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LATE STAGE RESPONSES OF LAYING CHICKENS FED DIETS SUPPLEMENTED WITH DIFFERENT SOURCES AND LEVELS OF VITAMIN D: 1- PRODUCTIVE AND REPRODUCTIVE PERFORMANCE.

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ABSTRACT: This experiment was conducted to study the effect of supplementing layer diets with two vitamin D (VD) sources included three levels from each of vitamin D_3 (VD_3) and 25-hydroxycholecalciferol (25-OHD_3) on egg production and reproduction performance. At 11 month of age 189 layers and 21 cocks were chosen and distributed equally into 9 groups. Each group contained 3 replicates contained 7 hens and 1 cock each. Nine experimental diets were formulated, the first one was control diet that contained 2500 IU VD_3/kg diet. The 2nd and 3rd diets formed by supplemented control diet with 1250 and 2500 IU VD_3/kg diet respectively. The previous three diets supplemented with 0.04 mg 25-OHD_3/kg diet to create the 4th, 5th and 6th diets respectively. To perform 7th, 8th and 9th diets the first three diets supplemented with 0.08 mg 25-OHD_3/kg diet respectively. The experiment was extended for four months and egg production performance, reproduction performance and liver enzymes were measured. The following results were obtained:

Supplementation of 25-OHD_3 improved significantly egg production% during the 2nd month and entire experimental period. Increasing total VD content than NRC (1994) increased significantly ($P < 0.05$) egg production% than control treatment after the 2nd month. Hen's persistence showed clear improvement especially with diets contained total VD from 0.125 to 0.17375 mg/kg. Egg mass per hen per day was improved as seem as egg production%.

Feed intake of entire experimental period was increased significantly ($P < 0.05$) by increasing VD than NRC 1994. Significant improvement ($P < 0.05$) was observed in feed conversion when diet contained 0.17375 VD mg/kg compared with control treatment. Neither reproduction performance nor liver function influenced significantly by VD source and levels.

We recommend that 25-OHD_3 should combine with VD_3 in layer diets at late stage but not more than 0.17375 mg/kg.

Keywords: 25-hydroxycholecalciferol-vitamin D_3-egg production.

INTRODUCTION

Increasing vitamin D (VD) content in layer diets than NRC (1994) recommendation was applied to increase human consumption from VD (Persia *et al.*, 2013) , where VD transfer from diet to yolk and use as a source of VD for human. Mattila *et al.* (1992) found strong correlation between source and levels of VD in diets and concentration of VD in egg yolk. During winter in open side system when daylight and light intensity decreased the ultraviolet was restricted and VD must be adequate (Holick, 2005). Nascimento *et al.* (2014) reported that supplemented different VD sources to aged hens diets influenced ($p<0.05$) egg production percent, egg mass, feed intake, feed efficiency and Haugh unit. Han *et al.* (2017) reported that vitamin D_3 (VD_3) (cholecalciferol) commercially is used in poultry diets as a source of VD_3 then it hydroxylated to 25-hydroxycholecalciferol (25-OHD_3) in the liver and converted in the kidney to 1,25 dihydroxycholecalciferol (1,25-OHD_3).

The rate of VD_3 absorption from diets ranged from 60 to 70% (Stanford, 2006) but absorption of 25-OHD_3 was higher than VD_3 (Angel *et al*., 2005). On the other hand 25-OHD_3 has longer half-life compared with other features of VD (Stanford 2006). Moreover transference rate of 25-OHD_3 into eggs was higher than vitamin D_3 and lead to reduced progeny skeletal disorders, improve hatchability (Torres *et al*., 2009) and chicks quality (Saunders-Blades and Korver, 2015). Many authors have studied the relative biological value of VD_3 compared with 25-OHD_3 where Han *et al.* (2017) reported that the relative biological value of 25-OHD_3 was higher than VD_3. Soares *et al.* (1995) and Han *et al.* (2016) reported that VD_3 possess half relative biological value of 25-OHD_3, furthermore Atencio *et al.* (2005) increased evaluation of 25-OHD_3 relative biological value compared with VD_3 up to 400%. On the other hand when calcium requirement decrease than requirements, effectiveness of 25-OHD_3 clearly appear (Ledwaba and Roberson, 2003) and as hens aged as renal 25-OHD_3 – lhydroxylase activity decreased (Elaroussi *et al.,* 1994), so hydroxylation of D_3 to 25-OHD_3 decreased.

The major VD role appears in regulating mineral absorption (Stanford, 2006), specially its pivotal role in calcium metabolism (Li *et al.*, 2018). The ability of bird for metabolizing calcium is a factor that responsible for determining persistence of egg production (Taylor, 1970). On the other hand (1,25-OHD_3) play important role in regulation neuroendocrine (Sonnenberg *et al.*, 1986) and reduce gonadotrophin secretion that lowered persistence of laying hen (Sharp *et al.,* 1992).

So the present study aimed to prolong persistence stage and recover peak of egg production during late stage of egg production in local chickens by supplemented diet with different VD sources and levels higher than NRC (1994) recommendation.

