EFFECT OF IN OVO INJECTION OF VITAMIN D3 ON BONE GROWTH AND SOME BLOOD PARAMETERS IN FAYOUMI CHICKENS.

Zenat A. Ibrahiem

Poultry Production Department, Facult of Agriculture, Zagazig Universit, Zagazig, Egypt.

(Received 2 /6/ 2012, Accepted 15/8/ 2012)

SUMMARY

The effect of in ovo injection with vitamin D3 on subsequent performance traits and some blood constituents of hatched chicks was studied. A total of 300 egg from Fayoumi laying hens were used. They were divided into five treatments groups representing a control group, sham- injected at 7, 14 days and vitamin injected at 7, 14 days respectively. The injected dose was 16.000 IU of 1,25-cholecalciferol D3 (1,25 (OH)2-D3). Chicks were weighed to the nearest gram at hatching then were weighed weekly up to 8 week of age. Also, Shank, keel, tibia and femur measurements were recorded from day old till 8wk of age. Blood samples were taken from six chicks per treatment at day old and 8wk of age to determine plasma parathormone (PTH) concentration and some blood parameters (total protein, albumin, calcium, phosphorous, alkaline and acid phosphatases enzymes activity). The obtained results showed that, the chick weight at hatch was significantly higher in vitamin D3 injected groups either at day 7 or 14 of incubation period. The highest hatchability percentage was recorded for vitamin D3 injected eggs at 14 days of incubation period followed by those injected at d7. There was no significant difference in body weight among vitamin injected groups, sham and un-injected birds at all experimental periods. At day old the keel length was longer for the in ovo vitamin D3 injected chicks either at 7 or 14 days of incubation period. At 2wks of age vitamin D3 injection at day 14 increased significantly shank and tibia lengthes compared with other treatments and the control group. In addition the results revealed that VD7 group had higher of keel, femur and tibia lengths followed by VD14 group than the others at 4 and 8 weeks of age. Parathyroid hormone (PTH) concentration was significantly higher in plasma of chicks that hatched from injected eggs either at 7 or 14 days of incubation period and at 8wks of age. Same was true for plasma Calcium and phosphorus levels at day old and 8wks of age. It was observed that plasma total protein significantly increase in vitD14 and vitD7 treatment groups than control one or sham 7 and 14 respectively, while total albumin was significantly higher in vit D7 followed by vit D14 than other sham injected and control groups. Both, ALP and ACP revealed the same trend in vit D7 followed by vit D14 than other groups. The histological observation confirm the previous findings, that vit.D3was critical for bone development at early ages of chicken growth. The best results occurred in the current research were observed for vit. D3 injection at day 7. It is concluded from these results that in ovo injection of vit. D3 could improve bone formation; stimulate PTH secretion from parathyroid gland.

Keywords: *In ovo ingection; cholecalciferol; bone growth; parathormone; tibia; blood parameters.*

INTRODUCTION

The subsequent development of avian embryos and hatched chicks are influenced by the yolk nutrient status (Al-Murrani, 1982). Many nutrients have important structural, physiological, and immunological roles in avian embryogenesis and growth performance. In-ovo injection of nutrients may help overcome any constraint of inadequate egg nutrition.

Vitamin D3 is considered to be pro hormone generated in the skin through Ultra violet irradiation of 7-dehydrocholesterol or absorbed from the diet in the intestinal tract (Deluca 2004). It has been reported that the supplementation of high levels of vitamin D3 reduced incidence of leg problem, such as TD and Ca rickets in broilers (Whitehead *et al.*, 2004, Atencio *et al.*, 2005, Driver et al., 2006). However, skeletal homeostasis maintenance and a lack of skeletal disorders are required (Driver *et al.*, 2006). Vitamin D compounds are best known for their role in stimulating intestinal Ca absorption, and thus contributing to optimal bone mineralization and improved quality of the skeletal system. 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); and then 25(OH) D3 is hydroxylated to form the biologically active form of vitamin D-1, 25(OH) 2 D3 (Dixon and Mason,

2009). It was reported that Ca and ph homeostasis disturbances may result from impaired hydroxylation of cholecalciferol and diminished formation of 25 (OH) 2 D3 and 1, 25(OH) 2 D3, especially in conditions of liver disease and poor liver function (Abe *et al.*, 1982; Whitehead, 2004). Thus, dietary provision of 25(OH) D3 may have beneficial effects on the maintenance of Ca homeostasis and skeletal system properties and function.

Many studies had indicated the effect of vitamin D3 and its metabolites on the embryonic development in chickens, the importance of vit D3 to hatchability and its role in calcium metabolism and transfer of calcium from the shell to the embryo was reviewed by Kubota *et al* (1981). In addition to causing the late embryonic mortality, acholecalciferol deficiency also causes beak deformities and inadequate skeletal formation. Henry and Norman (1978) fed hens 1, 25-(OH) 2 D3 as a sole source vit D3 and found that fertile eggs appear to develop normally without any malformation. On the other hand, Sunde *et al.* (1978) reported that laying hens (40 weeks of age) maintained on 1,25-(OH)2 D3 for 28 weeks produce eggs which appear normal, but produce some embryos having a defective appear mandible soares, occured between day 11 and 13 of incubation period. Hatchability is at least partially supported by 1,25-hihydroxy cholecalciferol by it's injection into the deficient egg. Sunde *et al.*, (1978), indicated that the hen does not transfer 1.25-dihydroxy-cholecalciferol to support embryonic development. Moreover, hens fed 1,25-dihydrocholecalciferol as the source of vitamin D produced normal eggs but they failed to hatch (Sunde *et al.*, 1978, Henry and Norman, 1978 and Abdulrahim *et al.*, 1979). Elaroussi *et al.* (1993) raised Japanese quail hens on 1, 25-(OH)2 D3 and injected their eggs, in the air sac prior to incubation, with 125ng\egg cholecalciferol or 100ng\egg 1,25 (OH)2 D3 or 300ng\egg 1,25-(OH)2 D3. They found that tibial total calcium in one-day old chick from egg injected with cholecalciferol had the highest level. Deng and Ha afa (2004) reported that in young hens cortical bone trabecula were normal at first finally there was different grade of re absorption from Lacune in cortical bone. Temporary addition of vitamin D3 may improve and play a role in prevention of osteoporosis (Atencio et al., 2005). Kim *et al.*, (2011) suggests that high levels of vitamin D3 can increase bone growth and mineral deposition in broiler chicks, while Tatara *et al.*, (2011) found positive influence on bone properties at advanced stage of the productive cycle.

Thus the purpose of the present work was to study the effect of in ovo injection of vit D3 on post-hatch chick weight, bone growth and some blood parameters in Fayoumi chickens. Results of this study should provide information regarding the time of vit D3 in ovo injection to aid in overcoming early onset rickets in chicks.

