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PRODUCTIVE AND PHYSIOLOGICAL RESPONSES TO VITAMIN D3 AND C SUPPLEMENTATION IN BANDARA HENS

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ABSTRACT

An experiment was conducted to determine the effect of dietary vitamin D3 (0, 300 or 600 IU) and vitamin C supplementation (0, 250 or 500 mg / kg of ration) on some physiological responses and productive performance of Bandara strain. A total number of 270 hens of Bandara local strain aged 22 weeks were selected and divided randomly into 3 experimental groups (each of 90 hens) according to vitamin D3 supplementation (0, 300 or 600 IU/ kg of ration) and each group was divided into 3 subgroups (each of 30 hens) according to vitamin C supplementation (0, 250 and 500 mg/kg of ration). Results obtained could be summarized as follows: Pullets fed combination of 300 IU vitamin D3 and 250 mg of vitamin C /Kg ration increased significantly egg Shell weight percentage and thickness (13.51% and0.34 mm, respectively) than those of control (11.85% and 0.31 mm, respectively).

Pullets fed 600 IU of vitamin D3 and 500 mg vitamin C /Kg ration improved significantly feed conversion (3.07 g feed/g egg), rate of egg production (67.8%/hen/day), egg mass (33.03 gm/hen/day), fertility percentage (94.79%), Calcium balance (68.53%), total calcium absorption, absorption/cm length or per gram dry matter / hour through the intestinal parts (22.89 mg/hr, 0.467 mg/cm and 8.33 mg/gm, respectively) and increased significantly plasma calcium and inorganic phosphorus (21.75 and 5.80 mg/dl, respectively) compared with those of other treatments or control (18.74 and 5.65 mg/dl, respectively). Pullets fed 300 IU vitaminD3 and 500mg vitamin C had significantly the highest percentage of hatchability (94.59%) compared with those of control (88.90%). Pullet's age had significant effect on feed intake, feed conversion, egg production, egg weight, mass, proportional shell weight, shell thickness, plasma calcium and inorganic phosphorus. Supplemented diet with Vitamins D3 and C at a level of (600 IU and 500 mg/kg, respectively) seemed to be adequate to achieve the favorable results and may be recommended for the economic point view.

INTRODUCTION

Ascorbic acid (AA) has an important metabolic role as a result of its reducing properties and function as an election carrier, it's not an essential nutrient for poultry species because they possess the gulonolactone oxidase enzyme which is part of the biosynthetic pathway and is lacking in humans, other primates and some other species. However, there has been considerable interest in a possible nutritional role for AA on the basis that (I) endogenous synthesis may not be adequate to meet the full needs of poultry at all time or (II) requirements (AA) may be increased under certain circumstances (e.g. stressful conditions such as environmental, nutritional, pathological) Keshavarz (1996) and Whitehead and Keller (2003). Many inconsistent reports appear in the literature concerning the effect of supplemental ascorbic acid on the performance of laying hens. For example, several investigators reported improvement in shell quality (Volker and Weiser, 1993; Zapata and Gernat, 1995 and Whitehead and Keller, 2003). Albumen quality (Benabdeljelil and Jensen, 1990; and Whitehead and Keller, 2003). Egg production (Sushil *et al.,* 1998a,b; El-Gendi *et al.,* 1999). Calcium metabolism (Orban *et al.,* 1993). Feed intake and feed conversion (Ubosi and Gandu, 1995 and Balnava and Muheereza, 1997). Mortality (Bell and Marion, 1990; Ubosi and Gandu, 1995 and Sushil *et al.,* 1998a,b) due to supplemented the hens with ascorbic. The mechanism that underlies the beneficial effect of vitamin C on shell quality has not been elucidated. Volker and Weiser (1993) speculated that, the beneficial effect of vitamin C may be due to its involvement in hydroxylation of 25–hydroxycholecalicferol (25 -OH-D_3) to 1,25 dihydroxycholicalciferol 1,25 $(OH)_2 D_3$ and hydroxylation of proline and lysine, which are involved in collagen biosynthesis. Also, it is well documented that total calcium absorption deposition is controlled by the active metabolite of cholicalciferol 1,25 dihydroxycholicalciferol. Cholicalciferol (vitamin D_3 undergoes two sequential hydroxylation in liver and kidney to form 25 hydroxycholicalciferol and secondarily 1,25 dihydroxycholicalciferol (Abe *et al.,* 1982 and Soares, 1984). 1,25 $(OH)_2 D_3$ is required for the regulation of calcium absorption and excretion and can initiate the mobilization of calcium reserves from the bones to provide adequate calcium for the production of egg shell if needed (Abe *et al.,* 1982). It may be accepted that dietary cholicalciferol can play an important role in controlling blood level of 1,25 dihydroxycholicalciferol and sequentially in stimulating calcium absorption and deposition. In laying hens, many reports on the influence of supplemental vitamin D_3 on weight gain, feed consumption, feed conversion, egg weight and egg mass. Some reports indicated no effect in the previously mentioned parameters (Keshavarz, 1996 and El-Afifi and Abu Taleb, 2002). Whereas others have shown improvement in egg shell quality and calcium absorption (Abe *et al.,* 1982; Soares, 1984 and Wood *et al.,* 1998). The present study was aimed to improve productive performance, calcium utilization and the rate of calcium absorption in Bandara local strain by vitamin D3 and C supplementation.