MATERIALS AND METHODS

Birds and experimental treatments:

At eleven months of age 189 hens and 27 cocks of Golden Montazah (GM) chickens were selected and distributed randomly into 9 experimental groups. Each experimental group consisted of 3 replicates with 7 hens and 1 cock per each replicate. Nine experimental diets were formed where, the first diet was control basal diet that contains 2500 IU vitamin D_3/kg diet, where the 2^{nd} and 3^{rd} treatments formed by supplemented basal diet with 1250 and 2500 IU vitamin D_3/kg diet respectively. The previous three diets in the same arrangement supplemented with 0.04 mg 25-OHD_3/kg diet to create 4^{th}, 5^{th} and 6^{th} diets. To perform 7^{th}, 8^{th} and 9^{th} diets the first three diets supplemented with 0.08 mg 25-OHD_3/kg diet in the same order.

Preparing diets:

Layer control basal diet was formed depending on expected feed intake for local chickens (Table, 1) where it satisfies local laying hen requirements and contains 2500 IU VD_3. Diets were prepared weekly to avoid destroyed of VD_3 and 25-OHD_3 where feed was offered daily to experimental birds. According to European Food Safety Authority EFSA (2009) the total mixture of VD_3 with 25-OHD_3 when combined and provided to poultry must not exceed than 0.8 mg/kg diet. Table, 2A illustrates converting VD_3 from international unit to milligram while, the total content of VD_3 plus 25-OHD_3 for different experimental diets were illustrated in Table 2B that ranged from 0.0625 to 0.205 mg/kg diet. The upper limit (0.205 mg/kg diet) that used in this experiment was lower than the upper limit (0.8 mg/kg) of EFSA (2009) so the limits of all diets in this experiment were in the safe limit of European food safety authority.

Management conditions and measurements:

Each 7 hens within each replicate were housed individually in a single cage successively and one cock was inserted randomly within each replicate. To evaluate production performance, the experiment was extended for four months from 11^{th} to 14^{th} month of age, where hens fed *ad libitum* and water was available all the time. All treatments received 16 hours continuous light daily, where artificial light was supplemented before sunrises during winter months to complete daylight length.

Eggs were gathered separately per each replicate and weighed daily then egg production percent, egg mass per hen per day and average egg weight were calculated according to Bonekamp *et al.* (2010). For each weekend residual feed was weighed and subtracted from offered daily feed to calculate feed intake. Feed conversion ratio was calculated according to EL-Husseiny *et al.* (2008), where feed intake per hen per day was divided by egg mass per hen per day.

At the last week of production period six eggs from each replicate were chosen to estimate egg quality. Egg width and length was measured to the nearest millimeter, hence shape index was calculated by dividing width by length multiplied by 100. Yolk height and width was measured, hence yolk index was calculated by dividing height by width multiplied by 100. Yolk and shell were weighed, hence albumin weight was calculated by subtraction, hence yolk and albumin weight percent were calculated. The measurements of egg shell along with strength and its ultrastructure will be study in another paper.

Three blood samples were withdrawn from each treatment from wing vein in heparinized test tubes and centrifuged for 15 at 3000 rpm to separate plasma. Live enzyme activity in terms of AST (Aspartate aminotransferase) and ALT (Alanine Transaminase) were measured using colorimetric method.

At the end of egg production period each replicate as it is (7 hens + 1 cock) get down and housed separately in one pen. Each group continued in consuming its experimental diet, after three weeks eggs were collected daily and storage at 20 C°

for five days to conduct reproduction assessment. Fifteen eggs were selected from each replicate to get 45 eggs from each treatment that incubated in hatch master, where fertility and hatchability per fertile eggs were calculated.

Statistical analysis:

General linear model "univariate model" procedure of statistical software package (SPSS, 2007) version 16 was used to apply two-way ANOVA according to the following statistical model:

$Yij = \mu + Hi + Dj + (H \times D)\ ij + eij$

Where,

Yij = dependent observation.

μ = overall mean.

Hi = effect of 25-OHD_3 (i = I, II and III).

Dj = effect of VD_3 (j = 1, 2 and 3).

(Hi × D) ij = effect of interaction between 25-OHD_3 and VD_3.

eij = the residual error.

Post Hoc multiple comparisons for observed means procedure of (SPSS, 2007) was used to compare means at F-test ($P \leq 0.05$) according to (Duncan, 1955).

For detecting persistence response, means of egg production percent for a control treatment was used as standard and compared with other eight treatments. Each treatment from the eight experimental treatments was compared separately with control by inserting egg production percent (4 months of experiment) in line chart with linear trendline that symbolized as persistence response according to (Vai *et al.*, 2014).

RESULTS AND DISCUSSION

Egg production performance:

Egg production percent was significantly improved by feeding diets supplemented with different 25-OHD_3 levels regardless VD_3 during the second month and for entire experimental period. However, this improvement was not significant during other months (Table, 3). On the other hand supplementing layers diets with VD_3 levels higher than NRC (1994) recommendation regardless to 25-OHD_3 did not significantly affect egg production percent during experimental period. Regarding experimental treatments during the 2nd, 4th and entire experimental period supplemented basal diet with different VD sources and levels higher than NRC (1994) recorded significantly ($P < 0.05$) higher egg production percent compared with control diet. The highest mean of egg production percent during entire period was recorded for hens that fed diet supplemented with 0.08 mg 25-OHD_3 combined with 2500 IU VD_3/kg diet (0.1425 mg/kg diet total VD).