MATERIALS AND METHODS

The present study was carried out at poultry Farms, Agricultural Experiment and Researches Unit, Faculty of Agriculture, Zagazig University, during the year of 2011.

Experimental design:

Three hundred fertile eggs from Fayoumi (Fay) laying hens were obtained from El-Azab Poultry Station at El-Fayoum Governorate, Animal Production Research Institute, Ministry of Agriculture, Egypt. The eggs were weighed and distributed into five groups of 60 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 (0.5ml sterile water\egg) at day 7and 14 respectively (Sh7, Sh14). 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 rest two groups (Fourth and Fifth) were injected at day 7and 14 of incubation period(VD7, VD14) with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3) which purchased as 50ml bottle from RNA International Food Secures Company, Ontario Canada, each ml containing 160×10^6 IU of vitamin. 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 seated with sterile paraffin and eggs were returned to the incubator. On the 19th d the eggs were shifted to the Hatcher and kept in the respective pedigree hatching boxes. On the day of hatch chicks were weighed, wing banded and transferred to the battery brooders for growth performance study. The chick weight to egg weight (pre-incubated) ratio and hatching percentages were compared among treatment groups to see the effect of vitamin injection.

Bird housing and management:

The chicks hatched from the respective treatment group were distributed in battery brooder cages with provisions for separate feed, water and droppings trays. Chicks were fed *ad libitum* on a commercial basal diet which was formulated to cover the recommended requirements for the local chicken strains The composition, calculated and chemical analyses of the basal diet are shown in (Table 1) .Chicks were maintained on a light cycle of 16L: 8D.

Table (1): Composition, calculated and chemical analysis of the basal diets

Ingredients	%
Yellow corn	60.00
Soybean meal (44%)	27.75
Wheat bran	5.25
Lime stone	0.5
Bone meal	2.75
Sand	2.16
Cotton seed oil	1.00
NaCl	0.25
Vitamin and Min. Mix*	0.25
DL-methionine	0.09
Total	100.00
*Calculated analysis***	
CP %	18.11
ME kcal/kg	2850
Crude fiber %	3.69
Crude fat %	2.86
Calcium %	0.98
Available phosphorus %	0.7
Lysine %	0.92
Methionine %	0.35
Methionine + Cysteine %	0.67
Sodium %	0.13

**Vitamin and mineral mix contain/1 kg vit A 4 000 000, vit D3 500 000 IU, vit E 16.7 g, Vit K3 0.67 g, vit B1 0.67 g, vit B2 2 g, vit B6 0.67 g, vit B12 0.004 gm, pantothenic acid 6.67 g, Niacin 16.7 g, Biotin 0.7 g, Folic acid 1.67 g, Choline chloride 400 g, Selenium 0.33 g, Copper 1.67 g, Iron 25 g, Manganese 10 g, Zinc 23.3 g, Iodine 0.25 g, magnesium 133.4 g. **According to Egyptian Feed Composition Tables for Animal and Poultry Feedstuffs (2001)*

Data collection:

All chicks of the treatment groups were weighted weekly up to 8 weeks of age old, some measurement of bone (mm) were taken, such as Tibia (TL), shank (ShL) and keel (KL) lengths, Tibia weight (TW) was also recorded for day old. At 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 centrifugated at 4000rpm for 15 minutes, Another six chicks per treatment groups were slaughtered at 8wk of age and blood samples were also collected, then centrifugated as described previous. Plasma samples were stored at -20°C until analysis. plasma samples were assigned for determination of total protein, albumin, Calcium, phosphorus, alkaline phosphates (ALK) and acid phosphates (ACP) using available commercial kits. The hormone of Parathormone (PTH) was determined by RIA technique as reported by Woodhead (1990).

Statistical analyses

Data were subjected to analysis of variance using general linear model described in SAS User's Guide (SAS Institute, 1994). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

Histological observation

The right dissected tibia were decalcified by ll-formic acid solution and placed in Bowan's fixative. Six tibia from each treatment group were chosen at random for histological analysis. One-half of the proximal tibiae was dehydrated with ethyl alcohol and embedded in paraffin. Two 5-µmicron sections were taken from each Sample, and stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

Egg weight, hatchability and embryonic mortality:

The effects of in ovo injection with vitamin D3 on egg weight hatchability percentage, chick weight at hatching and residual yolk weight of Fayoumi strain are presented in Table 2.

It is clear from the results that the differences between all treatments in egg weight were not significant. It is worth to mention, however, that egg weight was recorded before the injection of eggs, since no effect of treatments was obtained. Concerning the effect of different treatments on chicks weight at hatch, the present results show that chick weight was significantly higher in vitamin D3 injected groups either at day 7 or 14 of incubation period. It appears that this increase in hatching weight might be due to an increase in calcium and phosphorus utilization by embryos and enhancing bone development which in turn influence hatching weight. On the other hand, some authors 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 (Gore and Qureshi 1997, Tako, *et al.*, 2004, Uni and Ferket, 2004, Foye, *et al.*, 2006, Lopes *et al.*, 2006, Pedroso, *et al.*, 2006 and Dos Santos, *et al.*, 2010).

Various factors play important role in influencing hatchability efficiency and growth performance during embryonic and post-hatch life, such as genetic make-up, egg characteristics and incubation environment (Narushin and Romanov, 2002; Petek *et* al., 2003; Abiola *et al.*, 2008). Hatching drastically changes the way chicks retrieve nutrients. In the embryo, yolk lipids are transported directly into the blood by endocytosis (dos Santos *et al.*, 2010), but after hatching, the yolk content is absorbed through both the yolk sac membrane and the Meckel's diverticulum, and is digested and absorbed from the intestinal tract. At hatch the energy reserves in the yolk sac may not be sufficient to supply in the maintenance energy requirements of a chick (Dibner *et al.*, 1998), and fasting effects may develop before the bird is removed from the hatchery. However, despite being capable of ingesting feed, the intestinal tract of the chick is still immature (Uni *et al.*, 2003). Ohta *et al.* (2001) suggested that the increase in hatching weight of 7-day-old embryos injected with amino acids may have been owing to a higher content of amino acids in the yolk or the better utilization of amino acids by the embryo. Foye *et al.* (2006) observed a higher body weight and thigh and breast weights in day-old turkeys when they were inoculated at 23 d of incubation with egg-derived protein.

Table (2): Effects of in ovo injection with vitamin D3 on egg weight hatchability percentage, chick weight at hatching and residual yolk weight of Fayoumi strain.

