EFFECT OF *IN OVO* INJECTION OF 25 HYDROXYCHOLECALCIFEROL DURING EMBRYONIC DEVELOPMENT ON BROILERS PERFORMANCE, BONE STRUCTURE AND SOME BLOOD PARAMETERS

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SUMMARY

This study was conducted to investigate the effect of *in ovo* injection of vitamin D3 during different embryogenesis periods on post hatch productive performance, some blood parameters, bone growth and tibia bone histology of Hubbard chicks. A total number of 500 eggs with average weight of 65 g were obtained from a commercial broiler breeder flock (Hubbard) at 55 weeks of age. Eggs were divided into five groups of 100 eggs in four replicates, 25 eggs each. The first one was served as negative control group (un-injected), while the second and third groups were used as sham control which were injected with (0.1 ml sterile water\egg) into air cell at day 7and 14, respectively (SE7, SE14). The Fourth and Fifth groups were injected with a dose of 0.1 ml vit.D3 (Containing 500 IU) into air cell at day 7 and 14 of incubation period respectively (DE7, DE14). The present findings revealed that chicks hatched from eggs injected with vit. D3 has significantly better productive performance (higher LBW and BWG, less FI and better FCR) than control and sham groups. Plasma level of PTH, Ca, P, ALK-P and ACP were significantly increased with *in ovo* injection with vit. D3. Also, Tibia weight, length and width were significantly increased for the in ovo injected with vit.D3. Femur weight, length and width were insignificantly increased for the *in ovo* injection with vit.D3. Shank length was significantly increased for the *in ovo* injection with vit.D3, while shank width was insignificantly increased compared with the control and sham groups. Furthermore, the histological structure of bone was enhanced with *in ovo* injection with vit. D3 either at 7 or 14 days of incubation period. In conclusion from the present study shows that *in ovo* injection with a dose of 0.1 ml vit.D3 (Containing 500 IU) in Hubbard broiler chicks at 7^{th} or 14^{th} day of incubation period enhanced productive performance, blood parameters, bone development and histological structure.

Keywords: *in ovo, vitamin D3, growth performance, bone, histology, and broiler.*

INTRODUCTION

In ovo injection, which is the administration of exogenous nutrients into the egg, enhance egg nutriture and early growth performance by improving pre-hatch chicken embryo energetic and nutritional status, maturation and function of the intestine. This technology allows supplementing nutrients to broiler embryos, as it is known that the nutritional supply from yolk sac is not sufficient to support the fast grow rate broiler chicks present during their neonatal phase (Willemsen *et al*., 2010).

The major hormonally active product of the vitamin D endocrine system is 1,25-dihydroxycholecalciferol (1,25 (OH)2 D3). 1,25-dihydroxycholecalciferol is formed in the organism in two stages; In the first stage; cholecalciferol (vitamin D3) is converted into 25-hydroxycholecalciferol (25(OH) D3) in the liver; and then 25(OH) D3 is hydroxylated to form the biologically active form of vitamin D-1,25(OH)2 D3 in the kidney (Deluca, 2004 and Dixon and Mason, 2009). 1,25-Dihydroxyvitamin D3 acts through a nuclear receptor to express its numerous functions including stimulating intestinal Ca absorption, Ca mobilization in bone, Ca reabsorption in the kidney, bone homeostasis, and improved quality of the skeletal system (Deluca, 2004 and Zamani *et al.*, 2018 and Salim *et al*., 2019).

Vignale *et al.,* (2015) studied the effect of vit.D3 on growth performance of broiler chicks and found significant increases in body weight and body weight gain with high diet concentrations of cholecalciferol (5520 IU/kg feed), or a diet with 25(OH)D_3 (5520 IU/kg feed) for 42 day of age. Ahmed *et al.* (2015) observed a synergetic effect between phytase and vit.D3, and reported significant increases in live body weight and live weight gain in phytase and vitamin D3 supplemented groups particularly, supplementation of 500 phytase unit /kg plus 5000 IU vitamin D3/kg. Han *et al.* (2012) found significant increases in body weight and body weight gain, while found significant decreases in feed intake and improve feed conversion of broilers with addition of 5 or 10 micro gram 1α-OH D3 //kg basal diet for 21 days. Pesti and Shivaprasad (2010) reported that dietary supplementation of 25 µg/kg diet of 1α-OH-D3 significantly decreased feed intake and improved feed conversion of broiler chicks.