Improvement of egg production by supplemented different 25-OHD_3 levels agree with (Käppeli *et al.,* 2011) who presented data demonstrated that supplemented 25-OHD_3 improved egg production percent. The highest levels of 25-OHD_3 recorded higher egg production percent compared with the highest levels of VD_3, this may be due to the relative biological values of 25-OHD_3 for egg production compared with D3 was 138% (Atencio *et al.*, 2005). Improvement that achieved by 25-OHD_3 supplementation may be occur because the experiment was done in a late stage of egg production where 25-OHD3 is effective during the late stages of egg production (Keshavarz, 2003). Moreover as hens aged as the renal 25-OHD_3 -l-hydroxylase activity decreased (Elaroussi *et al.,* 1994). The insignificant response of egg production percent for increasing VD_3 during the entire period agree with Mattila *et al.* (2004) who reported that hens received 6,000 and 15,000 IU VD_3/kg diet didn’t possess different rate of lay compared with control treatment that contained 2,500 IU VD_3/kg diet.

The insignificant decrease in egg production percent when diet contain the highest level of 25-OHD_3 combined with the highest level of VD_3 agree with Coto *et al.* (2010) who reported that 25-OHD_3 had more noticeable effect on performance at low levels of VD_3 but had no difference with higher levels of VD_3.

Figure 1 illustrates that linear trendline of control treatment showed the lowest persistence response where it started at the 1st month with (52.78%) and finished 4th month of experiment with (42.86 %). When 25-OHD_3 was supplemented in a high level (0.8 mg/kg) combined with 2500 IU/kg diet brought the best persistence response where it started 1st month with (59.92%) and finished 4th month of experiment with (55.95 %). So we can suppose that supplemented 25-OHD_3 in a high level combined withVD_3 but not contain total VD more than 0.17375 mg/kg diet prolonged peak of egg production and possessed ability to improve persistence response.

Supplemented basal diets with different 25-OHD_3 levels increased significantly ($P < 0.05$) egg mass per hen per day compared with diets without 25-OHD_3 during 2nd, 4th months and for the entire period (Table, 4). Nevertheless supplemented basal diets with VD_3 levels higher than NRC (1994) recommendations did not significantly affect egg mass per hen per day during different months and for entire experimental period. Egg mass results of 25-OHD_3 supplementation were in agreement with Soto-Sealanova, and Molinero (2005) who observed an increase in egg mass by about 2.1% when 25-OHD_3 supplemented to control diets. Insignificant effect off VD_3 agree with Persia *et al.* (2013) who presented data illustrated that there were insignificant difference in egg mass when diets contain 2200, 9700, 17200, 24700 and 102200 IU VD_3/Kg diet. This may be due to 25-OHD3 had better potency compared with VD3 at low levels of supplementation (Atencio *et al.*, 2005). The main effects of 25-OHD_3 and VD_3 (singly) on average egg weight were insignificantly for entire experimental period but significant effect was observed for experimental treatments. Regarding effect of experimental treatments on average egg weight the results showed unclear trend which is difficult to be explained as many physiological and nutritional factors can affect egg weight.

Feed intake and feed conversion

Significant effect for 25-OHD_3 was observed in feed intake regardless VD_3 for all months and entire period, where supplemented basal diet with 25-OHD_3 increased feed intake (Table, 5). In contrast VD_3 with regardless 25-OHD_3 affected significantly feed intake during the 3rd and 4th and entire experimental period, moreover the insignificant effect of VD_3 disappeared when it increased to the upper limit. The conflict results in feed intake for the different sources of VD may be due to the different metabolites forms of VD affected in feed intake (Garcia *et al.*, 2013). Supplementation of 25-OHD_3 effected insignificantly feed conversion so we can expect that increasing in feed intake may be attributed to increasing egg mass per hen per day. Regarding combination of 25-OHD_3 with VD_3, the results of feed intake didn't agree with Rivera *et al.* (2014) who reported that incorporation of 25-OHD_3 with VD_3 lowered feed intake than VD_3 when supplemented individually.

Slightly improvement was observed in feed conversion when total content of VD in diets ranged from 0.09375 to 0.17375 mg/kg diet for entire experimental period. The results agree with Mattila *et al.* (2004) who found slightly improvement in feed conversion during the period from 36-67 weeks of age when total diet content from VD increased. Increasing total VD content than 0.205 mg/kg diet delayed feed conversion but not less than control treatment.

Egg qual

Both of shape index and albumen weight percent recorded significant response to 25-OHD_3 supplementation (Table, 6). Increasing 25-OHD_3 level to 0.04 mg/kg significantly increased ($P<0.01$) shape index compared with zero and 0.08 mg/kg. On the other hand albumen weight percent significantly increased ($P<0.01$) by increasing 25-OHD_3 level to 0.08 mg/kg.

Except for shape index increasing VD_3 didn't bring significant effect on egg quality. The result agrees with Yao *et al.* (2013) who reported that increasing VD_3 in layer diets didn't affect egg quality. Nevertheless effectiveness of shape index by VD sources and levels but it still around the normal range, where the values of shape index of sharp and round eggs were 71.9 and 82.8 respectively (Duman *et al.*, 2016).