Treatment\ Traits	Egg weight (gm)	Chick weight at hatch (gm)	Chick weight Percent %	residual yolk weight	Hatchability percent %	Embryonic mortality %
C	$38.52^{a}\pm1.17$	$30.11^{b}\pm1.29$	$78.11^{a}\pm1.83$	$7.37^{a}\pm0.49$	76.45	8.25
Sh7	$39.00^{a}\pm1.19$	$29.67^{b}\pm0.76$	$76.07^{a}\pm1.04$	$8.98^{a}\pm0.50$	76.82	10.50
Sh14	$40.03^{a}\pm2.40$	$30.31^{b}\pm1.61$	$75.72^{a}\pm1.98$	$5.20^{a}\pm0.42$	78.40	11.65
VD7	$41.66^{a}\pm1.08$	$32.23^{ab}\pm0.73$	$77.35^{a}\pm0.64$	$8.90^{a}\pm0.45$	83.36	6.80
VD14	$43.06^{a}\pm2.75$	$34.81^{a}\pm1.01$	$80.22^{a}\pm4.58$	$10.58^{a}\pm0.65$	91.24	8.70

C= Control (Un-injected), Sh7 = Sham control which injected with (0.5ml sterile water\egg) at day 7 of incubation period, Sh14 = Sham control which injected with (0.5ml sterile water\egg) at day 14 of incubation period, VD7 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3) and VD14 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3).

Means within a column with different superscripts are significantly different ($P< 0.05$).

Concerning, the hatchability of Fayoumi eggs, the results from Table 2 show that in ovo vitamin D3 injection increased hatchability percentage as compared with those sham injected or control eggs. It appears, however, that vitamin D3 injection either at day 7 or d14 of incubation period enhanced significantly the residual yolk which may, suggest a subsequent enhancement and\or sparing effect of the treatment on nutrients profile of yolk. In addition, the results show that the highest hatchability percentage was recorded for vitamin D3 injected eggs at 14 days of incubation period followed by those injected at day7. It is of interest to observe that the hatchability (%) of sham-injected eggs was lower as compared to the other treatment groups. This result could be explained by the fact that the percentage of embryonic mortality in the sham-injected eggs was the highest as show in Table (2).

Our results are in the same line of several previous finding 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.*, 2007).

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 (0.5ml sterile water\egg) at day* 7and 14 respectively (Sh7, Sh14). 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 rest two groups (Fourth and Fifth) were *injected at day 7and 14 of incubation period(VD7, VD14) with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3)* which purchased as 50ml bottle from RNA International Food Secures Company, Ontario Canada, each ml containing 160×10^6 IU of vitamin.

Post-hatch chick weight

Table (3) shows the chick weights from hatching tell 8 weeks of age (WOA) as affected by in ovo injection with vitamin D3. It was observed that, there were no significant differences in body weight among vitamin injected groups, sham control and un-injected birds at all experimental periods except at hatching our results are in full agreement with those obtained by Michalczuk *et al.*, (2010), who reported that at the end of rearing period a statistically significant differences were no longer found in body weight values.

Table (3): Effect of prehatching in ovo injection with vitamin D3 (VD) on chick weight (gm) of Fayoumi strain from hatching to 8 weeks of age.

Treatment\ Traits	Chick weight at hatch	2wk	4wk	8wk
C	30.113^{b}±1.290	148.030^{a}±3.181	304.626^{a}±10.401	827.5^{a}±33.008
S7	29.673^{b}±0.762	153.990±4.516	285.618^{a}±10.935	848.0^{a}±14.628
S14	30.313^{b}±1.607	146.273^{a}±4.079	323.300^{a}±33.403	832.5^{a}±52.500
VD7	32.231^{ab}±0.727	146.427^{a}±5.953	320.505^{a}±13.801	892.5^{a}±82.500
VD14	34.811^{a}±1.013	143.300^{a}±6.398	287.200^{a}±6.170	858.0^{a}±30.886

C= Control (Un-injected). Sh7 = Sham control which injected with (0.5ml sterile water\egg) at day 7 of incubation period. Sh14 = Sham control which injected with (0.5ml sterile water\egg) at day 14 of incubation period. VD7 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3) and VD14 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3).

Means within a column with different superscripts are significantly different (P< 0.05)

Also Tatara *et al.*, (2011) stated that no statistically significant differences in body weight values were found in any age-differentiated groups of turkeys when compared with the subgroups receiving two different forms of vitamin D3 in the diet. However, Uni and Ferket (2004) reported that in ovo feeding treatment increased body size by 3% over control during 0-7 days of age. Ohta *et al.* (1999) reported 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.

Bone characteristics

Table 4 shows some bone measurements of day-old Fayoumi chicks as influenced by in ovo injection with vitamin D3 at two different periods of embryogenesis. It is clear from the results that keel length was longer for the pre in ovo vitamin D3 injected chicks either at 7 or 14 days of incubation period. Vitamin D3 injection at day 14 increased significantly shank length compared with other treatments and the control group.

Data in Table (4) reveal also that, in ovo injection of vitamin D3 has a significant influence on both tibia length and weight as compared with other treatment. It is clear from the results that femur length at hatching did not significantly affected by different treatments.

From the previous results, it seems likely that bone formation during the incubation period could be enhanced by in ovo injection of eggs at day14 of embryogenesis. These results confirm the previous findings by Hurwitz (1992) and Elaroussi, *et al.* (1993) which stated that vit D3 is very important for bone formation at early ages of chicken growth. Also, the results obtained by Kim *et al.*, (2011) suggested that high levels of vitamin D3 can increase bone growth and mineral deposition in broiler chick's bones.

Table (4): Effect of prehatching in ovo injection with vitamin D3 (VD) on post hatching bone development of Fayoumi chicks at one day of age.

Treatment\Traits	ShL	TL	TW	FL	KL
C	$1.920^{b}\pm0.048$	$2.520^{b}\pm0.048$	$0.232^{ab}\pm0.018$	$1.860^{a}\pm0.060$	$1.720^{b}\pm0.066$
Sh7	$1.920^{b}\pm0.073$	$2.360^{b}\pm0.092$	$0.188^{b}\pm0.024$	$1.820^{a}\pm0.086$	$1.800^{b}\pm0.077$
Sh14	$1.720^{b}\pm0.120$	$2.420^{b}\pm0.11i$	$0.224^{ab}\pm0.024$	$1.700^{a}\pm0.100$	$1.840^{b}\pm0.143$
VD7	$1.960^{b}\pm0.163$	$2.580^{ab}\pm0.037$	$0.274^{a}\pm0.050$	$1.880^{a}\pm0.128$	$1.880^{a}\pm0.086$
VD14	$2.400^{a}\pm0.122$	$2.780^{a}\pm0.096$	$0.244^{ab}\pm0.015$	$1.980^{a}\pm0.239$	$2.000^{a}\pm0.070$

C= Control (Un-injected), Sh7 = Sham control which injected with (0.5ml sterile water\egg) at day 7 of incubation period, Sh14 = Sham control which injected with (0.5ml sterile water\egg) at day 14 of incubation period, VD7 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3) and VD14 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3).