MATERIALS AND METHODS

The present study was carried out at Gemmizah Poultry Research Station, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt.

A total number of 270 hens of Bandara local strain aged 22 weeks were housed in individual cages (45 x 30 x 42 cm). Hens were randomly divided into 3 experimental groups (each of 90 hens) according to vitamin D3 level (0, 300 or 600 IU/ kg) on top of the control level which is 2200 IU vit D3/ kg diet (Table1) and each group divided into 3 subgroups (each of 30 hens) according to vitamin C level (0, 250 and 500 mg/kg). Birds were fed ad-libtum on basal layer ration of nutritive value indicated in (Table, 1) according to NRC (1994).

Table 1: Composition and calculated analysis of the basal laying ration.

Ingredients	%
Yellow corn	63.14
Soybean meal (44%)	27.10
Vitamins & Minerals (premix)*	0.30
DL- Methionine	0.06
Limestone	7.60
DI-calcium phosphate.	1.50
Salt	0.30
Total	100.00
Calculated analysis:	
Crude Protein %	17.52
M.E (kcal / kg)	2741
Calcium %	3.3
Total phosphorus %	0.64
Available phosphorus %	0.41
Lysine %	0.89
Methionine %	0.34
Methionine + Cystine %	0.61

*** each one kilogram of diet contains : vit A 12000 IU; vit D3 2200 IU; vit E 10 mg ; vit K3 2 mg; vit B1 1mg; vit B2 4 mg; vit B6 1.5 mg; vit B12 10 µg; Niacin 20 mg; Pantothinic acid 10 mg; Folic acid 1 mg; Biotin 50 µg; Choline 250 mg; Copper 10 mg; Iron 30 mg; Manganese 55 mg; Ziinc 50 mg ; Iodine 1 mg; Selenium 0.1 mg and Cobalt 0.1 mg.**

The productive performance parameters estimated were egg production, egg weight, egg mass, feed intake and conversion. Shell weight percentage and thickness were considered as parameters detecting the egg shell quality. Data were calculated at 28th, 36th , 44th 52nd weeks of pullet's age. Egg produced were collected and incubated at 36 and 52 weeks of pullet's age. Fertility percentage was estimated as percentage of fertile eggs to the number of set eggs. Incubated eggs were weighed at 0 and 18 days of incubation for the determination of egg weight loss percentage. Total of 20 newly hatched chicks were randomly chosen from each experimental group and weighed at hatch.

At the peak of egg production, five pullets from each treatment were randomly chosen for the determination of calcium balance. The proximate analysis of tested materials, experimental diets and dried excreta were carried out according to the official methods (A.O.A.C, 1990).

Calcium retention as percentage of ingestion was calculated as Ca intake (g) – Ca in excreta (g) / Ca intake X 100.

Plasma calcium and inorganic phosphorus were colorimetrically determined at 28th, 36th, 44th and 52nd week of age using kits.

Calcium absorption rate in-vitro throughout the various intestinal parts (duodenum – jejunum and ileum) was estimated at the end of the experimental period using the intestinal sac methods (Madge, 1975) with some modification applied by El-Gendi (1985). The data were then calculated as rate of calcium absorption per gm dry matter or 1 cm length of various intestinal parts.

Data were analyzed using program (SPSS, 1997). A linear model including the effect of treatments, pullet's age and all possible interactions were used for analyzing such data. Significant tests were verified by **Duncan**'s new multiple range test (1955).