Reproductive performance

Neither 25-OHD_3 nor vitamin D_3 affected significantly reproductive performance (Table, 7). The results of reproductive performance agree with (Torres *et al.*, 2009) who found similar reproductive performance for hens that fed diets contained 25-OHD_3 and vitamin D3. Supplemented diets with 0.04 and 0.08 mg 25-OHD_3 /kg improved numerically fertility percent. This result agree with (Nadeau and Korver, 2013) who observed increasing in fertility percent when diets of broiler breeder supplemented with 25-OHD_3. The improvement of fertility by 25-OHD_3 supplementation may be due to 25-OHD_3 able to improve sperm quality (Rosa *et al.*, 2010). Hatchability of fertilized eggs for diets contained 0.08 mg 25-OHD_3/kg increased by about 4% compared with diets without 25-OHD_3. This is may be due to 25-OHD_3 reduced early embryonic mortality by 30% (Saunders-Blades and Korver, 2014) and increased hatchability (Saunders-Blades and Korver, 2015). Increasing vitamin D_3 than 2500 led to slightly increase (1%) in hatchability percent compared with control treatment (Table, 7). This may be that the best values of embryonic mortality and hatchability percent were obtained when vitamin D_3 levels was 2750 IU/kg during postpeak of egg production respectively (Atencio *et al.*, 2006). Increasing hatchability percent for 25-OHD_3 compared with vitamin D_3 may be due to 25-OHD_3 has higher ability to transport to eggs and support high embryonic development (Atencio *et al.*, 2005). Coto *et al.* (2010) reported that when 25-OHD_3 performed the major source of vitamin D_3 in hen diets hatchability percent of fertilized eggs was improved.

Liver enzymes

The results of liver enzymes (Table, 7) showed that different levels of vitamin D_3 and 25-OHD_3 didn't affect significantly AST and ALT. liver enzymes values found in low concentrations and AST and ALT were the most obvious indicators for hepatocyte injury and damage (Wang *et al.*, 2015). The normal values of AST and ALT of demonstrated that liver state was in a normal case. The results of AST and ALT agree with (Rath *et al.*, 2007) who reported that there were no clear differences in AST and ALT for vitamin D3 and 25-OHD3 treatments.

The slightly increase in liver enzymes values of vitamin D_3 and 25-OHD_3 compared with control treatment may be due to increasing egg production percent. Increasing egg production percent (Table ,3) corresponded with increasing feed intake (Table ,5) and certainly feed ingredients digestion and metabolism hence liver activity increased to promote the higher metabolic rate (Onagbesan *et al.*, 2004). Moreover Hocking *et al.,* (2002) reported that higher AST may be associated with increasing rates of lay.

CONCLUSION

In conclusion supplemented layer diets with VD_3 and 25-OHD_3 in a combination higher than NRC recommendations and less than 0.17375 mg/kg diet improved egg production performance during late stage of egg production and prolonged layers persistenc

Table (1): Composition and calculated analysis of control diet.

Ingredients	**Control diet**
Yellow corn	65.00
Soybean meal (44% CP)	5.30
Corn gluten (60% CP)	9.00
Wheat bran	12.00
Di-calcium phosphate	2.39
Lime stone	5.63
Salt	0.37
Premix	0.30
DL- methionine	0.01
L-Arg	0
Total	100
Calculated analysis	
CP	15.14
ME.	2786.49
Ca	3.45
Av.P	0.37
Lys.	0.70
Met.	0.31
SAA	0.61
Na	0.17
Vtamin D_3	2500 IU

Premix contain per 3kg vit A: 12 000 000, vit D3: 2500 000 IU, vit E: 50000mg, vit K3: 3000mg , vit B1: 2000mg, vit :B2 7500mg, vit B6 :3500 mg, vit B12: 15mg, Pantothenic acid :12000mg, Niacin: 30000mg, Biotin: 150mg, Folic acid: 1500mg, Choline: 300gm, Selenium: 300mg, Copper: 10000mg, Iron: 40000mg, Manganese: 80000mg, Zinc: 80000mg, Iodine: 2000mg, Cobalt: 250 mg and CaCO3 to 3000g.

Table (2A): Converting different levels of vitamin D_3 from IU to mg.

VD_3*IU	VD_3** ng	VD_3 mg
2500 × 25	62500 ng	0.0625 mg
3750 × 25	93750 ng	0.09375 mg
5000 × 25	125000 ng	0.125 mg

* International unit (IU) multiplied by 25 to turn out to be nanogram (ng).
** Nanogram (ng) divided on 10^6 to turn out to be milligram (mg).
VD_3:vitamin D_3

Table (2B): Total content of experimental diets from 25-OHD_3 and VD_3 mg/kg diet.

Experimental diets	25-OHD_3 and VD_3 mg/kg diet		Total content of VD sources	
	25-OHD_3	VD_3	mg/kg diet	IU/kg diet
I * 1	zero	0.0625	0.0625	2500
I * 2	zero	0.09375	0.09375	3750
I * 3	zero	0.125	0.125	5000
II * 1	0.04	0.0625	0.1025	4100
II * 2	0.04	0.09375	0.13375	5350
II * 3	0.04	0.125	0.165	6600
III * 1	0.08	0.0625	0.1425	5700
III * 2	0.08	0.09375	0.17375	6950
III * 3	0.08	0.125	0.205	8200

25-OHD_3: 25 Hydroxycholecalciferol VD_3: vitamin D_3 VD: vitamin D

Table (3) : Effect of 25-OHD$_3$, vitamin D$_3$ and their combination on egg production percent.