Means within a column with different superscripts are significantly different (P< 0.05)

The effect of in ovo injection with vitamin D3 on posthatching bone development of Fayoumi chicks at 2weeks of age are shown in Table (5). It was observed that, in ovo injection at day7 and day 14 of incubation significantly increased shank length compared with those sham injected groups.

Concerning keel length, no significant differences between treatments were observed. However VD 7 and VD14 treatments had the higher tibia length compared with the other groups.

Table (5): Effect of prehatching in ovo injection with vitamin D3 (VD) on posthatching bone development of Fayoumi chicks at 2weeks of age.

Treatment\Traits	ShL	KL	TL
C	$3.860^{ab}\pm0.092$	$3.200^{a}\pm0.100$	$4.620^{c}\pm0.106$
Sh7	$3.720^{b}\pm0.086$	$3.300^{a}\pm0.054$	$4.366^{c}\pm0.117$
Sh14	$3.860^{ab}\pm0.102$	$3.440^{a}\pm0.067$	$4.700^{bc}\pm0.114$
VD7	$4.060^{a}\pm0.050$	$3.566^{a}\pm0.163$	$4.980^{ab}\pm0.96$
VD14	$4.120^{a}\pm0.086$	$3.560^{a}\pm0.128$	$5.140^{a}\pm0.102$

C= Control (Un-injected), Sh7 = Sham control which injected with (0.5ml sterile water\egg) at day 7 of incubation period, Sh14 = Sham control which injected with (0.5ml sterile water\egg) at day 14 of incubation period, VD7 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3) and VD14 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3).

Means within a column with different superscripts are significantly different (P< 0.05).

The results in Tables 6 and 7 revealed that VD7 group had higher of keel, femur and tibia lengths followed by VD14 group than the other ones at 4 weeks and 8 weeks of age. The results agreed with those obtained by Tatara *et al.*, (2011) who found benefits resulting from administration of 25(OH) D3 to the diet in the skeletal formation of turkeys.

Table (6): Effect of prehatching in ovo injection with vitamin D3 (VD) on posthatching bone development of Fayoumi chicks at 4weeks of age.

Treatment\Traits	KL	FL	TL
C	$33.816^{d}\pm0.808$	$43.400^{a}\pm2.403$	$59.766^{b}\pm3.212$
Sh7	$39.950^{c}\pm0.540$	$41.366^{bc}\pm1.424$	$57.00^{b}\pm1.750$
Sh14	$52.516^{b}\pm1.313$	$36.300^{c}\pm3.137$	$64.55^{ba}\pm0.050$
VD7	$59.850^{a}\pm2.308$	$52.200^{a}\pm0.922$	$71.42^{a}\pm1.325$
VD14	$52.133^{b}\pm2.351$	$48.466^{ab}\pm2.832$	$64.75^{ab}\pm2.000$

C= Control (Un-injected), Sh7 = Sham control which injected with (0.5ml sterile water\egg) at day 7 of incubation period, Sh14 = Sham control which injected with (0.5ml sterile water\egg) at day 14 of incubation period, VD7 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3) and VD14 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3).

Means within a column with different superscripts are significantly different (P< 0.05).

Table (7): Effect of prehatching in ovo injection with vitamin D3 (VD) on posthatching bone development of Fayoumi chicks at 8weeks of age.

Treatment\ Traits	KL	FL	TL
C	70.875^{b}±3.233	63.392^{b}±0.789	93.60^{b}±4.513
S7	76.608^{ab}±1.609	65.366^{ab}±1.219	93.65^{b}±1.617
S14	70.875^{b}±3.233	63.700^{ab}±0.890	93.683^{b}±4.519
D7	81.275^{a}±3.671	69.766^{a}±3.757	108.283^{a}±4.060
D14	79.258^{ab}±2.279	66.325^{b}±1.655	106.866^{ab}±3.803

C= Control (Un-injected), Sh7 = Sham control which injected with (0.5ml sterile water\egg) at day 7 of incubation period, Sh14 = Sham control which injected with (0.5ml sterile water\egg) at day 14 of incubation period, VD7 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3) and VD14 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3).

Means within a column with different superscripts are significantly different (P< 0.05).

Blood parameters

Table (8) represents some blood parameters responses to in ovo vit D3 injection in day old chicks. It is clear from the results that parathyroid hormone (PTH) concentration was significantly higher in plasma of chicks that hatched from injected eggs either at 7 or 14 days of incubation period. The vit D3 injection at day 7 revealed the highest values for plasma calcium levels, then at day 14 respectively. This trend was not observed for the plasma phosphorus concentration. The data indicated that there were significant differences among treatment at the two different incubation periods and control and sham injected at the same two periods. It appears from the previous results that, in ovo injection with vitamin D3 at days 7 or 14 of embry6ogenesis could improve or stimulate PTH secretion from parathyroid gland in Fayoumi chickens, since the consequences were the observed increases in plasma concentration of calcium and phosphorus. Concerning plasma protein and albumin the results in Table (8) shows no significant differences among treatment groups.

There were significant differences between control and sham groups from one hand, and between vit. D 7 and Vit. D14 from the other hand, where vit. D7 had the higher values of ACP followed by Vit D14. The opposite was observed for ALP levels where the higher values were observed in vit. D14 followed by vit. D7. Also sham 7 and control had the same trend followed by sham 14, treatments.

Table (8): Effect of in ovo injection with vitamin D3 (VD) on some blood constituents in day old Fayoumi chicks.

Treatment\Traits	TP	ALB	ALP	ACP	PTH	Ca	Pi
C	4.45^{a}	2.13^{a}	36.83^{c}	3.68^{b}	4.03^{b}	8.83^{b}	5.30^{b}
	±0.19	±0.09	±1.75	±0.19	±0.19	±0.20	±0.16
Sh7	4.33^{a}	2.10^{a}	41.50^{c}	3.80^{b}	4.00^{b}	8.77^{b}	4.90^{b}
	±0.03	±0.15	±1.06	±0.31	±0.17	±0.30	±0.21
Sh14	4.53^{a}	2.10^{a}	44.77^{bc}	5.37^{a}	4.80^{b}	12.37^{a}	5.27^{b}
	±0.12	±0.06	±7.73	±0.22	±0.85	±0.09	±0.28
VD7	4.57^{a}	2.00^{a}	57.47^{ab}	4.73^{ab}	7.40^{a}	10.27^{ab}	6.90^{a}
	±0.33	±0.06	±5.73	±0.74	±0.60	±1.67	±0.15
VD14	4.25^{a}	2.30^{a}	67.20^{a}	4.75^{ab}	8.10^{a}	11.30^{ab}	6.35^{a}
	±0.05	±0.10	±2.40	±0.05	±1.10	±0.50	±0.55

C= Control (Un-injected), Sh7 = Sham control which injected with (0.5ml sterile water\egg) at day 7 of incubation period, Sh14 = Sham control which injected with (0.5ml sterile water\egg) at day 14 of incubation period, VD7 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3) and VD14 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1,25 (OH)2 cholecalciferol (vitamin D3).