RESULTS AND DISCUSSION

1. Egg production, egg weight and egg mass:

Data obtained in table (2) showed that Pullets fed (600IU vitamin D3+500mg vitamin C /Kg ration) were significantly increased in the rate of egg production (67.8%/hen/day) and egg mass (33.03 gm/hen/day) when compared with other treatments applied or control group (600.5%/hen/day and 29.08 gm/hen/day, respectively). Similar results were observed by Keshavarz (1996), Newman and Leseon (1999) and Abdel Galil and Abdel Samad (2004), they found that, vitamin C + D_3 supplementation improved egg production. However, analysis of variance showed insignificant effect on average egg weight due to treatments applied. These results agreed with those reported by Kassim and Norzihe (1995) and Keshavarz (1996) who did not find any significant effect of dietary supplementation with vitamin C and D_3 on egg weight.

Table 2: Means and standard error of egg production % (hen/day) , egg weight and egg mass as affected by the different dietary treatments.

Item		Egg/hen/day %	Egg weight (gram)	Egg mass (gram/hen/day)
Treatments:				
Vit. D3(IU)	Vit. C (mg)	***	NS	***
0	0	$60.5^{e} \pm 0.01$	48.71 ± 0.33	$29.08^{d} \pm 0.39$
0	250	$63.1^{d} \pm 0.01$	48.71 ± 0.41	$31.47^{bc} \pm 0.47$
0	500	$64.1^{cd} \pm 0.01$	48.67 ± 0.39	$31.29^{c} \pm 0.46$
300	0	$65.3^{abcd} \pm 0.01$	48.55 ± 0.34	$31.48^{bc} \pm 0.41$
600	0	$66.7^{abc} \pm 0.01$	48.62 ± 0.41	$32.28^{abc} \pm 0.49$
300	250	$64.9^{bcd} \pm 0.01$	48.22 ± 0.38	$31.41^{bc} \pm 0.40$
300	500	$67.5^{ab} \pm 0.01$	48.69 ± 0.39	$32.87^{ab} \pm 0.47$
600	250	$65.3^{abcd} \pm 0.01$	48.80 ± 0.31	$31.67^{abc} \pm 0.38$
600	500	$67.8^{a} \pm 0.01$	49.13 ± 0.41	$33.03^{a} \pm 0.41$
Pullets' age (week):		***	*	***
28		$62.1^{c} \pm 0.01$	$44.65^{d} \pm 0.19$	$28.36^{c} \pm 0.28$
36		$73.4^{a} \pm 0.01$	$47.30^{c} \pm 0.17$	$36.04^{a} \pm 0.26$
44		$65.9^{b} \pm 0.01$	$51.60^{b} \pm 0.16$	$34.55^{b} \pm 0.22$
52		$58.6^{d} \pm 0.01$	$51.70^{a} \pm 0.19$	$37.62^{c} \pm 0.31$

Means with different superscripts in the same column are significantly different.

Egg production rate and egg weight significantly increased by advancing age reaching their maximum values at the 36^{th} and 52^{nd} weeks of pullet's age (73.4%/hen/day and 51.70 gm, respectively).These results agreed with those of Samak (2001) who observed that, bird's age had highly significant effect on both of egg production rate and egg weight.

Average egg mass mostly increased as pullets grew older reaching its maximum value at the 36th week of pullet's age (36.04 gm/hen/day), thereafter it decreased up to the end of the experimental period (27.62 gm/hen/day).

2. Feed intake and conversion:

Data presented in table (3) showed insignificant variation between treatments in feed intake, however, highly significant differences were found in feed conversion due to treatment applied. feed conversion was significantly (P<0.001) improved in Pullets fed 300IU or 600 of vitamin D3 with 500 mg of vitamin C /Kg ration (3.07 gm ration / gm egg) when compared with other treatments. These results agree with the findings of Aburto and Britton (1996) who stated that feed conversion improved as the level of vitamin D3 increased. Results also supported by those of Abdel Galil and Abdel Samad, 2004). They found that ascorbic acid supplementation improved feed intake and feed conversion efficiency.

Table 3: Means and standard error of feed intake and conversion as affected by the different dietary treatments.