Items	Factor	Egg production percent 1st month	2nd month	3rd month	4rd month	Entire period
25OH D$_3$	zero mg/kg diet (I)	56.35	51.59^{c}	54.89	46.30	52.28^{b}
	0.04 mg/kg diet (II)	60.98	55.16^{b}	55.29	50.93	55.56^{a}
	0.08 mg/kg diet (III)	59.39	58.07^{a}	57.28	49.87	56.15^{a}
	SE±	1.67	1.53	1.54	1.43	0.80
	P. values	NS	0.012	NS	NS	0.001
Vitamin D$_3$	2500 IU/kg diet (1)	57.94	54.37	55.69	47.88	53.97
	3750 IU/kg diet (2)	60.05	53.70	56.88	50.79	55.36
	5000 IU/kg diet (3)	58.73	56.75	54.89	48.41	54.70
	SE±	1.67	1.53	1.54	1.43	.80
	P. values	NS	NS	NS	NS	NS
Interaction	I * 1 (0.0625 mg/kg diet)*	52.78	48.02^{d}	48.41^{b}	42.86^{d}	48.02^{c}
	I * 2 (0.09375 mg/kg diet)	60.71	44.05^{cd}	57.14^{a}	46.83^{bc}	52.18^{b}
	I * 3 (0.125 mg/kg diet)	55.56	62.70^{a}	59.13^{a}	49.21^{ab}	56.65^{a}
	II * 1 (0.1025 mg/kg diet)	61.11	56.35^{ab}	59.52^{a}	44.84^{c}	55.46^{ab}
	II * 2 (0.13375 mg/kg	58.73	54.76^{abc}	54.37^{ab}	55.95^{a}	55.95^{ab}
	II * 3 (0.165 mg/kg diet)	63.09	54.37^{abc}	51.98^{ab}	51.98^{ab}	55.36^{ab}
	III * 1 (0.1425 mg/kg diet)	59.92	58.73 ^{ab}	59.13^{a}	55.95^{a}	58.43^{a}
	III * 2 (0.17375 mg/kg	60.71	62.30^{a}	59.13^{a}	49.60^{ab}	57.94^{a}
	III * 3 (0.205 mg/kg diet)	57.54	53.17b^{c}	53.57^{ab}	44.05^{c}	52.08^{b}
	SE±	1.67	2.65	2.66	2.47	1.38
	P. values	NS	0.001	0.004	0.001	0.001

$^{a,b,..}$ Means within the same column and parameters with different superscripts are significantly differ ($P \leq 0.05$)

NS: not significant. 25OH D$_3$: 25 Hydroxycholecalciferol *: Total vitamin D content

Table (4): Effect of 25-OHD_3, vitamin VD_3 and their combination on Egg mass per hen per day and average egg weight (g).

Items	Factor	Egg mass per hen per day (gm)					Average egg weight (gm)				
		1st mont	2nd month	3rd month	4rd month	Entire period	1st mont	2nd mont	3rd	4rd mont	Entire period
25OHD_3	zero	27.79	25.26^{c}	27.99	23.59^{b}	26.16^{b}	49.38	48.89	50.94	51.00	50.05^{c}
	0.04	30.54	27.27^{b}	28.60	26.20^{a}	28.15^{a}	50.18	49.41	51.76	51.36	50.68^{b}
	0.08	29.97	29.61^{a}	29.79	25.86^{a}	28.81^{a}	50.47	50.91	51.98	51.74	51.28^{a}
	SE±	0.85	0.78	0.80	0.75	0.41	0.41	0.26	0.27	0.28	0.16
	P.	NS	0.001	NS	.031	0.001	NS	0.000	.0001	NS	0.000
Vitamin D_3	2500	29.04	26.85	28.54	24.64	27.27	50.31	49.31	51.20	51.36	50.43
	3750	30.13	26.91	29.87	26.35	28.32	50.09	49.99	52.57	51.81	51.11
	5000	29.14	28.37	27.98	24.65	27.53	49.64	49.91	50.92	50.94	50.35
	SE±	0.85	0.78	0.80	0.75	0.41	0.41	0.26	0.27	0.28	0.16
	P.	NS	NS	NS	NS	NS	NS	NS	.015	NS	NS
Interaction	I * 1	26.20	23.46^{d}	24.28^{d}	22.22^{c}	24.04^{e}	49.71	48.72	50.05	51.94	50.11^{b}
	I * 2	29.81	21.26^{cd}	29.43^{a}	23.69^{bc}	26.05^{d}	49.03	48.55	51.66	50.55	49.95^{c}
	I * 3	27.35	31.04^{ab}	30.26^{a}	24.87^{bc}	28.38^{ab}	49.41	49.41	51.10	50.50	50.10^{b}
	II * 1	30.50	27.40^{bc}	30.57^{a}	22.70^{bc}	27.79^{ab}	50.59	48.72	51.53	50.48	50.33^{b}
	II * 2	30.09	27.49^{bc}	28.95^{b}	29.33^{a}	28.97^{ab}	51.00	50.18	53.20	52.30	51.67^{a}
	II * 3	31.02	26.92^{bc}	26.29^{c}	26.56^{ab}	27.70^{bc}	48.96	49.33	50.56	51.30	50.04^{c}
	III * 1	30.42	29.70^{ab}	30.78^{a}	28.99^{a}	29.97^{a}	50.64	50.48	52.02	51.64	51.19^{a}
	III * 2	30.49	31.98^{a}	31.22^{a}	26.05a	29.94^{a}	50.25	51.25	52.84	52.57	51.73^{a}
	III * 3	29.01	27.15b	27.38^{b}	22.53^{bc}	26.52^{cd}	50.54	51.00	51.10	51.00	50.91^{a}
	SE±	1.47	1.36	1.38	1.31	0.71	0.71	0.44	0.46	0.48	0.28
	P.	NS	0.001	.002	0.001	0.001	NS	NS	NS	NS	0.017

[a,b,..] Means within the same column and parameters with different superscripts are significantly differ ($P \leq 0.05$)

NS: not significant. 25OH D_3: 25 Hydroxycholecalciferol *: Total vitamin D content

Table (5) : Effect of 25-OHD_3, vitamin VD_3 and their combination on feed intake and feed conversion.