Means within a column with different superscripts are significantly different (P< 0.05)

The data in Table (9) illustrate some blood parameters response to in ovo injection with vitamin D3 in eight weeks old Fayumi chicks.

It was observed that plasma total protein significantly increase in vitD14 and vitD7 treatment groups than control one or sham 7 and 14 respectively, while total albumin was significantly higher in vit D7

followed by vit D14 than other sham in injected and control groups. The higher concentration of plasma protein in the vit. D3 treated groups may reflect the positive role of blood proteins in bone matrix formation. In this respect, Weber (1999) have reported that plasma protein are necessary substances for collagen building and bone calcification.

The results also showed that both, ALP and ACP revealed the same trend in vit D7 followed by vit D14 then other groups. This mean that there were significant differences between the injected treatment either at day 7 or day 14 and un injected control and sham groups at the same period of vitamin injected.

Table (9): Effect of in ovo injection with vitamin D3 (VD) on some blood constituents at 8weeks of age in Fayoumi chicks.

Treatment\Traits	TP	ALB	ALP	ACP	PTH	Ca	Pi
C	5.43^{b}	2.40^{c}	245.43^{ab}	24.93^{b}	7.63^{c}	10.35^{c}	5.87^{c}
	±0.09	±0.16	±11.74	±0.87	±0.54	±0.31	±0.22
Sh7	4.77^{c}	2.43^{bc}	225.63^{b}	24.73^{b}	8.43^{c}	11.17^{bc}	5.63^{c}
	±0.09	±0.09	±3.47	±3.75	±0.42	±0.52	±0.19
Sh14	4.67^{c}	2.50^{bc}	240.33^{ab}	33.63^{ab}	9.07^{c}	11.93^{b}±0.	6.33^{bc}
	±0.09	±0.58	±27.55	±7.54	±0.43	45	±0.33
VD7	5.87^{a}	2.93^{a}	278.07^{a}	39.90^{a}	10.80^{b}	13.63^{a}	6.87^{ab}
	±0.18	±0.13	±5.25	±3.76	±0.36	±0.12	±0.18
VD14	5.85^{a}	2.65^{ab}	257.30^{ab}	38.10^{ab}	12.70^{a}	13.85^{a}	7.63^{a}
	±0.05	±0.05	±9.10	±3.50	±0.10	±0.75	±0.52

C= Control (Un-injected), Sh7 = Sham control which injected with (0.5ml sterile water\egg) at day 7 of incubation period, Sh14 Sham control which injected with (0.5ml sterile water\egg) at day 14 of incubation period, VD7 Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1.25 (OH)2 cholecalciferol (vitamin D3) and VD14 = Sham control injected at day 7 of incubation period with a dose of 0.1ml containing 16000 IU of 1.25 (OH)2 cholecalciferol (vitamin D3).

Means within a column with different superscripts are significantly different (P< 0.05)

Concerning PTH hormone the highest value was in vit D14, followed by vit D7, while the other groups showed the similar lowest values. On the other hand, it was noticed that the plasma calcium was significantly increased in vit D14 and vit D7 (injected groups), whears, there were no significant difference between un-injected groups for serum Pi levels at 8wks. These results agreed with those obtained with Narbaitz (1979) who reported that, in ovo injection with 200 p mol 1,25-dihydroxy-cholecalciferol significantly increased the concentration of plasma calcium. Our results agreed with those obtained by Khan *et al.* (2010) who showed that the concentrations of calcium and phosphorus minerals in the serum increased progressively with the high level of VIT-D3 supplementation to birds at both 21 and 42 days of age. Bilal *et al.* (2010) found that, there is strong evidence indicating that both parathyroid hormone and 1.25-dihydroxycholecaciferol (1.25-(OH)2 D3) play a role in the regulation of calcium metabolism in the chick embryo. Thus the embryonic parathyroid glands show ultra structural signs of activity (Narbaitz, 1972) and when maintained in vitro respond to variations in the concentration of calcium in the medium with corresponding ultra structural changes (Narbaitz and Tiller, 1974). Plasma phosphate was progressively elevated between days 11 and 15 while increased calcium accumulation in the skeleton, yolk sac, and allantoic fluid occurred between days 12 and 15 in vit. D enriched embryos. In this respect about 75% of the total body calcium of newly hatched chicks was obtained from the eggshell. Thus the dissolution and/or transport of eggshell calcium is dependent on vitamin D in quail embryos. In addition, both parathyroid hormone and (1.25-(OH) 2 D3) are known to produce significant hypercalcemia when injected into the chick embryo (Narbaitz and Tolnai, 1978).

It is well known that large amounts of calcium are transported from the egg shell to the embryonic skeleton (Johnston and Comar, 1974). The re absorption of the shell mineral increases abruptly around the 13th day of incubation and this fact has been linked to the differentiation in the chorioallantoic membrane of specialized cells known as "intercalate", "calcium-absorbing", or "villus-cavity" cells (Skalinsky and kondalenko, 1963; Owczarzak, 1971; Coleman and Terepka, 1972; Narbaitz, 1972; Narbaitz and Tellier, 1974). Previous results (Narbaitz, 1979; Narbaitz and Tolnai, 1978) have shown that parathyroid hormone and 1.25-(OH)2 D3 can produce hypercalcemia in 15-to 17-day-old chick embryos which is in close agreement with our results. Concerning acid and alkaline phosphatases activity, the results show that both enzymes were significantly higher in both vit. D7 and D14 groups than other treatments either at day old or at 8 weeks of age. It is well known that ALP plays an important role in bone mineralization. The osteoblasts in bone increase ALP activity in response to poor bone mineralization which leads to elevation in bone serum ALP levels. It appears from these results that phosphatases activity was greatly

changed with age and in ovo injection with vit. D3. This was also related to the parathyroid hormone level which significantly increased in treated groups. The increased levels of ALP and ACP may be attributed to the higher osteoblasts activity associated with medullay bone formation and PTH stimulatory effect. It is generally accepted that both ALP and ACP are used as physiological markers for the mature phenotypes of osteoplasts, and since their activities in bone formation and resorption may be regulated by PTH, estrogen, thyroxine and Insulin-like Growth factors. This confirms the results of Bishop *et al.* (2000) and Hassan *et al.* (2006). Moreover when dietary phosphorus decreased ALP decreased as reported by Koch *et al.* (1984). On the other hand, increasing cholecalciferol levels have been shown to increase intestinal phytase and ALP activity in chicks (Davies *et al.*, 1970). Dwyer *et al.* (1997) stated that the treated groups in broiler chicks was no significantly different than controls for ALP levels. In the present study our results not similar to the findings of above researchers, there were significant differences between all groups for serum ALP activity.

