PHYSIOLOGICAL ROLE OF VITAMIN D3 IN BONE GROWTH DURING PRE AND POST HATCHING DEVELOPMENT

BY

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RESULTS AND DISCUSSION

1- Egg weight, hatchability and embryonic mortality:

The effects of *in ovo* injection with vitamin D3 on hatching and residual yolk weight of Fayoumi and Dandarawy strains are presented in (Table-2). It is clear from the results that Dandarawy eggs were heavier than Fayoumi ones, although the differences between there were not significant.

These differences in egg weight is due mainly to the genetic background of each strain which undoubtedly different. 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.

The differences between strains in chick weight at hatch were not significant. However , data revealed that body weight of chicks from heavier eggs was higher throughout the study , irrespective of the applied treatment or the strain used. In this respect **sklan *et al*., (2003)** observed that heavier eggs produced chicks with heavier hatching weights and heavier broilers at market age. This is consistent with the results obtained in the present study, in which eggs of different weights exhibited different hatching weight.

This was also in close agreement with **Vieira and Moran (1998)** who found that younger broiler breeders produced lighter eggs and consequently, lighter chicks at hatch.

Table 2: Effect of *In ovo* injection by vit.D3 on some hatching traits of Fayoumi and Dandarawy strains

Strain (S)	Treatments (T)					*Overall*	Significance		
	C	Sh7	Sh14	D7	D14		S	T	S * T
				Egg Weight (gm)					
F	38.518 ±1.1745	41.663 ±1.081	39.00 ±1.187	44.7283 ±1.204	41.532 ±1.446	41.088			
D	44.67666 ±2.11988	41.745 ± 3.052	43.808 ±1.076	41.8200 0±2.6825	42.020 ±1.646	42.983			
Overall	41.598	41.696	40.923	43.565	41.727	-	NS	NS	NS
				Chick Weight (gm)					
F	30.1133 ±1.2904	30.2316 ±0.7275	29.673 ±0.7621	34.812 ±1.014	33.147 ±1.399	31.095			
D	32.203 ±1.637	31.230 ±2.298	31.90 ±1.162	30.42 ±1.37	31.38 ±1.181	31.651			
Overall	31.158^{b}	30.6^{b}	30.565^{b}	33.054^{a}	32.439^{a}	-	0.043	NS	NS
				Residual yolk Weight (gm)					
F	7.372 ±0.488	8.902 ±0.446	8.5833 ±0.651	8.977 ±0.499	9.197 ±0.578	9.006^{b}			
D	9.998 ±0.77	9.043 ±0.914	10.923 ±2.128	10.99 ±0.778	10.420 ±0.645	10.249^{a}			
Overall	8.686 ^{b}	8.958 ^{b}	9.071^{b}	9.780^{a}	.686^{a}		0.03	0.0173	NS

a,b... means within treatment with no common superscripts are significantly different (P≤0.05)

C=control , Sh7=Sham injected at 7 or 14, D7, D14, Vitamin D3 injected at d 7 or14 of incubation period. NS=Not significant at > 0.05

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 authers 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 posthatch development (**Gore and Qureshi, 1997; Tako, *et al*., 2004; Uni and Ferket, 2004; Lopes *et al*., 2006; Pedroso, *et al*., 2006 and Dos Santos, *et al*., 2010).**

The hatchability of Fayoumi eggs was higher as compared with Dandarawy eggs (Table 3) . Morever, results show that *in ovo* vitamin D3 injection increased hatchability percentage for both strains as compared with those sham injected or control eggs. It was also observed that the hatchability (%) of Dandarawy eggs was lower than that of Fayoumi eggs regardless the applied treatments. It is also noticed that sham injection of aggs has reduced the hatchability percent of Dandarawy eggs to a great extent when compared to Fayoumi eggs . This may be to attributed the higher residual yolk weights of Dandarawy chicks indicative of low bioavilability of egg yolk nutrients by Dandarawy embryos .