Bello *et al.* (2014a) reported that *in ovo* injection of 25(OH)D3 dosage levels above 1.20 µg at day 18 of incubation may have the potential to promote the post hatch bone mineralization of broilers. It is hypothesized that in ovo administration of 25(OH)D_3 in commercial diluent on day 18 of incubation may be capable of supporting improved post hatch bone development in broilers. Moreover, Bello *et al.* (2014b) showed that the *in ovo* injection of 0.20, 0.60, or 1.80 µg 25(OH)D_3 day at 18 of incubation increased the bone breaking strength of male broilers on day 28 post hatch age when compared to those received diluent injections without added 25(OH)D_3. They were suggested that the *in ovo* administration of supplemental 25(OH)D_3 may promote skeletal formation in the broiler embryo and post hatch chick by stimulating the absorption of Ca reservoirs in the yolk. Levels above 1.20 µg on day 18 of incubation may have the potential to promote the post hatch bone mineralization of broilers. Yair *et al.* (2013) showed that the injection of an enrichment solution containing organic micro minerals and vitamins, including vitamin D_3, into the amnion of broiler hatching eggs on day 17 of incubation period, improved the mineralization and mechanical properties of bones and increased the medullary area of long bones.

Vitamin D is essential for regulation of calcium absorption from the digestive tract and for the deposition of calcium in and withdrawal of calcium from bones. El-Shazly (2012) suggested that injecting eggs from two local strains of chickens with vitamin D3(16000 IU) at day 7 or day 14 of incubation significantly increased in plasma calcium and phosphorus level at 8 weeks of age. Kim *et al.* (2011) suggests that dietary supplementation of vitamin D_3 at 250 µg/kg diet for 3 week-old broiler chicks can increase bone growth and mineral deposition in broiler chick at the marketing age (42 day of age). Bolu *et al.*(2006) showed that serum calcium, phosphorus and Plasma alkaline phosphatase significantly increased when commercial broilers were fed a diet supplemented with cholecalciferol (800 IU/kg diet) for 8 weeks compared with birds fed the normal recommended dietary cholecalciferol inclusion level (200 IU/kg diet).

Thus the purpose of the present work was to study the effect of in ovo injection of vitamin D3 during different embryogenesis periods on post hatch productive performance, some blood parameters, bone growth and histology of Hubbard chicks

MATERIALS AND METHODS

The present study was carried out at the Department of Poultry Production, Fac. of Agric. Ain Shams Univ. from March to May, 2018.

***Experimental Procedures**:*

A total number of 500 eggs with average weight of 65 g were obtained from a commercial broiler breeder flock (Hubbard) at 55 weeks of age. Eggs were divided into five groups of 100 eggs in four replicates, 25 eggs each. All eggs were normally incubated at 37.6°C and 65% RH in an automatic incubator. The first one was served as negative control group (un-injected), while the second and third groups were used as sham control which were injected with (0.1 ml sterile water\egg) into air cell at day 7and 14, respectively. (SE7, SE14). Sterile water injection was included as sham control primarily to rule out a possible negative response caused by the stress of injection and handling. The Fourth and Fifth groups were injected with a dose of 0.1 ml vit.D3 (Containing 500 IU) into air cell at day 7 and 14 of incubation period, respectively (DE7, DE14). At the 18 day of incubation, all eggs were transferred to the hatcher and kept till hatching at 37.2°C and 70% RH. The hatched chicks from all groups were fed *ad libitum* on commercial basal diet which was formulated to cover recommended requirements of the broiler chickens (NRC, 1994). The composition and calculated analysis of the basal diet are shown in Table (1).

Table (1): Composition and calculated analysis of the basal starter and finisher diets.