Items	Factor	Feed intake (g/hen/d)					Feed conversion				
		1st month	2nd month	3rd	4rd month	Entire period	1st mont	2nd mont	3rd mont	4rd mont	Entir e
$25OHD_3$	zero	97.08^{b}	92.97^{b}	90.22^{b}	84.08^{b}	91.09^{b}	3.80	4.00^{a}	3.49	3.82	3.78
	0.04	98.60^{b}	97.83^{a}	92.78^{b}	93.03^{a}	95.56^{a}	3.49	3.97^{a}	3.47	3.87	3.70
	0.08	104.47^{a}	98.36^{a}	96.06^{a}	85.58^{b}	96.12^{a}	3.87	3.63^{b}	3.50	3.64	3.66
	SE±	0.96	0.93	1.06	0.84	0.56	0.12	0.12	0.10	0.11	0.06
	P.	0.000	0.0001	0.001	0.0001	0.0001	NS	.048	NS	NS	NS
Vitamin D_3	2500	101.06	95.83	91.36	84.97^{b}	93.31^{b}	3.77	3.89	3.47	3.77	3.73
	3750	100.22	95.97	95.19^{a}	90.58^{a}	95.49^{a}	3.64	3.98	3.44	3.72	3.69
	5000	98.88	97.36	92.50^{a}	87.14^{b}	93.97^{ab}	3.74	3.74	3.56	3.83	3.72
	SE±	0.96	0.93	1.06	0.84	0.56	0.12	0.12	0.10	0.11	0.06
	P.	NS	NS	0.033	0.0001	0.017	NS	NS	NS	NS	NS
Interaction	I * 1	96.08^{de}	87.42^{e}	81.58^{c}	80.17^{ef}	86.31^{e}	3.96	3.99	3.65	3.87	3.87^{a}
	I * 2	101.00^{abc}	88.33d^{e}	94.08^{a}	82.17^{ef}	91.40^{d}	3.69	4.45	3.46	3.72	3.83^{ab}
	I * 3	94.17^{d}	103.17^{a}	95.00^{a}	89.92^{bc}	95.56^{ab}	3.74	3.57	3.36	3.86	3.63^{ab}
	II * 1	101.42^{abc}	98.75^{ab}	97.00^{a}	83.67^{de}	95.21^{bc}	3.60	3.99	3.39	4.01	3.75^{ab}
	II * 2	97.83^{bcde}	98.50^{ab}	93.58^{a}	102.33	98.06^{ab}	3.54	4.04	3.47	3.78	3.75^{ab}
	II * 3	96.56^{cde}	96.25^{bc}	87.75^{b}	93.08^{b}	93.41^{cd}	3.34	3.88	3.57	3.82	3.65^{ab}
	III * 1	105.67^{a}	101.33^{a}	95.50^{a}	91.08^{bc}	98.40^{a}	3.77	3.69	3.36	3.44	3.57^{ab}
	III * 2	101.83^{ab}	101.08^{a}	97.92^{a}	87.25^{cd}	97.02^{ab}	3.68	3.45	3.38	3.66	3.54^{b}
	III * 3	105.92^{a}	92.67^{cd}	94.75^{a}	78.42^{f}	92.94^{cd}	4.15	3.75	3.75	3.81	3.87^{a}
	SE±	1.657	1.619	1.83	1.45	0.96	0.21	.021	0.17	0.18	0.10
	P.	0.006	0.0001	.0001	0.0001	0.0001	NS	NS	NS	NS	.029

$^{a,b,..}$ Means within the same column and parameters with different superscripts are significantly differ ($P \leq 0.05$)

NS: not significant. $25OHD_3$: 25 Hydroxycholecalciferol. *: Total vitamin D content

Table (6) : Effect of 25-OHD_3, vitamin VD_3 and their combination on egg quality traits .

Items	Factor	Shape index	Yolk index	Albumen weight%	Yolk weight %	Haugh unit
$25OHD_3$	zero mg/kg diet (1)	76.50[b]	39.95	57.79[ab]	32.06	105.76
	0.04 mg/kg diet (2)	78.01[a]	39.11	57.22[b]	32.38	104.79
	0.08 mg/kg diet (3)	76.53[b]	39.73	58.27[a]	31.66	105.40
	SE±	0.44	0.28	0.28	0.27	0.63
	P. values	0.022	NS	0.031	NS	NS
Vitamin D_3	2500 IU/kg diet (I)	75.72[b]	39.31	58.15	31.71	104.71
	3750 IU/kg diet (II)	77.76[a]	39.33	57.53	32.23	106.01
	5000 IU/kg diet (III)	77.56[a]	40.15	57.60	32.16	105.23
	SE±	0.44	0.278	0.28	0.27	0.627
	P. values	0.002	NS	NS	NS	NS
Interaction	I * 1 (0.0625 mg/kg diet)*	74.45	39.84	57.85[a]	32.19[b]	105.68
	I * 2 (0.09375 mg/kg diet)	77.60	39.86	57.97[a]	31.76[b]	106.15
	I * 3 (0.125 mg/kg diet)	77.46	40.13	57.53[a]	32.22[b]	105.47
	II * 1 (0.1025 mg/kg diet)	77.37	38.95	58.29[a]	31.29[b]	103.57
	II * 2 (0.13375 mg/kg	77.62	38.59	55.96[b]	33.62[a]	105.51
	II * 3 (0.165 mg/kg diet)	79.04	39.78	57.43[a]	32.24[b]	105.29
	III * 1 (0.1425 mg/kg diet)	75.35	39.13	58.30[a]	31.63[b]	104.89
	III * 2 (0.17375 mg/kg	78.06	39.54	58.67[a]	31.33[b]	106.37
	III * 3 (0.205 mg/kg diet)	76.18	40.53	57.85[a]	32.03[b]	104.93
	SE±	0.76	0.48	0.48	0.47	1.09
	P. values	NS	NS	0.032	0.021	NS