This holds true where the overall mean of residual yolk weight was significantly higher for Dandarawy compared with Fayoumi embryos (Table 2). It appears , however, that vitamin D3 injection either at day 7 or d 14 of incubation period enhanced significantly the residual yolk overall weight which may suggest a subsequent enhancement and (or) a sparing effect of the treatment on nutrients profile of yolk.

Table 3: Hatchability and embryonic mortality of all incubated eggs

	Treatments (T)				
	C	Sham		D7	D14
		7	14		
	Hatchability %				
F	78.4	76.0	78.6	83.3	91.2
D	65.5	55.0	45.0	68.0	70.5
overall	71.86	63.61	61.8	75.60	80.80
	Embryonic mortality%				
Early (1-13d)	8.12	10. 2	10.58	11.37	10.75
Late (13-21d)	13.80	13.1	15.42	9.85	6.10
Pipped	6.18	10.2	10.36	3.13	2.35

Significant level: NA= Not applicable as single hatch was taken

Concerning the effect of different *in ovo* treatments on the overall hatchability and embryonic mortality of all incubated eggs, the results in Table-3 show that the highest hatchability percentage was recorded for vitamin D3 injected eggs at 14 days of incubation period. Followed by those injected at d 7 and the control treatment eggs. It is of interest to observe that the hatcability (%) of sham injected eggs was the lowest 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 , especially the late dead embryos (%) as shown in (Table-3). It is worthy to mention that this group contained both 7 and 14 day sham-injected eggs.

On the other hand, results revealed that *in ovo* injections increased early embryonic mortality percentages of all treatment groups as compared to the control one. Morever, during the late incubation period the dead embryos (%) was increased in the control and the sham-injected eggs.

The percentage of pipped chicks was higher for the sham-injected group but numerically lower for the vitamin-injected groups. These results support the previous findings which claimed that vitamin D3 injection could improve embryonic viability which in turn decrease the percentage of dead embryos (**Ohta *et al*.,1999; Bhanja *et al*.,2007).**

2- Bone characteristics of day chicks:

Table-4 shows some bone measurements of day-old Fayoumi and Dandarawy chicks as influenced by *in ovo* injection with vitamin D3 at two different periods of embryogenesis. It is clear from the results that Dandarawy chicks had significantly longer overall mean keel length compared to Fayoumi chicks. Morever, keel length was significantly longer for the pre *in ovo* vitamin D3 injected chicks either at 7 or 14 days of incubation period,

regardless the strain of chicks. The interaction between strains and treatments was not, However, significant. A similar trend was noticed for shank length but Fayoumi chicks had significantly longer shanks than Dandarawy ones.

Vitamin D3 injection increased significantly shank length compared with sham injected or the control treatments. The differences between in ovo injection at 7 and 14 days of incubation were not significant. The interaction between strain and treatment was significantl indicating either genetic or treatment influences. Data in (Table-4) reveal also that the overall means of both tibia length and femur length were significantly influenced by the strain of chicks, where Fayoumi chicks had almost always higher values compared with Dandarawy ones.

Also *in ovo* injection of vitamin D3 has a significant influence on both tibia and femur lengths as compared with other treatments. The interaction between strain and treatment was highly significant. Concerninig the effect of strain and in ovo treatments on tibia weight , the present data (Table-4) indicate that Fayoumi chicks has significantly heavier tibia weights compared to Dandarawy ones.

However, tibia weight of the sham-7 injected eggs was the lowest as compared to other treatments. It appears from the previous results that *in ovo* injection with vitamin D3 either at day 7 or 14 of incubation period could improve long bones measurements of day old chicks. The elongation of keel, tibia and femur of chicks may be due to the physiological role of vitamin D3 on bone formation during embryogenesis.