Ingredient (%)	starter diet	finisher diet
yellow corn	52.03	61.38
soybean meal (44%)	29.60	22.50
corn gluten meal (60%)	7.15	6.25
vegetable oil	4.00	4.00
wheat bran	2.80	2.60
bone meal	3.30	2.15
Limestone	0.14	0.14
Premix*	0.30	0.30
NaCl (salt)	0.50	0.50
L-Lysine-HCl	0.18	0.18
Total	100	100
Calculated Analysis:		
Crude Protein (%)	23.07	20.03
ME/Kcal/Kg	3100	3207
Crude Fiber (%)	3.80	3.32
Ether extract	6.50	6.73
Calcium(%)	0.92	0.90
Available P(%)	0.48	0.46
Lysine (%)	1.22	1.05
Methionine (%)	0.55	0.49
Meth+cystine (%)	0.92	0.81

Each 3 Kg contains: vit.A 12000000 IU, vit.D3 25000000 IU, vit.E 10g , vitK 3g , vit.B_2 5 g , vit.B6 1.5g , vit.B12 0.01g , niacin 30g, folic 1g Biotin 0.05 g , Pantothenic acid 10g, Copper 10 g , Iodine 1g, Selenium 0.1, Iron 30g, Manganese 60g , Zink 50g , Cobalt 1g.

Growth performance:

Chicks were individually weighed at hatch and at the end of the experimental period (42 DOA) and their live body weight (LBW) and feed intake (FI) were recorded. The body weight gain (BWG) and feed conversion ratio (FCR), (FI/BWG) were calculated.

Bone growth:

Tibia, femur, shank and keel measurements were recorded , at slaughtering time, at the end of the experimental period (42 DOA).

Biochemical Analysis:

Blood samples were collected from five chicks per treatment at slaughtering time centrifuged (4000 rpm/5min). Plasma was decanted and stored frozen at -20ºC until the biochemical analysis of some blood parameters was done.

Parathyroid hormone (PTH) was measured by using radioimmunoassay techniques according to the method described by Woodhead (1990).

Plasma calcium and phosphorus were assayed by the method of Tietz (1990) and Henry (1974), respectively.

Alkaline phosphatase and acid phosphatase were assayed by the methods described by Reitman and Frankel (1957).

Tibia bone histology:

Representative samples from the tibia bone of 42 day chicks during the slaughtering time were carefully dissected. 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 Zylol and it embedded in paraffin wax.Transverse sections (4-5 microns, thickness) were taken, mounted on glass slides and stained with Haematoxylene and Eosin stains (H.E). All sections were exanimated under electric microscope provided with a computerized camera.

Statistical analysis:

Data were subjected to one-way analysis of variance by using the general linear models procedure (GLM) of the statistical analysis system (SAS, 2003). Differences among treatments means were detected by using Duncan multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance:

Results presented in Table (2) showed the effect of *in ovo* injection of Hubbard broiler eggs with vitamin D3 during different embryonic stages on the productive performance of chicks at the end of the experimental period. The highest ($p<0.01$) value of body weight at 1 day of age (DOA) was of DE7 followed by E14 compared to the other groups. The same trend was recorded at 42 DOA. The cumulative weight gain of chicks from 1 to 42 days showed significant ($p<0.01$) increase in vitamin D3 chicks, either from eggs that injected at 7 and 14 of incubation. The DE7 chicks group had significantly consumed less feed than the other treatments. Feed conversion ratio was significantly ($p<0.05$) improved in DE7 chicks during the whole growing period (1-42 day). In addition, *in ovo* injection of eggs at day 7 of incubation (DE7) had insignificantly improved feed conversion ratio during the whole experimental period compared with *in ovo* injection of eggs at day 14 of incubation (DE14).

Table (2): Effects of *in ovo* injection with vitamin D3 on productive perfprmance of Hubbard broiler chicks.

Item	Treatment					SEM	Sig
	C	SE7	SE14	DE7	DE14		
Live Body weight (gm)							
1 day	43.32^{b}	42.75^{b}	43.68^{b}	46.29^{a}	45.21^{a}	0.023	**
42 day	1820.00^{c}	1821.70^{c}	1822.62^{c}	2005.00^{a}	1977.96^{b}	0.015	**
Body weight gain (gm)							
1-42 day	1776.68^{b}	1778.94^{b}	1778.90^{b}	1959.43^{a}	1932.84^{a}	0.025	**
Feed intake (g)							
1-42 day	3400.0^{a}	3394.33^{a}	3413.01^{a}	3300.62^{b}	3390.12^{a}	120.3	**
Feed conversion ratio (g)							
1-42 day	1.91^{a}	1.91^{a}	1.92^{a}	1.68^{b}	1.75^{ab}	0.01	*