[a,b,..] Means within the same column and parameters with different superscripts are significantly differ ($P \leq 0.05$)

NS: not significant. $25OHD_3$: 25 Hydroxycholecalciferol *: Total vitamin D content.

Table (7) : Effect of 25-OHD$_3$, vitamin VD$_3$ and their combination on liver enzymes activity and some reproduction parameters.

Items	Factor	Reproduction		Liver enzymes	
		Fertility	**Hatchability per fertile**	**AST U/L**	**ALT U/L**
25OHD$_3$	zero mg/kg diet (1)	89.63	93.40	17.78	8.67
	0.04 mg/kg diet (2)	91.85	95.34	13.78	13.56
	0.08 mg/kg diet (3)	93.33	97.67	18.89	12.11
	SE±	1.66	1.40	2.69	2.90
	P. values	NS	NS	NS	NS
Vitamin D$_3$	2500 IU/kg diet (I)	89.63	95.04	21.78	9.22
	3750 IU/kg diet (II)	93.33	95.35	15.22	9.44
	5000 IU/kg diet (III)	91.85	96.02	13.44	15.67
	SE±	1.66	1.40	2.69	2.90
	P. values	NS	NS	NS	NS
Interaction	I * 1 (0.0625 mg/kg diet)*	84.45	92.10	27.00	4.67
	I * 2 (0.09375 mg/kg diet)	93.33	92.86	14.33	6.67
	I * 3 (0.125 mg/kg diet)	91.11	95.24	12.00	14.67
	II * 1 (0.1025 mg/kg diet)	93.33	95.40	13.67	12.67
	II * 2 (0.13375 mg/kg diet)	91.11	95.56	10.33	5.33
	II * 3 (0.165 mg/kg diet)	91.11	95.06	17.33	22.67
	III * 1 (0.1425 mg/kg diet)	91.11	97.62	24.67	10.33
	III * 2 (0.17375 mg/kg	95.55	97.62	21.00	16.33
	III * 3 (0.205 mg/kg diet)	93.33	97.78	11.00	9.67
	SE±	2.87	2.431	4.66	5.02
	P. values	NS	NS	NS	NS

NS: Not Significant.

AST: Aspartate transaminase ALT: Alanine transaminase

25OHD$_3$: 25 Hydroxycholecalciferol *: Total vitamin D content

25-hydroxycholecalciferol-vitamin D_3-egg production.