Histological observation:

The histological structure of the tibia bone from day old Fayoumi chicks are shown in Figures 1 to 5. It is clear from the histological sections that the general structure of the tibia bone was similar in all treatments with great changes associated with the previous in ovo vitamin D3 injection during embryogenesis. In general, the compact bone (cb) layer in Fig. 1 (control) is composed of collagen fibers and ground irregular-shaped osteocytes (os). These osteocytes are an osteoblasts that has been surrounded by calcified matrix. A similar structure was also observed in the cross sections of sham injected treatments either at 7 or 14 day of embryogenesis (Fig. 2 and3). The proliferative zone (P) of the pervious section is well developed with many chondrocytes (ch) being in different developing stages. In tibia sections of vitamin D3 injected groups (Fig. 4 and 5), the proliferative zone is characterized by many large-sized chondrocytes with an irregular hypertrophic pattern especially in the vit. D3 injected treatment at day 7 of incubation period (Fig. 4). The same holds true for the VD 14 treatment although the number and size of the osteocytes and chondrocytes was lower than vit. D7 injected group. In the latter sections (Fig. 4 and 5) several osteocytes were also observed to be fully enclosed within a Lacunae of different size and shape. These changes may suggest the beneficial effect of in ovo vitamin D3 injection either at 7 or 14 days of incubation period. The best results occurred in the current research were observed for vit. D injection at day 7.

Previous data concerning the effect of in ovo injection of vit. D3 on the histological structure of bones in day old chicks were scarce. However, the present findings confirm many previous results dealing with bone formation resorption and remodeling in chickens, especially in the modern strains of economic importance to prevent bone fracture in broilers and osteoporosis in laying hens. In this respect, Ali, (1992); Fleming *et al.* (1998); Bishop *et al.* (2000); Deng and Haiafa (2004), Yan, *et al.* (2005); and Kim *et al.* (2011) have reported that bone growth depends upon many factors including nutrition, age, maternal and physiological status of birds.

It could be concluded that in ovo injection of vit. D3 can be regarded as a possible method to improve hatchability body weight at hatch, bone growth and stimulate PTH secretion from parathyroid gland. Further investigations are needed to highlight their role in bone development. This preliminary study may open a window for future research in this respect especially in local chicken strains.

Control

Fig. (1): T. S. of tibia from the control day old chicks (H&Ex40)

Sh7

Fig. (2): T.S. of tibia from sham injected (d7) chicks (H&Ex20)

Sh 14

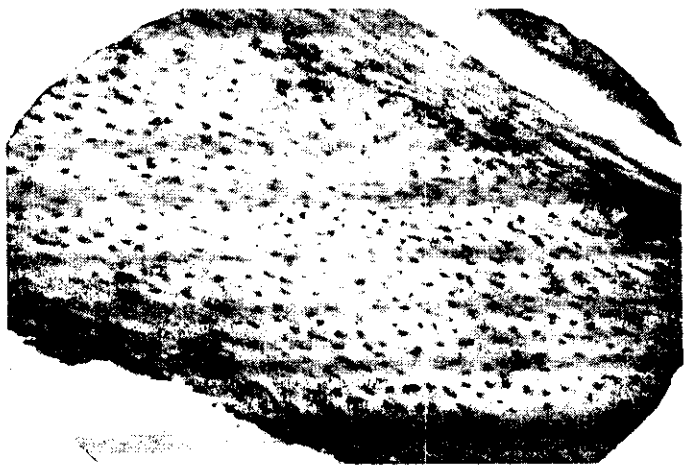

Fig. (3): T.S. of tibia from the sham injected (14 d) chicks (H&Ex20)

vit. D7

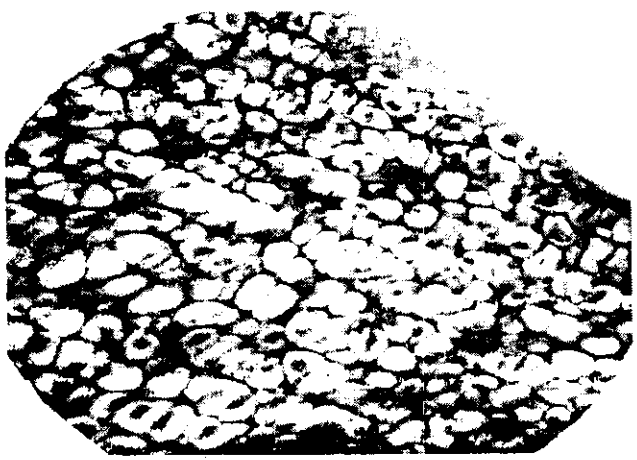

Fig. (4): T.S. of tibia from the in ovo injected (at day 7) chicks (H&Ex40)

Fig. (5): T.S. of tibia from the in ovo injected (at d 14) chicks (H&Ex40)

REFERANCES

Abdulrahim, S. M., M. B. Patel and J. McGinnis (1979). Effects of vitamin D3 and D3 metabolites on production parameters and hatchability of eggs. Poultry Sci., 58: 858-863.

Abe, E., H. Horikawa, T. Masumura, M. Sugahara, M. Kubota and T. Suda (1982). Disorder of cholecalciferol metabolism in old egg-laying hens. The Journal of Nutrition, 112: 436-446.

Abiola, S.S., O.O. Meshioye, B.O. Oyerinde and M.A. Bamgbose (2008). Effect of egg size on hatchability of broiler chicks. Arch. Zootech., 57: 83-86.

Al-Murrani, W. K (1982). Effect of injecting amino acids into the egg on embryonic and subsequent growth in the domestic fowl. Br. Poult. Sci., 56: 1691.

Ali, S.Y. (1992). Matrix formation and mineralization of bone. In: Bone Biology and Skeletal Disorders in Poultry. Carfax Publishing Comp., pp. 19-38.

Atencio, A., H. M. Edwards Jr, and G. M, Pesti (2005). Effect of the level of cholecalciferol supplementation of broiler breeder hen diets on the performance and bone abnormalities of the progeny fed diets containing various levels of calcium or 25-hydroxycholecalciferol. Poultry Sci., 84(10): 1593-1603.

Bhanja, S.K, A.B. Mandal, S.K. Agarwal, S. Majumdar and A. Bhattacharyya (2007). Effect of in ovo injection of vitamins on the chick weight and post-hatch growth performance in broiler chickens. World Poultry Science Association, Proceedings of the 16th European Symposium on Poultry Nutrition, Strasbourg, France, 26-30 August, 143-146.