C: Negative control (un injected), SE7: sham injected at 7 day of incubation, SE14: sham injected at 14 day of incubation, DE7: Vit.D3 injection at 7 day of incubation, DE14:Vit.D3 injection at 14 day of incubation.
$^{a,b,c.....}$ Means within rows with different superscripts are significantly different ($P\leq 0.05$)
** : $P\leq 0.05$ **: $P\leq 0.01$.*

It clearly noted from the present results that *in ovo* injection time with vitamin D3 increased LBW and body weight gain of broiler chicks. This may be due to that chicken fibroblast proliferation and bone calcification can be strongly stimulated by the addition of vitamin D3 either in the diet or via *in ovo* feeding. 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 enhancing bone development which in turn influence hatching weight. 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 results were also obtained by El-Shazly (2012) who used vit. D3 in ovo injections and Vignale *et al.,* (2015) using a diet with 25(OH)D3 (5520 IU/kg feed) for 42 DOA and they reported enhanced BW and breast meat yield due to increase the fractional rate of protein synthesis by 3- fold compared with the control diet. These results confirm and support our findings in the present study. It is appears from the results that the effect of the injection time of hatching eggs with D3 improved feed conversion ratio of broiler chicks during the whole experimental period. This effect may be related to the positive effects of D3 in improving nutrients utilization. This was also related to the improvement in both live body weight (LBW) and body weight gain (BWG) of chicks during the whole experimental period, since, FCR is a function of BWG and feed consumption. It is suggested by many authors that addition of vitamins, amino acids or carbohydrates to chicks embryos at different incubation periods had a positive influence on intestine development and digestive enzymes expression at

hatch and subsequent growth post hatching, thereby allowing more efficient utilization of feed which in close agreement of our results (Tako *et al.*, 2004); Uni and Ferket (2003); Pedroso *et al.*, (2006); Ali (2015) and Salim *et al*., (2019).

Plasma PTH, Ca and P concentration:

The effect of *in ovo* injection with vit.D3 at day 7 or 14 of incubation period on plasma parathyroid hormone (PTH), Ca and P concentration of broiled chicks is illustrated in Table (3). It is clear from the results that plasma level of PTH was significantly higher in chicks of DE7 followed by DE14 injected groups compared with control and sham groups.

Plasma Ca level, *in ovo* vit.D3 injection at day 14 (DE14) recorded ($p<0.05$) the highest value followed by the DE7 injected group at 42 DOA compared with control and sham groups. The same trend was recorded for the plasma P concentration, *in ovo* vit.D3 injection at day 7 (DE7) and day 14 (DE14) had significantly increased plasma P level compared with the control and sham groups.

It appears from these results that *in ovo* injection with vit.D3 either at 7 or 14 of embryogenesis could improve parathyroid gland function and stimulate PTH secretion. The consequence of such treatment in the increase in plasma Ca and P levels. Previous results showed that vit.D3 is very important in both absorption and resumption of bone, since the administration of large quantities of vit.D3 may help bone remodeling in much the same way that PTH does. Moreover, some investigators reported that, in the absence of vit.D3 the effect of PTH on bone resorption is greatly reduced, which turn causes bone problems and imposes marked physical stress on broiler legs (dischondroplasia syndrome). They added that administration of vit.D3 would help in restoring osteoblastic activity (Nys, 1993; Dake, 2000; El-Shazly, 2012; Ali, 2015 and Salim *et al*., 2019).

El-Shazly (2012) suggested that injecting hatching eggs with vitamin D3 at 7 or 14 day of incubation significantly increased of plasma calcium and phosphorus level at 8 weeks of age. On the other hand, Ahmed (2015) showed significant increases in plasma calcium and phosphorous level with dietary supplementation of 500 phytase unit /kg diet plus 5000 IU vitamin D3/kg diet, compared to the control groups of broiler chicks. Also Nawaz *et al*. (2008) showed that blood calcium and phosphorus contents increased with increasing levels of cholecalciferol in the diet of broiler chicks. It is clear from the previous results that in ovo injection of vit.D3 at day 7 or 14 of incubation period had improved the mechanism of Ca and P regulation in broiler chicks under the conditions of the present study. Since, this mechanism include PTH, Ca and P the results showed significant increases in response to vit.D3 treatments. It is postulated that vit.D3 promotes bone calcification via increasing the Ca and P concentration in the extra cellular fluids and by enhancing the transport of Ca ions through osteocytes membranes for bone formation, This was confirmed by the findings that vit.D3 facilitates bone formation by inducing biosynthesis of osteocalcin (a vit.D-bind protein) which is a specific product of the osteoblasts during bone formation and or remodeling. These results are in close agreement with those reported by Dake (2000); Hassan *et al*. (2006); Ali (2015) and Salim *et al*. (2019). It is worth to mention that the concentration of plasma Ca, P and PTH are related to the growth pattern and the growth period of the hatched chicks rather than the genetic and metabolic demands of the growing chicks. It is likely, however, that plasma Ca and P level are a function of metabolic activities associated with the physiological demands for bone formation and remodeling. Such metabolic activates seemed to be controlled by hormones, nutrition, vitamins and the strain of chicks.