Item		Feed intake (gm/hen/day)	Feed conversion (gm feed/ gm egg)
Treatments:		NS	***
Vit. D3(IU)	Vit. C(mg)		
0	0	98.45 ± 0.37	3.55 [a] ± 0.06
0	250	98.65 ± 0.34	3.26 [b] ± 0.06
0	500	98.60 ± 0.33	3.32 [b] ± 0.06
300	0	98.19 ± 0.39	3.20 [bc] ± 0.06
600	0	98.35 ± 0.36	3.20 [bc] ± 0.06
300	250	98.94 ± 0.35	3.22 [bc] ± 0.06
300	500	98.52 ± 0.35	3.07 [c] ± 0.06
600	250	98.04 ± 0.38	3.18 [bc] ± 0.06
600	500	98.25 ± 0.35	3.07 [c] ± 0.06
Pullets' age (week):		*	***
28		93.60 [d] ± 0.12	3.57 [a] ± 0.04
36		101.20 [b] ± 0.13	2.90 [d] ± 0.04
44		102.40 [a] ± 0.06	3.02 [c] ± 0.04
52		96.37 [c] ± 0.04	3.43 [b] ± 0.04

Means with different superscripts in the same column are significantly different.

Analysis of variance revealed significant differences (p<0.05) in the amount of feed intake due to pullet's age. Feed intake increased gradually as pullets grew older reaching its maximum value at the 44th week of pullet's age (102.40 gm/hen/day) then decreased toward the end of the experimental period reaching (96.37gm/hen/day) at the 52nd week. In addition inspection of data obtained indicated that feed conversion improved consistently with increasing bird's age reached its best value at the 44th week of their age (4.04g feed/g egg) after which its slightly and insignificant increased. This may be attributed to the decrease in egg production compared with the amount of feed consumed.

3.Proportional egg shell weight and shell thickness:

Results obtained (Table, 4) showed that pullets fed 300 IU vitamin D3 and 250 mg of vitamin C/ kg ration significantly increased proportional egg Shell weight and shell thickness (13.51% and0.34 mm, respectively) followed by those fed 300 IU vitamin D3 (13.14% and 0.34 mm, respectively) compared with other treatments applied or those of control group (11.85% and 0.31 mm, respectively). This may be attributed to that ascorbic acid is involved in calcium metabolism, possibly enhancing intestinal calcium absorption or reabsorption of bone to release calcium ions. Similar results were observed by Dorr and Balloun, 1976 and Weiser *et al.,* (1988 a, b) who indicated that, ascorbic acid may be stimulatory or synergistic with 1.25 dihydroxycholecaliferol (1,25 $(OH)_2$ D_3) production and also may be involved in bone metabolism of calcium.

Table 4: Means and standard error of proportional egg shell weight and shell thickness as affected by the different dietary treatments.

Item		Shell weight (%)	Shell thickness (mm)
Treatments:		**	**
Vit. D3 (IU)	Vit. C (mg)		
0	0	11.85 ^{d} ± 0.66	0.31 ^{b} ± 0.004
0	250	12.58 ^{cd} ± 0.46	0.31 ^{b} ± 0.005
0	500	12.77 ^{c} ± 0.30	0.31 ^{a} ± 0.009
300	0	13.14 ^{b} ± 0.25	0.34 ^{b} ± 0.004
600	0	12.37 ^{cd} ± 0.29	0.32 ^{a} ± 0.009
300	250	13.51 ^{a} ± 0.58	0.34 ^{ab} ± 0.007
300	500	12.04 ^{d} ± 0.51	0.33 ^{ab} ± 0.007
600	250	12.14 ^{cd} ± 0.48	0.33 ^{ab} ± 0.007
600	500	12.33 ^{cd} ± 0.53	0.32 ^{ab} ± 0.009
Pullets' age (week):		**	**
28		13.42 ^{b} ± 0.20	0.34 ^{a} ± 0.005
36		13.98 ^{a} ± 0.18	0.33 ^{a} ± 0.005
44		12.60 ^{c} ± 0.17	0.32 ^{b} ± 0.005
52		10.09 ^{d} ± 0.33	0.31 ^{b} ± 0.005

Means with different superscripts in the same column are significantly different.

Analysis of variance showed high significant effect ($p<0.01$) on either egg shell percentage or thickness due to pullet's age.

Proportional egg shell weight reached its maximum value at 36th week of pullet's age (13.98%) then decreased gradually reached its minimum value at the end of the experimental period (10.09%). However, egg shell thickness was (0.34mm) at the 28th week of age then decreased by advancing age reached its minimum value at the end of the experimental period (0.31mm).

4.Egg weight loss percentage, Fertility, hatchability and chick's weight:

Data obtained (Table, 5) showed insignificant variation between different treatments applied on either egg weight loss percentage or chick's weight at hatch. However, eggs of control group had the highest rate of egg weight loss (16.39%) and the lowest average of hatched chick's weight (32.62gm). Eggs of pullets fed 300IU vitamin D3 and 250mg of vitamin C/kg ration showed the lowest value of egg weight loss (12.17%). On the other hand, the highest averages of hatched chick's weight were recorded in eggs of pullets fed 250mg of vitamin C (34.02gm).