Table 4: Effect of prehatching *In ovo* injection with vitamin D3 on posthatching bone development of Fayoumi and Dandarawy chicks at day old

Strain (S)	Treatments (T)					*Overall*	Significance		
	C	Sh7	Sh14	D7	D14		T	S	T*S
				keel length (cm)					
F	1.72 ±0.066	1.76 ±0.051	1.63 ±0.084	1.80 ±0.078	2.00 ±0.071	1.777[b]			
D	1.94 ±0.040	1.74 ±0.051	1.72 ±0.037	2.08 ±0.04	1.94 ± 0.117	1.884[a]			
Overall	1.830[bc]	1.750[bc]	1.677[c]	1.940[a]	1.970[a]		0.004	0.02	NS
				Shank length (cm)					
F	1.96 ±0.163	1.64 ±0.143	1.75 ±0.102	2.64 ±0.120	2.84 ±0.040	2.150[a]			
D	2.08 ±0.058	1.88 ±0.086	1.72 ±0.037	2.16 ±0.133	2.04 ±0.06	1.976[b]			
Overall	2.020[c]	1.760[b]	1.736[b]	2.400[a]	2.440[a]		0001	0.06	0001
				Tibia length (cm)					
F	2.52 ±0.048	1.86 ±0.074	1.98 ±0.130	2.44 ±0.128	2.78 ±0.097	2.304[a]			
D	2.04 ±0.04	2.02 ±0.146	1.82 ±0.037	2.44 ±0.075	2.48 ±0.0490	2.160[b]			
Overall	2.280[b]	1.940[c]	1.909[c]	2.440[a]	2.630[a]		0001	0.01	0.01
				Femur length (cm)					
F	1.86 ±0.060	2.10 ±0.141	1.65 ±0.091	2.58 ±0.139	2.50 ±0.114	2.119[a]			
D	1.76 ±0.04	1.68 ±0.073	1.72 ±0.058	1.80 ±0.044	2.16 ±0.0748	1.824[b]			
Overall	1.810[bc]	1.890[b]	1.681[c]	2.190[a]	2.330[a]		0001	001	0.004
				Tibia weight (gm)					
F	0.23 ±0.018	0.18 ±0.007	0.23 ±0.019	0.25 ±0.016	0.25 ±0.007	0.229[a]			
D	0.19 ±0.002	0.16 ±0.005	0.18 ±0.004	0.19 ±0.004	0.202 ±0.019	0.188[b]			
Overall	0.209[a]	0.170[b]	0.212[a]	0.223[a]	0.224[a]		0.0009	001	NS

a,.... means within treatment with no common superscripts are significantly different (P≤0.05)

C=control , Sh7=Sham injected at 7 or14, D7, D14, Vitamin D3 injected at d 7 or14 of incubation period. NS=Not significant at P≤0.05

This concept was also proposed and examined by many authors who found that vitamin D3 enrichement of eggs either by direct *in ovo* injection or by using some directory manipulations may enhance bone remodeling and improving osteogenesis in birds **(Kubota, *et al*., 1981;Hurwitz, 1989; Elaroussi, *et al*.,1993 and Gay,1996).** It seems that the increase in tibia weight of vitamin D3 injected chicks could reflect the enhanced bone mineralization of these groups as a result of vitamin D3 effects on bone calcification via its role on calcium metabolism and or by the role played on parathyroid hormone stimulation.

In this respect **Dacke (2000)** reported that vit.D3 facilitates bone formation by inducing biosynthesis of osteocalcin (a non-collagenous vitamin D binding protein) which is a specific product of osteoblasts during bone formation. This is evidence that PTH receptors are located on osteoblasts surfaces but have been considered absent in osteoclasts which suggest that PTH can induce rapid changes in Ca transfer by osteoblasts and osteocytes for excessive bone mineralization **(Pandala and Gay, 1990; May *et al*.,1993 and Bronner,1996).** This confirms and support our results about the beneficial effect of *in ovo* vit.D3 injection during embryogenesis.

3- Bone characteristics of 2 weeks old chicks:

Regardless the strain effect, the present results show that the *in ovo* injection with vitamin D3 at day seven of incubation period significantly increased chicks weight at 2 weeks of age (Table-5). This was also observed for the vit.D3 injected group at day-14 of embryogenesis. The lowest chick weight was recorded for chicks that hatched from sham-injected eggs either at 7 or 14 days of incubation period.