Bilal T., H. Eseceli, A. Altiner and O. Keser (2010). Effect of vitamin D3 and\or zeolite supplementation to laying hen rations added microbial phytase on some blood indices. 1. Calcium and inorganic phosphorus levels and alkaline phosphatase activity, Journal of Animal and Veterinary Advances, 9 (10): 1458-1462.

Bishop, S.C., R.H. Fleming, H.A. McCormack, D.K. Flock and C.C. Whitehead (2000). Inheritance of bone characteristics affecting osteoporosis in laying hens. Br. Poult. Sci., 41: 33-40.

Coleman, J.R., and A.R. Terepka (1972). Fine structural changes associated with the onset of calcium, sodium and water transport by the chick choriaoallantoic membrane. J. Membrane Biol., 7: 111-127.

Davies, M.I., G.M. Ritcey and I. Motzok (1970). Intestinal phytase and alkaline phosphatase of chicks influence of dietary calcium inorganic and phytate phosphorus and vitamin D3. Poult. Sci., 49: 1280-1286.

Deluca, H. F. (2004). Overview of general physiological features and functions of vitamin D. Am. J. Clin. Nutr., 80: 1689S-1696S.

Deng Yi and Hou Haiafa (2004). Effect of vitamin D3 concentration in caged layer diets on bone mineral content and microstructure. Chinese Journal of Veterinary Science and Technology, 34(10): 19-22.

Dibner, J.J., Knight, C.D., Kitchell, M.L., Atwell, C.A., Downs, A.C. & Ivey, F.J. (1998). Early feeding and development of the immune system in neonatal poultry. J. Appl. Poult. Res., 7: 425-436.

Dixon, K.M. and R.M Mason, (2009). Vitamin D. The International Journal of Biochemistry & Cell Biology, 41: 982-985.

Dos Santos ,T. T., A. Corzo , M. T. Kidd , C. D. McDaniel , R. A. Torres Filho , and L. F. Araújo (2010). Influence of in ovo inoculation with various nutrients and egg size on broiler performance, J. Appl. Poult. Res., 19: 1–12

Driver, J. P., A. Atencio , G. M. Pesti, H. M. Edwards Jr., and R. I. Bakalli. (2006). The effect of maternal dietary vitamin D3 supplementation on performance and tibial dyshondroplasia of broiler chicks. Poult. Sci., 85: 39-47.

Duncan, D.B. (1955). Multible range and multible F tests. Biometrics, 11:1-42.

Dwyer, M.R., L.F. Kubena, R.B. Harvey, K. Mayura and A.B. Sarr (1997). Effects of inorganic adsorbents and cyclopiazonic acid in broiler chickens. Poult. Sci., 76: 1141-1149.

Elaroussi, M. A., H. F. Deluca, L. R. Forte, and H. V. Biellier (1993). Survival of vitamin D-deficient embryos: time and choice of cholecalciferol or its metabolites for treatment in ovo. Poultry Science. 72(6): 1118-1126.

Fleming,R.H., H.A. McCormack and C.C. Whitehead (1998). Bone structure and strength at different ages in laying hens. Br.Poult. Sci., 39: 434-440.

Foye, O; T.Z Uni, and P. R. Ferket (2006). Effect of in ovo feeding egg while protein, β-hydroxy-β-methylbutyrate and carbohydrates on glycogen status and neonatal growth of turkeys. Poult. Sci., 85: 1185-1192.

Gore, A. B and M. A. Qureshi (1997). Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. Poult. Sci., 76: 984-991.

Hassan, M.S., S.M.El-Soudany and Kh.M. Roushdy (2006). Relationship between parathyroid, calcitonin hormones and productive, physiological and immunological performance of some local strains. Egypt. Poult. Sci., 26(1): 297-317.

Henry, H.L. and A.W. Norman (1978). Vitamin D2 dehydroxylated metabolites are required for normal chicken egg hatchability. Science, 201: 835-837.

Hurwitz, S. (1992). The role of vitamin D in poultry bone bone biology. In: poultry Sci., Symp. Bone Biology and skeletal disorders in poultry, Carfax Pub. Company. Ovo. Poultry Science, 72 (6): 1118-1126.

Johnston, P. M. and C. L. Comar (1974). Distribution and contribution of calcium from the albumen, yolk and shell to the developing chick embryo. Amer. J. Physiol., 183: 365-370.

Khan, S. H., R. Shahid, A. A. Mian, R. Sardar and M. A. Anjum (2010). Effect of the level of cholecalciferol supplementation of broiler diets on the performance and tibial dyschondroplasia. Journal of Animal Physiology and Animal Nutrion. 94: 584-593.

Kim, W.K., S.A. Bloomfield and S.C. Ricke (2011). Effect of age, vitamin D3, and fructooligosaccharides on bone growth and skeletal integrity of broiler chicks, Poult. Sci., 90: 2425-2432.

Koch, M.E., D.C. Mahan and J.R. Corley (1984). An evaluation of various biological characteristics in assessing low phosphorus intake in weanling swine. J. Anim. Sci., 59: 1546-1556.

Kubota, A., E, T. Shinki and T, Sunde (1981). Vitamin D metabolites and its possible role in the development of chicks embryo. J. Physiol., 119: 103-109.

Lopes, K. L. A. M: A.A pedroso, N. S. M. Leandro, J. H. Stringhini, and C. E. Barbosa (2006). Glutamine in ovo inoculation effect at the starter performance of broilers. Braz; J. Avian Sci. Camp., 8: 103.

Michalczuk M., D.Pietrzak, J. Niemiec, J. Mroczek (2010). Effectiveness of vitamin D3 and calcidiol (25-OH-D3) application in feeding broiler chickens-production performance and meat quality, Pol. J. Food Nutr. Sci., 60(2): 121-126.

Narbaitz R., (1979). Response of shell-less cultured chick embryos to exogenous parathyroid hormone and 1,25-Dihydroxycholecalciferol, General and comparative endocrinology, 37: 440-442.

Narbaitz, R. (1972). Submicroscopical aspects of chick embryo parathyroid glands. Gen. Comp. Endocrinol., 19: 253-258.

Narbitz, R. and P. P. Tiller (1974). The Differentiation of the chick chorionic epithelium: an experimental study. J. Embryol. Exp. Morphol., 32: 365.

Narbitz, R. and S. Tolani (1978). Effects produced by the administration of high doses of 1,25-dihydroxy-cholecalciferol to the chick embryo. Calcif. Tissue Res., 26: 221-226.

Narbaitz, R. (1979). Response of Shell-less Cultured Chick Embryos to Exogenous Parathyroid Hormone and 1,25-Dihydroxycholecalciferol. General and Comparative e Endocrinology, 37: 440-442.

Narushin, V.G. and M.N. Romanov (2002). Egg physical characteristics and hatchability. J. Wrld's Poult. Sci., 58: 297-302.