Table 5: Means and standard error of rate of hatching egg weight (gm), egg weight loss % , fertility % , hatchability % and hatched chick's weight (gm) as affected by the different dietary treatments.

Item		Egg weight (gram)	Egg weight loss (%)	Fertility (%)	Hatchability (%)	Chick's weight (gram)
Treatments:						
Vit. D3 (IU)	Vit. C (mg)	NS	NS	***	***	NS
0	0	54.03 ± 1.71	16.39 ± 2.40	86.36 ^{d} ± 2.15	88.90 ^{d} ± 0.70	32.88 ± 1.34
0	250	51.87 ± 2.67	12.55 ± 1.34	89.68 ^{c} ± 0.99	89.68 ^{d} ± 0.66	34.02 ± 0.58
0	500	51.40 ± 1.37	14.91 ± 1.22	94.72 ^{a} ± 0.73	93.05 ^{b} ± 0.61	33.44 ± 0.15
300	0	49.27 ± 1.38	15.50 ± 1.75	92.56 ^{b} ± 1.37	92.77 ^{b} ± 1.81	32.62 ± 0.40
600	0	50.92 ± 1.90	13.17 ± 0.58	92.21 ^{b} ± 0.44	94.50 ^{a} ± 0.73	32.98 ± 0.14
300	250	50.82 ± 1.51	12.17 ± 1.06	89.53 ^{c} ± 3.74	88.80 ^{d} ± 1.54	33.24 ± 0.15
300	500	54.73 ± 0.95	16.17 ± 1.69	92.40 ^{b} ± 0.77	94.59 ^{a} ± 0.40	33.83 ± 0.14
600	250	51.43 ± 1.64	14.28 ± 0.85	92.13 ^{b} ± 0.46	91.51 ^{c} ± 0.53	33.05 ± 0.44
600	500	52.10 ± 1.48	14.28 ± 0.88	94.79 ^{a} ± 0.47	89.03 ^{d} ± 1.02	33.82 ± 0.40
Pullets' age (week):		NS	NS	NS	NS	NS
36		51.33 ± 0.86	13.77 ± 0.54	91.93 ± 0.64	91.56 ± 0.88	33.13 ± 0.32
52		52.35 ± 0.72	14.99 ± 2.93	91.27 ± 0.69	91.13 ± 0.82	33.51 ± 0.70

Means with different superscripts in the same column are significantly different.

Table 6: Calcium retention (gm/day) and calcium balance (%) as affected by the different dietary treatments.

Item		Ca Intake (gm/day)	Ca Out (gm/day)	Ca Retained (gm/day)	Ca balance %
Treatments:					
Vit. D3 (IU)	Vit. C (mg)				*
0	0	3.28	1.67	1.61	49.10 ^{a} ± 1.820
0	250	3.25	1.34	1.91	58.80 ^{bc} ± 1.640
0	500	3.46	1.31	2.15	62.13 ^{bcd} ± 2.080
300	0	3.60	1.53	2.07	57.50 ^{b} ± 0.145
600	0	3.38	1.22	2.10	62.10 ^{bc} ± 0.628
300	250	3.88	1.44	2.44	62.80 ^{bcd} ± 0.967
300	500	2.97	1.02	1.95	65.65 ^{cd} ± 1.052
600	250	3.76	1.28	2.46	65.95 ^{cd} ± 0.655
600	500	2.86	0.90	1.96	68.53 ^{d} ± 0.678

Means with different superscripts in the same column are significantly different.

Regardless of the effect of treatments applied, pullets fed 600IU of vitamin D3 and 500mg of vitamin C/kg of ration had significantly the highest value of fertility percentage (94.79%), followed by those fed 500mg of vitamin C/kg of ration (94.72%) when compared with other treatments applied, while pullets of control group had significantly the lowest value (86.36%).). Similar results were obtained by Abdel Galil and Abdel Samad (2004) who reported that supplementing vit. C increased fertility and hatched chick's weight.

Pullets fed either 300IU vitaminD3 and 500mg vitamin C or 600IU of vitamin D3/kg ration had significantly the highest percentages of hatchability (94.59 and 94.50%, respectively) compared with those of other treatments or control (88.90%).

Analysis of variance showed insignificant effects on egg weight losses, fertility, hatchability percentages and chick's hatched weight.