Table (3): Plasma PTH, Ca, P concentration at 42 DOA.

Item	Treatment					SEM	Sig
	C	SE7	SE14	DE7	DE14		
PTH (ng/ml)	2.84^{b}	2.88^{b}	2.86^{b}	3.11^{a}	3.10^{a}	0.148	*
Ca (mg/dl)	9.80^{b}	10.14^{b}	9.69^{b}	11.76^{ab}	12.70^{a}	0.349	*
P (mg/dl)	3.77^{b}	3.64^{b}	3.88^{b}	4.28^{a}	5.05^{a}	0.263	*

C: Negative control (un injected), SE7: sham injected at 7 day of incubation, SE14: sham injected at 14 day of incubation, DE7: Vit.D3 injection at 7 day of incubation, DE14:Vit.D3 injection at 14 day of incubation.

^{a,b},:Means within rows with different superscripts are significantly different ($P\leq 0.05$)

**: $P\leq 0.05$*

Alkaline phosphatase and acid phosphatase activities:

The effect of *in ovo* injection with vit.D3 at day 7 or 14 of incubation period on alkaline phosphatase (ALK-P) and acid phosphatase (ACP) activities in blood plasma of broiled chicks is illustrated in Table (4). It is clearly observed that ALK-P activity was significantly increased in D3 treatment chicks compared with the other groups. The highest Alk-P activity was recorded in plasma of chicks from *in ovo* injected eggs at day 7 of embryogenesis (DE7), followed by those injected at day 14 of incubation (DE14). These results illustrate the pronounced influence of vit.D3 on Alk-P activity. Concerning acid phosphates activity in plasma, the highest value ($p<0.05$) obtained for the DE14 vit.D3 injected groups followed by DE7, then control and then both sham injected groups (C, SE7, SE14).

The increase in both ALK-P and ACP activity might be attributed to the higher osteoblastic activity associated with bone formation and mineralization .It is also well known that both ALK-P and ACP are used as markers for the mature phenotypes of osteoblasts and since their activities in bone formation and resumption may be regulated by Parathyroid hormone (PTH), estrogen, thyroid hormones and insulin-growth factors as reported by many authers (Bishop *et al.*, 2000; El-Ansary *et al*., 2007; Kim *et al*. 2011 and El-Shazly, 2012) . This was also demonstrated by the fact that chondrocytes is the source of ALK-P during late embryonic development and also during the fast growth period after hatching, where, ALK-P is important for bone calcification (Roberson and Edward, 1994) which support the present results**.**

Table (4): Alkaline phosphatase and acid phosphatase activities at 42 DOA.

Enzyme	Treatment					SEM	Sig
	C	SE7	SE14	DE7	DE14		
ALKP (U/L)	180.00^{b}	181.56^{b}	180.36^{b}	260.86^{a}	256.91^{a}	3.946	*
ACP (U/L)	33.10^{b}	34.94^{b}	31.56^{b}	43.48^{a}	55.92^{a}	2.747	*

C: Negative control (un injected), SE7: sham injected at 7 day of incubation, SE14: sham injected at 14 day of incubation, DE7: Vit.D3 injection at 7 day of incubation, DE14:Vit.D3 injection at 14 day of incubation.