It is clear that Fayoumi chicks were heavier than Dandarawy. ones, however, the differences between them were

not significant. At 4 weeks of age, Fayoumi chicks became significantly heavier than dandarawy ones.

At the same time, chicks that hatched from vitamin D3 enriched eggs were significantly heavier than the other treatments. This effect was more obvious for the 7 days *in ovo* injected group. The interaction between treatments and the strain of the chick was highly significant. It appears that Fayoumi chicks grow faster than Dandarawy during the first weeks of age. It seems likely that Fayoumi strain(breed), in general, have been subjected to intensive and continous trails from breeders and researcher for improving their productive performance as Fayoumi birds are considered the main native breed of chickens in Egypt **(El-Hossar *et al*.,1992 and Mahmoud, 2000).** Concerning the effect of vit.D3 injection on shank length the present results (Table-5) clearly show that in ovo injection at day-14 of incubation significantly increased shank length of both Fayoumi and Dandarawy chicks. This increase was also observed for the control and Sham-7 treatment groups, which may reflect genetical background by treatment effect. This holds true as the overall mean of shank length of Fayoumi chicks was higher than Dandarawy ones this results is supported by the significant interaction between strain of bird and treatment.

Table 5: Effect of prehatching *In ovo* injection with vitamin D3 on posthatching bone development of Fayoumi and Dandarawy chicks at 2 weeks of age

Strain (S)	Treatments (T)					*Overall*	Significance		
	C	Sh7	Sh14	D7	D14		S	T	S* T
				Body Weight (gm) 2 WOA					
F	173.828 ±4.846	168.678 ±2.389	172.104 ±2.134	192.803 ±7.74	184.562 ±2.764	178.39			
D	166.913 ±4.954	163.48 ±5.444	155.572 ±3.689	179.232 ±5.183	171.498 ±3.053	167.34			
Overall	170.372^{bc}	166.08^{bc}	163.84^{c}	186.11^{a}	178.02^{a}_{b}		0.032	NS	NS
				Keel length(cm)					
F	3.20± 0.100	3.56± 0.128	3.44 ±0.068	3.68 ±0.153	3.30 ±0.55	3.436^{a}			
D	2.80 ±0.55	3.22 ±0.073	2.94 ±0.11	3.100 ±0.45	3.020 ±0.0663	3.016^{b}			
Overall	3.00^{b}	3.390^{a}	3.19^{b}	3.390^{a}	3.160^{b}		0.004	0.001	NS
				Shank length(cm)					
F	3.860 ±0.093	4.120 ±0.0860	3.44 ±0.06	3.680 ±0.153	3.72 ±0.086	3.764^{a}			
D	3.575 ±0.144	3.220 ±0.073	3.440 ±0.075	3.100 ±0.447	3.620 ±0.120	3.383^{b}			
Overall	3.733^{a}	3.670^{a}	3.440^{b}	3.390^{b}	3.670^{a}		0.033	0.001	0.002
				Tibia length(cm)					
F	4.22 ±0.1140	4.140 ±0.103	4.180 ±0.097	4.800 ±0.1378	4.820 ±0.107	4.432^{a}			
D	4.15 ±0.0645	4.200 ±0.0134	4.360 ±0.129	4.500 ±0.105	4.6200 ±0.107	4.362^{b}			
Overall	4.165^{b}	4.173^{b}	4.272^{b}	4.651^{a}	4.733^{a}		0.014	0.037	0.053
				Tibia weight					
F	2.232 ±0.0183	2.244 ±0.0157	2.70 ±0.100	2.92 ±0.024	2.88 ±0.025	2.584^{a}			
D	2.176 ±0.0169	2.235 ±0.021	2.180 ±0.010	2.760 ±0.0158	2.65 ±0.019	2.401^{b}			
Overall	2.20^{b}	2.24^{b}	2.43^{b}	2.84^{a}	2.75^{a}		0.001	0.001	0.001

a,.... means within treatment with no common superscripts are significantly different ($P \leq 0.05$)