5.Calcium balance:

Table (6) showed that, the amount of Ca retained and Ca balance % were improved by supplementing laying hens diets with different levels of vitamin C, vitamin D_3 or mixture of both compared with control group. Hens fed diets supplemented with a mixture of vitamins D3 and C at levels of (300 IU + 250 mg), (300 IU + 500 mg), (600 IU + 250 mg) and (600 IU + 500 mg)/kg of ration were better in Ca balance (62.80, 65.65, 65.95 and 68.53%, respectively) followed by hens fed diets supplemented with vitamin C at a level of 250 mg (58.80) or 500 mg (62.13%), then by those fed 300 IU (57.50%) and 600 IU of vitamin D_3 (62.10%), respectively when compared with control group (49.10%).

The improvement in calcium absorption in vitamin D_3 + C feeding groups indicated the complementary effect between vitamin D_3 and vitamin C in enhancing calcium absorption therefore both of them must be present in the proper amount for increasing calcium absorption. These results were supported by Soliman (2002) who found that, supplementing laying hens diets with vitamin C improved the amount of Ca retained and Ca balance %. Orban *et al.,* (1993) and Zapata and Gernat (1995) reported that, ascorbic acid involvement in calcium metabolization by enhancing absorption of intestinal calcium. Other reports have indicated that, ascorbic acid promotes mineral mobilization resulting in increased plasma calcium (Door and Balloun, 1976). The improvement in calcium absorption due to cholicalciferol supplementation confirms that the level of cholicalciferol in the basal diet was insufficient for optimal calcium absorption of Bandara laying hens. Therefore increasing dietary content of cholicalciferol enhances calcium absorption. These results supported by Wood *et al.,* (1998) who reported that, the sub optimal cholicalciferol status may be an important factor in reducing plasma 1.25 dihydroxycholicalciferol and intestinal calcium absorption.

6.Mode of calcium absorption (in-vitro) throughout the different parts of the small intestine:

Data in (table, 7) showed significant variation between treatments applied in either total calcium absorption, the amount of calcium absorption/cm or per gram dry matter.

Total calcium absorption, absorption/cm length or per gram dry matter per hour through the intestinal parts were significantly higher in pullets fed 600 IU of Vitamin D_3 and 500 mg of vitamin C per kg of ration (22.89 mg/hr, 0.467 mg/cm and 8.33 mg/gm, respectively) compared with other treatments or control (16.67mg/hr, 0.313 mg/cm and 4.89 mg/gm, respectively). On the other hand, pullets fed 250 mg vitamin C/kg ration had the lowest values of total calcium absorption, absorption/cm length or per gram dry matter (15.33 mg/hr, 0.300 mg/cm and 4.07 mg/cm, respectively).

Pullets fed 600 IU of vitamin D_3 /kg ration were significantly higher in calcium absorption traits (19.89 mg/hr, 0.383 mg/cm and 5.14 mg/gm, respectively) when compared with those fed 300 IU vitamin D_3/kg (18.22 mg/hr, 0.372 mg/cm and 4.50 mg/gm, respectively) or control. These results indicated that, total calcium absorption per gram dry matter increased as dietary level of vitamin D_3 increased. Similar results were observed by Radwan (1990) and El-Gendi *et al.,* (1999). This may be lead to the conclusion that, vitamin D_3 increased the rate of diffusion of calcium across the intestinal wall (Radwan, 1980).

Ileum had significantly the highest values of total calcium absorption (26.26 mg/hr), absorption per cm (0.489 mg/cm) and per gram dry matter (6.78 mg/gm) than that of either duodenum or jejunum. This was quite true in all experimental groups. Similar results were observed by El-Sayed (1995) and Samak (2001). From the previously mentioned results it could be concluded that, ileum is the main site of calcium absorption since the amount of total calcium absorption and the rate of calcium absorption/cm length and per gm dry matter were found to be the highest in ileum portion compared to the other two portions of the small intestine (duodenum and jejunum). This may be due to the relative higher number of villi found in ileum compared with those in either duodenum or jejunum. This results agree with those of El-Sayed (1995) and Samak (2001). They found that, the total calcium absorption varied significantly between differed intestinal parts, being at its maximum rate in the ileum and jejunum and at the minimum rate in the duodenum.

Vitamin D_3 metabolites influence calcium transport by action on the permeability of cell surface to calcium (Wassermann and Taylor, 1969). They found that, the main sites of vitamin D_3 action on calcium absorption were duodenum and upon jejunum, respectively.