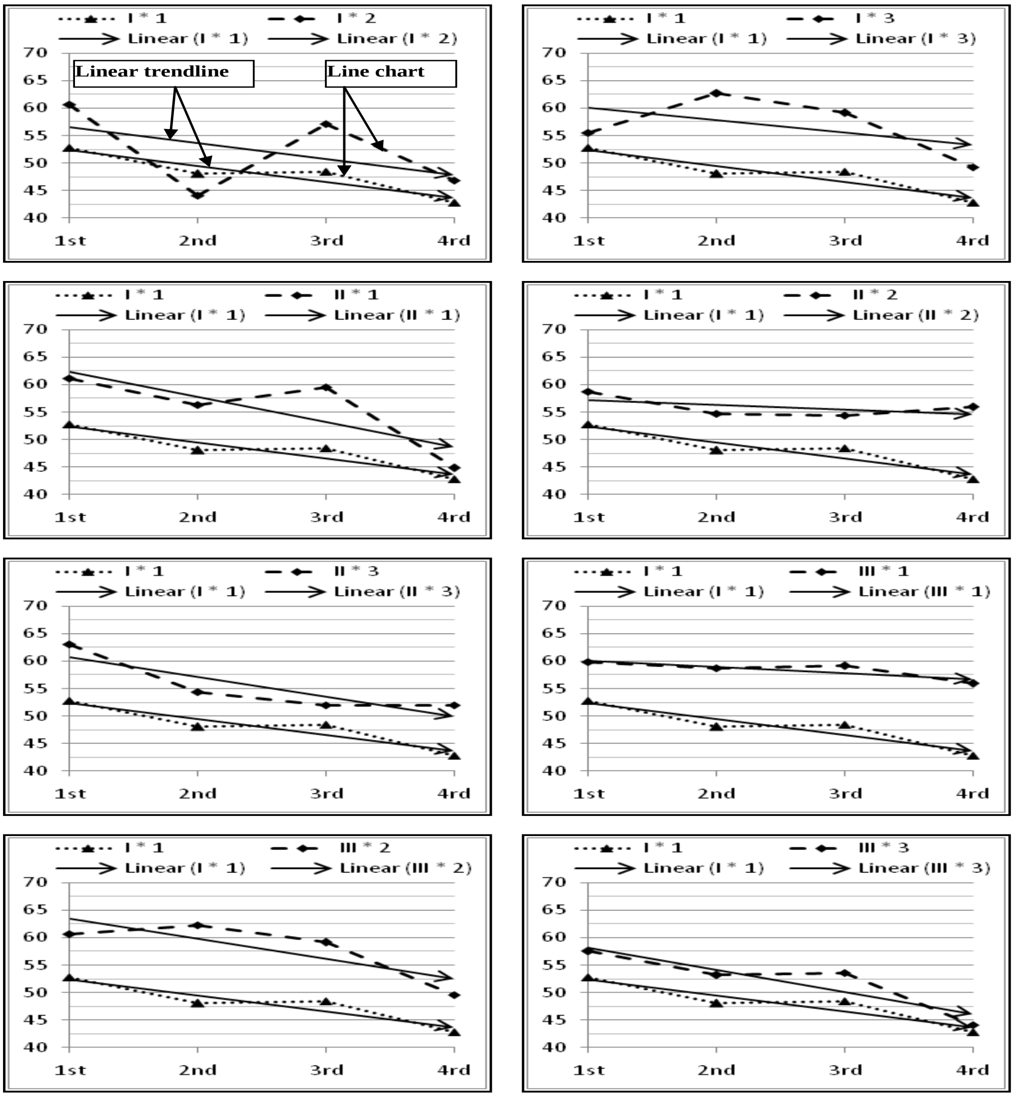

Figure (1): Line chart illustrated egg production percent of control compared with other treatments.

Linear trendline illustrated persistence response of control compared with other treatments.
25OH D_3 : Zero mg/kg diet (I) - 0.04 mg/kg diet (II) - 0.08 mg/kg diet (III)
Vitamin D_3: 2500 IU/kg diet (1) - 3750 IU/kg diet (2)- 5000 IU/kg diet (3)

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الملخص العربى

إستجابات المرحلة المتأخرة للدجاج البياض المغذى على علائق مضاف لها مستويات ومصادر مختلفة من فيتامين (د): 1- الأداء الإنتاجي والتناسلي.

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أجريت هذه التجربة لدراسة تأثير امداد علائق الدجاج البياض بمصادر ومستويات مختلفة من فيتامين (د) على الأداء الأنتاجي والتناسلي خلال مرحلة متأخرة من إنتاج البيض. تم استخدام 3 مستويات من مصدرين مختلفين من فيتامين (د) هما (د3) و 25-هيدروكسي د3. تم اختيار 189 دجاجة بياضة من دجاج المنتزه الذهبي عمر 11 شهر و21 ديك وزعت عشوائياً لـ 9 مجموعات معاملة. تحتوي كل مجموعة معاملة على 21 دجاجة وثلاث ديوك موزعة عشوائياً على 3 مكررات حيث يحتوي كل مكرر على 7 دجاجات وديك. تم تكوين 9 علائق تجريبية حيث كانت العليقة الاولي هي عليقة المقارنة القاعدية واحتوت على 2500 وحدة دولية فيتامين (د3)/كجم عليقة. تم تكوين العليقة 2و3 باضافة 1250و 2500 وحدة دولية من (د3)/كجم عليقة لعليقة المقارنة على الترتيب. تم اضافة 0.04مجم 25-هيدروكسي د3/ كجم عليقة للثلاث علائق السابقة على الترتيب لتكوين العلائق 4 و 5 و 6. ولتكوين العلائق 7 و 8 و 9 تم اضافة 0.08مجم 25-هيدروكسي د3/ كجم عليقة للثلاث علائق الأولى على الترتيب. وقد امتدت التجربة لمدة أربع شهور غذيت الدجاجات فيها تغذية حرة. تم تقدير قياسات الأداء الإنتاجي وصفات جودة البيض والأداء التناسلي ووظائف الكبد. وقد تم الحصول على النتاج التالية:

أدى اضافة 25-هيدروكسي د3 الى تحسين معنوي (عندا مستوى 5٪) في نسبة انتاج البيض خلال الشهر الثاني من التجربة والمدة الكلية للتجربة مقارنة بالعلائق التي لا تحتوي على 25-هيدروكسي د3. زيادة المحتوى الكلي من فيتامين (د) عن توصيات NRC (1994) سبب زيادة معنوية (عندا مستوى 5٪) في نسبة انتاج البيض خلال الشهر الثاني من التجربة مقارنة بعليقة الكنترول. تحسنت بوضوح مثابرة الدجاج البياض وخصوصاً عندما تراوح محتوى العليقة من فيتامين (د) (د3 و25-هيدروكسي د3) من 0.125 الى 0.17375 مجم/كجم عليقة. كما تحسنت صفة كتلة البيض/الدجاجة/اليوم بنفس تحسن النسبة المئوية لأنتاج البيض.

زاد الغذاء المستهلك زيادة معنوية (على مستوى 5٪) بإضافة مصادر مختلفة من فيتامين (د) بمستوى أعلى من توصيات NRC (1994) مقارنة بعليقة المقارنة. لوحظ تحسن معنوياً (على مستوى 5٪) في معامل تحويل الغذاء عندما كان المحتوى الكلي من فيتامين (د) 0.17375 مجم/كجم عليقة مقارنة بعليقة المقارنة. لم يتأثر كل من الأداء الإنتاجي ولا وظائف الكبد بمستوى أو مصدر فيتامين (د).

ونوصي بضرورة إضافة 25-هيدروكسي د3 لعلائق الدجاج البياض مخلوطاً مع فيتامين (د3) بحيث لا يزيد المحتوى الكلي عن 0.17375 مجم/كجم عليقة للمراحل المتأخرة من انتاج البيض.