Ohta, Y, N. Tushima, K. Koid, M. T. Kidd, and T. Ishibashi (1999). Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poult. Sci., 78: 1493-1498.

Ohta, Y, M. T. Kidd and T. Ishibashi (2001). Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after in ovo administration of amino acids. Poult. Sci., 80: 1430-1436.

Owczarzak, A. (1971). Calcium-absorbing cell of the chick chorio-allantoic membrane. I. Morphology, distribution and cellular interactions. Exp. Cell Res., 68: 113-129.

Pedrosa, A. A, L. S. Chaves. K. L. A. M. Lopes, N. S. M. Leandro M. B. Café, and J. H. Stringhini (2006). Nutrient inoculation in eggs from heavy breeders. Braz. J. Anim. Sci., 35: 2018-2026.

Petek, M., H. Baspinar and M. Ogan (2003). Effects of egg weight and length of storage on hatchability and subsequent growth performance of quail. S. Afr. J. Anim. Sci., 33: 242-247.

SAS (1994). SAS User's Guide; Statistics, Ver.6.04, 4th Edition, SAS institute. Inc., Carry, NC, U.S.A.

Skalinsky, E. I. and V. F. Kondalenko (1963). Electron microscopic studies of the chick chorioallantois during embryogenesis. Acta Morphol. Hung., 12: 247-259.

Sunde, M. L., C. M. Turk, and H. F. Deluca (1978). The essentiality of vitamin D metabolites for embryonic chick development. Science 200:1067-1069.

Tako, E., P.R. Ferket, and Z. Uni (2004). Effects of in ovo feeding of carbohydrates and β-hydroxy-β-methylbutyrate on the development of chicken intestine. Poult. Sci. 83:2023-2028.

Tatara, M.R, W. Krupski, M. Jankowski, Z. Zduńczyk, J. Jankowski and T. Studzinski (2011). Effects of dietary calcium content and vitamin D source on skeletal properties in growing turkeys. Brit. Poult. Sci., 52(6): 718-729.

Uni, Z. and P.R. Ferket (2003). Enhancement of Development of Oviparous species by *In ovo* Feeding. U. S. Regular Patent US 6,592,878 B2, July 15.

Uni, Z. and P.R. Ferket (2004). Methods of early nutrion and their potential. World's Poultry Science Journal 60:101-111.

Whitehead, C. C., H. A. McCormck, L. Meter, and R. H. Fleming (2004). High vitamin D3 requirements in broilers for bone quality and prevention of tibial dyschondroplasia and interactions with dietary calcium, available phosphorus and vitamin A. Br. Poult. Sci. 45:425-436.

Woodhead, J.S. (1990). The measurement of circulatory parathyroid hormone. Clinical Bioch., 23:17-21.

Yan, F., C. A. Keen, K. Y. Zhang and P. W. Waldroup (2005). Comparison of methods to evaluate bone mineralization. J. Appl. Poult. Res., 14: 492-498.

تاثير الحقن فى البيض بفيتامين D3 على نمو العظام وبعض خصائص الدم فى الدجاج الفيومى.

زينات عبد الجواد ابراهيم

قسم الدواجن- كلية الزراعة- جامعة الزقازيق- الزقازيق- مصر.

اجريت هذه الدراسة لتقييم تاثير الحقن في بيض الدجاج الفيومي بفيتامين D3 على مظاهر النمو وبعض خصائص الدم. استخدم في هذا البحث عدد 300 بيضة, قسم هذا العدد الى خمسة مجموعات تجريبية, استخدمت المجموعة الاولى كضابط سالب (لم تحقن) اما المجموعة الثانية والثالثة فقد تم حقنهما بالماء المقطر عند اليوم السابع والرابع عشر من التفريخ واستخدما (كضابط موجب). المجموعة الرابعة والخامسة تم حقنهما بفيتامين D3 عند اليوم السابع والرابع عشر من التفريخ وكانت الجرعة المستخدمة 16 الف وحدة دولية من الكولي كالسيفيرول (فيتامين D3). تم وزن الكتاكيت بعد الفقس مباشرة ثم اسبوعيا الى الاسبوع الثامن من العمر. كما تم قياس اطوال بعض العظام مثل عظمة الساق (shank) وعظمة القص (keel) وعظمة الفخذ (fumier) وعظمة الشظية (tibia) على فترات مختلفة بداية من الفقس الى الاسبوع الثامن كما اخذت عينات من البلازما عند الفقس وكذلك عند الاسبوع الثامن لتقدير لتركيز هرمون الباراثرمون وكذلك مستوى الكالسيوم والفسفور والبروتين الكلي والالبيومين ونشاط انزيمي الفوسفاتيز الحامضي والقلوي. كما تم عمل قطاعات هستولوجية في عظمة الشظية (tibia) عند الفقس. وتخلص اهم النتائج المتحصل عليها الى: زيادة معنوية في وزن الكتكوت عند الفقس في المجموعات التي تم حقنها بفيتامين D3 سواء عند اليوم السابع او الرابع عشر من التفريخ. اعلى نسبة فقس كانت في المجموعة التي تم حقنها بالفيتامين عند اليوم الرابع عشر يليها المحقونة عند اليوم السابع. لم تظهر النتائج تاثيرا معنويا لوزن الجسم في كل فترات التجربة في جميع المعاملات. طول عظمة القص (keel) كان اطول للمجموعات المحقونة بالفيتامين سواء في اليوم السابع او الرابع عشر عن المجموعات الاخرى. زاد طول عظمة الساق (shank) وعظمة الشظية (tibia) عند اسبوعين من العمر في المجموعة التي تم حقنها بالفيتامين عند اليوم الرابع عشر مقارنة بالمجموعات الاخرى. اما عند الاسبوع الرابع والثامن من العمر فقد اظهرت المجموعة التي تم حقنها بالفيتامين عند اليوم السابع زيادة في طول كل من عظمة الفخذ والقص وكذلك عظمة الشظية (tibia) ويليها التي تم حقنها عند اليوم الرابع عشر. كان تركيز هرمون الباراثرمون اعلى في المجاميع المحقونة بالفيتامين D3 عند فترتي التجربة وسلك مستوى الكالسيوم والفسفور نفس الاتجاه. اظهر انزيمي الفوسفاتيزالحامضي والقاعدي نشاطا اعلى في المجموعات التي تم حقنها بالفيتامين عند اليوم السابع يليه اليوم الرابع عشر. وقد عضدت نتائج الفحص الهستولوجي لعظمة الشظية (tibia) النتائج السابقة مما يعكس اهمية فيتامينD3 في تطور العظام.

وتخلص هذه التجربة الى ان حقن البيضة بفيتامين D3 يحسن من تكوين العظام وينبه افراز هرمون الباراثرمون من الغدة الجاردرقية.