7.Plasma levels of calcium and inorganic phosphorus:

Data presented in table (8) showed that, plasma calcium and inorganic phosphorus levels significantly affected by treatments applied. Pullets fed 600 IU of vitamin D3 and 500 mg of vitamin C Per Kg of ration had the highest levels of plasma calcium and inorganic phosphorus (21.75 and 5.80 mg/dl, respectively) compared with those of other treatments or control (18.74 and 5.65 mg/dl, respectively). These results agree with those of Stevens and Blair (1983). It could be suggested that vitamin D3 metabolites accelerate calcium absorption in the intestine of laying chicken. Analysis of variance showed highly significant effect ($p<0.001$) on plasma calcium and inorganic phosphorus.

Treatments applied had significant effect ($p<0.001$) on the ratio between calcium and inorganic phosphorus (C/P). Pullets fed 300 IU of vitamin D3 were significantly higher in C/P ratio (4.34) when compared with other treatments. On the other hand, pullets of control were significantly had the lowest value (3.34) followed by that fed 600 IU of vitamin D3 and 500 mg of vitamin C Per Kg of ration (3.79).

Plasma calcium level increased after the 28th week of age reached its maximum value (21.77 mg/dl) at the 36th week then decreased gradually reached its minimum value (19.03 mg/dl) at the 52nd week of age. However, plasma inorganic phosphorus reaching its maximum value (5.44 mg/dl) at the 52nd week of age. The ratio between calcium and inorganic phosphorus decreased with the advancing of age reaching its minimum value at the 52nd week of hen's age.

These results agreed with those of EL-Gendi *et al.,* (1999) and Samak (2001) who reported that, the serum calcium and phosphorus contents of laying hens were influenced by age.

8.Economical efficiency:

Results of economic efficiency for pullets of different treatments are shown in Table (9). Results obtained indicated that pullets fed mixture of vitamins D3 and C at a level of (300 or 600 IU and 500 mg, respectively) showed the highest economic efficiency (15%), followed by those fed 600 IU /kg of ration of vitamins D3 (13.7%) when compared with other treatments. Pullets of control group recorded the lowest value of the economic efficiency (6.9%).

From the previously mentioned results it could be concluded that, the inclusion rate of vitamins D3 and C at a level of (300 or 600 IU and 500 mg, respectively) per each kg of ration seemed to be adequate to achieve the favorable results and would be more economic.

Table 7: Means and standard error of total calcium absorption (mg/hr), calcium absorption (mg / cm length) and calcium absorption (mg/ gm dry matter) as affected by the different dietary treatment.

Item		Total calcium absorption (mg/hr)	Calcium absorption (mg/cm length)	Calcium absorption (mg/gm dry matter)
Treatments:		***	***	**
Vit. D3 (IU)	Vit. C (mg)			
0	0	16.67 ^{ef} ± 0.59	0.313 ^{ef} ± 0.022	4.89 ^{de} ± 0.21
0	250	15.33 ^{f} ± 0.59	0.300 ^{f} ± 0.022	4.07 ^{f} ± 0.21
0	500	17.78 ^{de} ± 0.59	0.340 ^{df} ± 0.022	5.89 ^{bc} ± 0.21
300	0	18.22 ^{cde} ± 0.59	0.372 ^{de} ± 0.022	4.50 ^{ef} ± 0.21
600	0	19.89 ^{bc} ± 0.59	0.383 ^{cd} ± 0.022	5.14 ^{de} ± 0.21
300	250	18.22 ^{cde} ± 0.59	0.431 ^{bc} ± 0.022	4.90 ^{de} ± 0.21
300	500	19.33 ^{cd} ± 0.59	0.393 ^{cd} ± 0.022	5.38 ^{cd} ± 0.21
600	250	21.44 ^{ab} ± 0.59	0.504 ^{a} ± 0.022	6.02 ^{b} ± 0.21
600	500	22.89 ^{a} ± 0.59	0.467 ^{ab} ± 0.022	8.33 ^{a} ± 0.21
Intestinal parts:		***	***	**
Duodenum		9.19 ^{c} ± 0.34	0.341 ^{b} ± 0.013	4.69 ^{b} ± 0.12
Jejunum		21.15 ^{b} ± 0.34	0.339 ^{b} ± 0.013	4.91 ^{b} ± 0.12
Ileum		26.26 ^{a} ± 0.34	0.489 ^{a} ± 0.013	6.78 ^{a} ± 0.12

Means with different superscripts in the same column are significantly different.

Table 8: Means and standard error of plasma calcium (mg / dl), inorganic phosphorus (mg/dl) and calcium phosphorus ratio as affected by the different treatments.