$^{a,b,\ldots}$:.Means within rows with different superscripts are significantly different ($P\leq 0.05$)

**: $P\leq 0.05$*

Bone development:

Results in Table (5) showed some bone measurements of 42 day old broiler chicks as influenced by *in ovo* injection with vitamin D3 at two different periods of embryogenesis. It was clear from the results that tibia weight, and tibia length were significantly increased for the *in ovo* injection of vit.D3 either at 7 or 14 days of incubation period (DE7 and DE14) compared to the control and sham groups (SE7 and SE14). Tibia width was not significant different. Femur weight, length and width were insignificantly increased for *in ovo* injection of vit.D3 either at 7 or 14 days of incubation period (DE7 and DE14) compared to the other groups. Shank length was significantly increased for *in ovo* injection of vit.D3 either at 7 or 14 days of incubation period (DE7 and DE14) compared to other groups. While shank width was insignificantly increased for vit.D3 groups compared with the control and sham groups. Concerning the effect of vit.D3 injection on keel length, the present results clearly showed that *in ovo* injection with vit.D3 either at 7 or 14 days of incubation significantly increased keel length compared with the other groups.

It appears from the previous results that the *in ovo* injection with vit.D3, especially at day 7 of embryonic development could significant improve long bones (tibia weight and length and shank length) measurements. The elongation of the long bones and also the breast bone (keel) of Vit. D3 *in ovo* injection may be due to the physiological role of vit. D3 on bone formation during embryogenesis. This concept was also proposed and examined by many authors (Elaroussi *et al.*, 1993; Kim *et al*., 2011; El-Shazly 2012; Zamani *et al.*, 2018 and Salim *et al.*, 2019) who found that vit.D3 enrichment of either by direct *in ovo* injection or by using higher levels of vitamins in the diet, to increase their contents in the yolk, may enhance bone remodelling and improving osteogenesis.

In this respect**,** Dake (2000) also found that vit.D3 facilitates bone formation by inducing biosynthesis of osteocalcin which is a specific collagenous protein product of osteoblasts during bone formation. These results support and confirm our the findings for the major role played by vit.D3 on bone quality of broilers, Since, our results are in close agreement with Vaiano *et al.* (1994) who reported that higher systemic concentrations of 1,25 (OH)2D3 between 7to 14 day of age will enhance the ability of broiler chickens to effectively mineralize the cartilaginous growth plates in the appendicular skeleton during

early bone maturation. Also (Kim *et al.*, 2011) suggested that high level of vitamin D3 (250 µg/kg) for 3 week-old chicks can increase bone growth and mineral deposition in broiler chick.

Table (5): Effect of *in ovo* vitamin D3 during embryonic development on post hatching bone development of broiler chicks.

Item	Treatment					SEM	Sig
	C	SE7	SE14	DE7	DE14		
Tibia Bone							
Wet weight (gm)	19.20^{b}	19.00^{b}	19.42^{b}	20.25^{a}	19.90^{a}	0.161	**
length (mm)	94.00^{b}	94.5^{b}	94.8^{b}	101.3^{a}	100.5^{a}	0.118	**
width (mm)	9.01	8.8	9.0	9.7	9.4	0.003	NS
Femur Bone							
Wet weight (gm)	12.90	12.50	13.75	14.75	14.38	1.432	NS
length (mm)	66.00	67.0	65.3	70.3	69.8	0.125	NS
width (mm)	10.5	10.3	10.8	11.5	11.0	0.019	NS
Shank Bone							
length (cm)	66.41^{b}	6.65^{b}	6.35^{b}	6.75^{a}	7.03^{a}	0.078	**
width (cm)	1.16	1.15	1.15	1.23	1.20	0.006	NS
Keel Bone							
length (cm)	12.16^{b}	12.05^{b}	12.10^{b}	13.15^{a}	12.95^{a}	0.078	**

C: Negative control (un injected), SE7: sham injected at 7 day of incubation, SE14: sham injected at 14 day of incubation, DE7: Vit.D3 injection at 7 day of incubation, DE14:Vit.D3 injection at 14 day of incubation.
^{a,b,}.:Means within rows with different superscripts are significantly different (P≤ 0.05).
***: P≤ 0.01; NS: not significant.*

Tibia bone histology:

The histological structure of the tibia bone of 42 DOA chicks as influenced by in ovo vit.D3 injection are shown in Figure (1). It appears that tibia bone section of 42 DOA chick from the control group (a, b and c). One can observe a small area of compact bone and then the greatest area of proliferative zone in which the cancellous bone tended to be well developed. There are many osteocytes, chondrocytes and haversion conals between the larg lacunae, in the bone matrix. There is also obvious vacuoles of resorped Osteoids and same lacunae appear elongated in shape, This appearance, indicative of bone resorption is due to the expected rapid bone calcification.