C=control , Sh7=Sham injected at 7 or14, D7, D14, Vitamin D3 injected at d 7 or14 of incubation period. NS=Not significant at $P \leq 0.05$

A similar trend was also observed for keel length where Fayoumi chicks had significantly longer keels than Dandarawy ones. However, the effect of *in ovo* injection on keel length was more effective in the 7d vit.D3-injection group. It is clear also that tibia length at 2 WOA was significantly influnced by different treatments, where vit.D3 injected groups had the highest values. Also, Fayoumi chicks had significantly long tibia than Dandarawy (Table-5). On the other hand,results reveal that tibia weight of Fayoumi chicks was significantly heavier than Dandarawy chicks at the same age (2WOA). The effect of *in ovo* vit.D3 injection was more pronounced either for vit.D3 injection at 7 or 14 days of age. The differences between treatments, strain and their interaction were highly significant. These results support the previous findings by **Hurwitz (1989); Elaroussi, *et al*. (1993); Roberson and Edwars (1994); Yan *et al*.(2005) and Kim *et al*.(2011)** who reported that the tibia bone is the fastest growing bone in the body and is considered as the most sensitive to any disturbances in Ca regulating systems including vit.D3 and parathyroid hormones secretion. This confirms our results where tibia length and weight were greatly changed by age strain and vit.D3 fortification of aggs during embryogenesis.

Blood constituents:

4- Blood paramerers of day-old chick:

Means of some blood constituents as influenced by pre-hatching in ovo injection with vitamin D3 in day old Fayoumi and Dandarawy chicks are presentedin (Tabl-6). It is clear from the results that total proteins were significantly higher for chicks that hatched from all treated group compared to the control one. the differences between Fayoumi and Dandarawy were not significant.

Table 6: Effect of *In ovo* injection with vitamin D3 (VD) on some blood constituents in day old Fayoumi and Dandarawy chicks

Strain (S)	Treatments (T)					*Overall*	Significance		
	C	Sh7	Sh14	D7	D14		S	T	T*S
				Total protein(g/dl)					
F	4.25± 0.54	4.86± 0.48	4.46± 0.62	4.18± 0.37	4.35± 0.82	4.40			
D	4.12± 0.36	4.50± 0.72	4.22± 0.18	4.80± 0.46	4.65± 0.54	4.43			
Overall	4.16^{c}	4.65^{a}	4.34^{b}	4.44^{ab}	4.48^{ab}	—	0.032	NS	NS
				Albumin (g/dl)					
F	2.16± 0.23	2.06± 0.18	2.21± 0.35	2.10± 0.12	2.25± 0.15	2.16			
D	2.30± 0.15	2.22± 0.10	2.14± 0.18	2.44± 0.26	2.18± 0.20	2.24			
Overall	2.21^{ab}	2.10^{b}	2.18^{ab}	2.23^{a}	2.19^{ab}	—	NS	0.024	NS
				Globulin (g/dl)					
F	2.09± 0.12	2.80± 0.22	2.25± 0.15	2.08± 0.16	2.10± 0.09	2.25			
D	1.82± 0.08	2.28± 0.13	2.08± 0.24	2.36± 0.10	2.47± 0.32	2.21			
Overall	1.95^{c}	2.56^{a}	2.17^{bc}	2.19^{bc}	2.24^{b}	—	NS	0.035	NS
				A/G ratio					
F	1.03± 0.02	0.74± 0.02	0.98± 0.03	1.01± 0.05	1.07± 0.02	0.96			
D	1.26± 0.03	0.97± 0.01	1.03± 0.02	1.03± 0.02	0.88± 0.02	1.01			
Overall	1.14^{a}	0.85^{b}	1.00^{ab}	1.02^{a}	0.97^{ab}	—	NS	0.042	NS

a,.... means within treatment with no common superscripts are significantly different ($P \leq 0.05$)

C=control , Sh7=Sham injected at 7 or14, D7, D14, Vitamin D3 injected at d 7 or14 of incubation period. NS=Not significant at $P \leq 0.05$