 66.9^{a}$ | $896 \pm 55.8^{a}$ | ** |
| **VW (µm)** | $82 \pm 6.6^{a}$ | $78 \pm 8.2^{b}$ | $86 \pm 10.5^{a}$ | $92 \pm 9.8^{a}$ | * |
| **CD (µm)** | $94 \pm 7.4$ | $102 \pm 6.8$ | $98 \pm 6.9$ | $90 \pm 8.1$ | NS |
| **VL:CD (%)** | $8.50 \pm 0.81^{b}$ | $7.06 \pm 0.76^{c}$ | $8.78 \pm 0.55^{b}$ | $9.96 \pm 0.73^{a}$ | ** |
| **VSA (mm2)** | $0.202 \pm 0.095^{b}$ | $0.176 \pm 0.128^{b}$ | $0.232 \pm 0.157^{a}$ | $0.259 \pm 0.241^{a}$ | ** |

*$^{a-c}$…Means with different superscripts within the same row differ significantly (*=P <0.05, **= P <0.01). NS= not significant. VL= villus length, VW= villus width, CD= crypt dept, VSA= villus surface area*

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## حقن فيتامين د3 في بيض التفريخ لتعزيز الأداء بعد الفقس، وهستولوجي الأمعاء، وخصائص العظام ومكونات الدم في دجاج انتاج اللحم.

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أجريت هذه الدراسة لتقييم دور فيتامين د3 [25 (OH) D3] على التطور الجنيني، الفقس، نمو العظام، وهستولوجى الأمعاء فى دجاج انتاج اللحم.

تم تقسيم عدد 400 بيضة تفريخ من دجاج انتاج اللحم (سلالة روص-308؛ متوسط وزن البيضة: 63.2 جم) إلى 4 مجموعات (100 بيضة في 4 مكررات لكل منها 25 بيضة) على النحو التالي: مجموعة الكنترول (غير المحقونة) والمذيب (ماء معقم): تم حقن البيض بالمادة الحاملة للفيتامين ومجموعة (فيتامين- 50) حيث تم حقن البيض بـ 50 ميكرولتر من فيتامين د3 ومجموعة (فيتامين-100) تم حقن البيض بـ 100 ميكرولتر من فيتامين د3. تم تحضين البيض في محضن أوتوماتيك عند درجة حرارة 37,6 درجة مئوية و60% رطوبة نسبية. تم حقن البيض الذي يحتوي على أجنة حية داخل كيس الامنيون بالجرعات المحددة سلفًا في اليوم الثامن عشر من التفريخ وعند الفقس تم تسجيل نسبة الفقس ووزن الكتكوت ومكونات البلازما. وعند عمر 35 يومًا، تم تسجيل الأداء الإنتاجي وتطور الأمعاء.

أوضحت النتائج : أن وزن الكتاكيت عند الفقس ونسبة الفقس (%) لم يتأثرا بالحقن بفيتامين د3. تحسن وزن الجسم والزيادة الوزنية ونسبة التحويل الغذائي بشكل ملحوظ عند عمر 35 يوم استجابةً لحقن البيض بفيتامين د3. كانت قيم البروتين الكلي والألبومين والجلوبيولين في البلازما للكتاكيت عمر يوم أعلى بشكل ملحوظ في الكتاكيت التي فقست من المعاملات المحقونة بالفيتامين مقارنة بالمجموعات الأخرى. ولوحظ هذا الاتجاه أيضًا عند عمر 35 يومًا. علاوة على ذلك، ارتفع مستوى الكالسيوم والفوسفور في البلازما بشكل ملحوظ في الدجاج في المجموعات المحقونة بـ D3 عند عمر يوم و 35 يوم. كان تركيز هرمون الغدة الجاردرقية (PTH) أعلى بكثير في الكتاكيت عمر يوم واحد والتي فقست من بيض الكنترول والبيض المحقون بالـ vehicle، ولكن تم تسجيل نتيجة معاكسة عند 35 يوم من العمر، حيث أظهرت المجموعات التي تم حقنها بالفيتامين مستويات عالية. كانت أوزان و أطوال عظمة الساق وعظم الفخذ والصدر اكبر معنويا ($P<0.05$) في دجاج انتاج اللحم الذي فقس من البيض المحقون بالفيتامين مقارنة بالمعاملة الكنترول. أدى حقن البيض بفيتامين د3 إلى تأثير واضح على الامعاء عند 35 يوم من العمر حيث لوحظ زيادة معنوية في طول الخملات وعرضها ومساحة سطح الخملات.

من النتائج السابقة نستنتج ما يلي: حقن بيض التفريخ في اليوم الثامن عشر من التحضين بكيس الأمنيون كان له تأثير إيجابي على الأداء الإنتاجي و كذلك تحسنت مستويات بروتينات البلازما، الكالسيوم، الفوسفور، وهرمون الغدة الجردرقية بشكل ملحوظ ، كما لوحظ تحسن وتطور لخصائص الأمعاء وجودة العظام ، ولكن لم يكن هناك تأثير معنوي على وزن الكتكوت ونسبة الفقس.