Tibia sections from vit.D3 groups showed nature state of bone formation (d, e). It is observed that the number and size of lacunae were diminished, while Osteocytes were abundant and the area of calcified bone is greatly increased. This was more obvious in the section from vit.D3 chicks groups of D7 treatment (d).

From the presence sections, it seems that the Osteogenesis process become faster by *in ovo* injection with vitamin D3 at the 7th day incubation of embryogenesis. This may be due to the role played by vitamin D3 in organs growth during incubation period. This is confirme by the fact that the development of embryonal somites (a mesoderm layer from which bones and muscles develop) takes place during the first 16 hours of incubation while the vertebral column of the embryo could be seen after 24 hours, and the beginning of bone calcification was at the 13th day of incubation period. In our study the *in ovo* injection was done at the 7th or 14th day of incubation.

In general, our results are in close agreement with those reported by Yan *et al. (*2005); Kim *et al.* (2011); El-Shazly (2012) and Ali (2015).

CONCLUSION

It could be concluded from the present study that, *in ovo* injection with a dose of 0.1 ml Vit.D3 (Containing 500 IU) in Hubbard broiler chicks at 7th or 14th day of incubation enhanced productive performance, Plasma level of PTH, Ca, P, ALK-P and ACP ,bone growth and histological structure of bone.

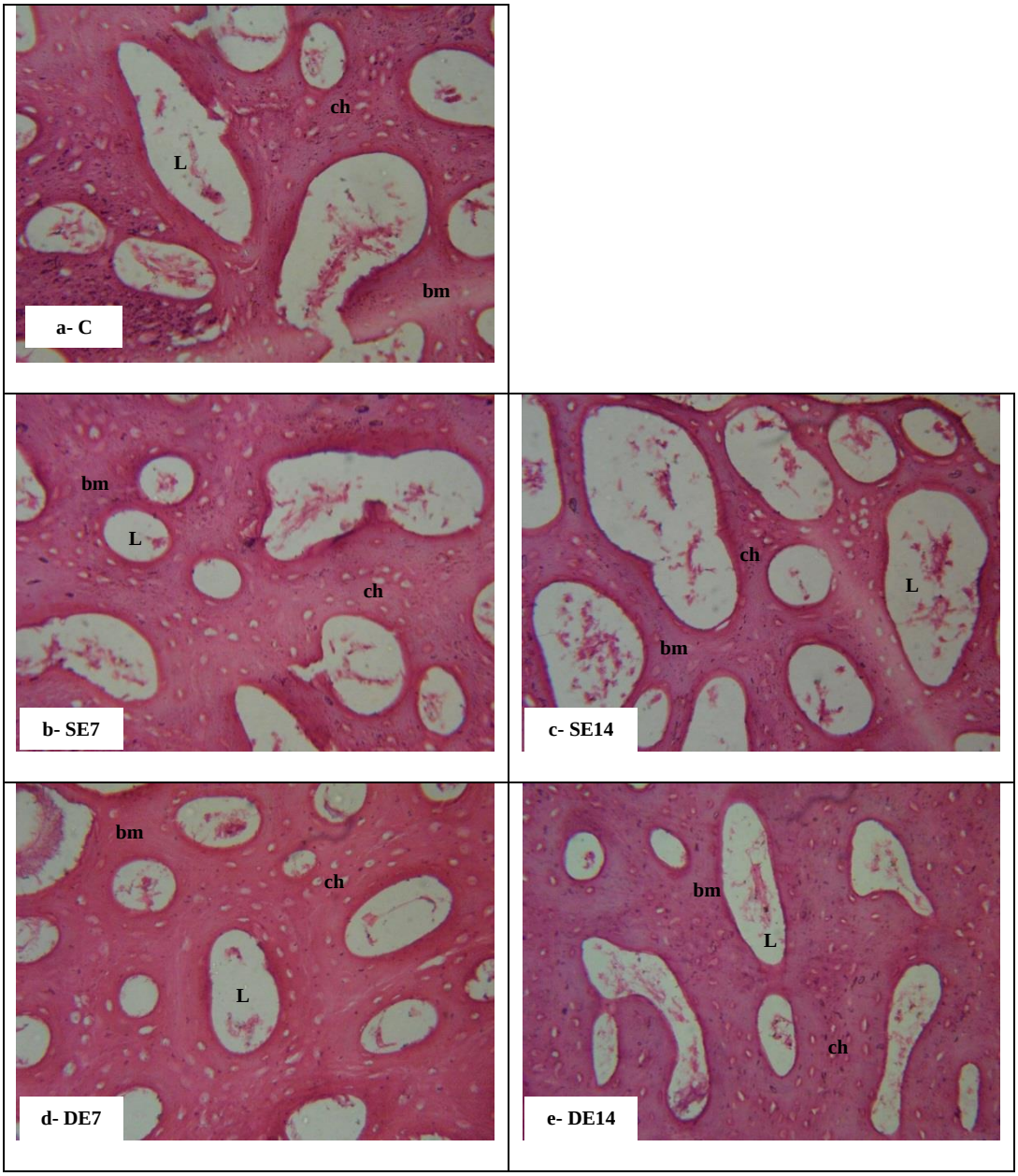

Figure (1): Transverse section through tibia bone from birds of different treatments at 42 days of age. Ch: chondrocytes, L: Lacunae, h: haversian canal, bm: bone matrix (H&E ×40).

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تأثير حقن البيض بمادة 25 هيدروكسي كولي كالسيفيرول أثناء التطور الجنيني على أداء دجاج اللحم وتركيب العظام وبعض قياسات الدم

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أجريت هذه الدراسة لمعرفة تأثير حقن فيتامين د$_3$ في البيض خلال فترات التطور الجنيني المختلفة على الأداء الإنتاجي بعد الفقس، بعض قياسات الدم، نمو العظام والتركيب النسيجي لعظمة الساق لكتاكيت هابرد. استخدم 500 بيضة بمتوسط وزن 65 جرام من قطيع لتربية الدواجن التجارية (هابرد) عند عمر 55 أسبوعًا. تم تقسيم البيض إلى خمس مجموعات كل منها 100 بيضة في أربع مكررات ، كل منها 25 بيضة. تم استخدام المجموعة الأولى كمجموعة مقارنة سلبية (غير محقونة) ، بينما استخدمت المجموعة الثانية والثالثة كمجموعة مقارنة شام حيث تم حقنها بـ (0.1 مل ماء مقطر/ بيضة) في الغرفة الهوائية في اليوم السابع و الرابع عشر على التوالي. و تم حقن المجموعتين الباقيتين (الرابعة والخامسة) بجرعة 0.1 مل من فيتامين د$_3$ (تحتوي على 500 وحدة دولية) في الغرفة الهوائية في اليوم السابع و الرابع عشر من فترة التفريخ على التوالي. أظهرت النتائج أن الكتاكيت الفاقسة من بيض محقون بفيتامين د$_3$ تتمتع بأداء إنتاجي أفضل بشكل ملحوظ (أعلى وزن جسم حي و زيادة وزنية ، وأقل غذاء ماكول وأفضل كفاءة تحويل غذائي) من مجموعات المقارنة. زاد مستوى كلا من الباراثيرون هرمون و الكالسيوم و الفوسفور و الكالين فوسفاتيز و الفوسفاتيزالحمضي في البلازما بشكل ملحوظ مع حقن البيض بفيتامين د$_3$. كما زاد كلا من الوزن والطول والعرض لعظمة الساق معنويا بحقن البيض بفيتامين د$_3$. وأيضا زاد كلا من الوزن والطول والعرض لعظم الفخذ بشكل طفيف نتيجة لحقن البيض بفيتامين د$_3$. كما زاد طول عظمة الساق معنويا نتيجة لحقن البيض بفيتامين د$_3$ بينما زاد عرض الساق بشكل طفيف مقارنةً بمجموعتي المقارنة والشام. علاوة على ذلك ، فقد تحسن التركيب النسيجي للعظام بحقن البيض بفيتامين د$_3$ سواء في اليوم السابع أو الرابع عشر من فترة التفريخ. نستنتج من هذه الدراسة أن حقن البيض بجرعة 0.1 مل من فيتامين د$_3$ (يحتوي على 500 وحدة دولية) في كتاكيت اللحم هابرد في اليوم السابع أو الرابع عشر من فترة التفريخ أدى إلى تحسين الأداء الإنتاجي ، قياسات الدم المختلفة وتطور العظام والتركيب النسيجي.