Item		Calcium (mg/dl)	Inorganic phosphorus(mg/dl)	Calcium/ phosphorus ratio
Treatments:				
Vit. D3 (IU)	Vit. C (mg)	***	***	***
0	0	18.74^{e} ± 0.18	5.65^{a} ± 0.14	3.34^{d} ± 0.11
0	250	19.28^{d} ± 0.18	4.90^{b} ± 0.14	4.04^{bc} ± 0.11
0	500	19.50^{d} ± 0.18	4.80^{b} ± 0.14	4.13^{abc} ± 0.11
300	0	20.71^{bc} ± 0.18	4.84^{b} ± 0.14	4.34^{ab} ± 0.11
600	0	20.54^{c} ± 0.18	5.05^{b} ± 0.14	4.13^{abc} ± 0.11
300	250	19.77^{d} ± 0.18	5.20^{b} ± 0.14	3.85^{c} ± 0.11
300	500	21.09^{b} ± 0.18	5.22^{b} ± 0.14	4.18^{abc} ± 0.11
600	250	20.94^{bc} ± 0.18	5.07^{b} ± 0.14	4.20^{abc} ± 0.11
600	500	21.75^{a} ± 0.18	5.80^{a} ± 0.14	3.79^{a} ± 0.11
Pullets' age (week):		***	*	***
28		20.74^{b} ± 0.12	5.14^{b} ± 0.10	4.13^{b} ± 0.07
36		21.77^{a} ± 0.12	5.00^{c} ± 0.10	4.44^{a} ± 0.07
44		19.48^{c} ± 0.12	5.09^{bc} ± 0.10	3.90^{c} ± 0.07
52		19.03^{d} ± 0.12	5.44^{a} ± 0.10	3.53^{d} ± 0.07

Means with different superscripts in the same column are significantly different.

Table 9: Economic efficiency as affected by treatments applied.

Treatment	Vit D_3 (IU)	0	0	0	300	600	300	300	600	600
Item	Vit C (mg)	0	250	500	0	0	250	500	250	500
Fixed cost / hen		12	12	12	12	12	12	12	12	12
Management / hen LE[1]		2	2	2	2	2	2	2	2	2
Total feed cost / hen LE		25.1	25	25.1	24.9	25.1	25.1	25	24.9	25
Total cost / hen LE		39.1	39	39.1	38.9	39.1	39.1	39	38.9	39
Total No. of egg / hen		110	115	117	119	121	118	123	119	123
Total egg price / hen LE[2]		33	34.5	35.19	35.7	36.3	35.4	36.9	35.7	36.9
Price of sold bird LE		9	9	44.1	9	9	9	9	9	9
Total revenue / hen LE		42	43.5	5	44.7	45.3	44.4	45.9	44.7	45.9
Net revenue / hen LE[3]		2.9	4.5	11.3	5.8	6.2	5.3	6.9	5.8	6.9
Economic efficiency %		6.9	10.3		13	13.7	11.9	15	13	15

1- Included medication vaccines and sanitation
2- The price of an egg at the time of experiment = 30 pt.
3- Net revenue per unit of total costs.
4- Economic efficiency % = Net revenue / total revenue X 100

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الاستجابة الإنتاجية والفسيولوجية لإضافة فيتامين د٣ و ج في دجاج البندرة

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أجريت هذه التجربة لدراسة تأثير إضافة كل من فيتامين د٣ بالمستويات صفر ، ٣٠٠ ، ٦٠٠ وحدة دولية وفيتامين ج بالمستويات صفر ، ٢٥٠ ، ٥٠٠ ملليجرام لكل كيلوجرام من العليقة المقدمة للطيور وعمر الطيور على الاستجابة الفسيولوجية والأداء الإنتاجي لدجاج البندرة تم اختيار عدد ٢٧٠ دجاجة عشوائيا من سلالة البندرة المحلية عند عمر ٢٢ أسبوع حيث غذيت حتى الشبع على عليقة إنتاجية ، قسمت الطيور إلى ثلاث مجموعات تجريبية متساوية (٩٠ دجاجة في كل مجموعة) تبعا لمستوى إضافة فيتامين د٣ (صفر ، ٣٠٠ أو ٦٠٠ وحده دولية) ، وقسمت طيور كل مجموعة إلى ثلاث مجموعات متساوية (٣٠ دجاجة في كل مجموعة) تبعا لمستوى إضافة فيتامين ج (صفر ، ٢٥٠ أو ٥٠٠ ملليجرام) وكانت أهم النتائج المتحصل عليها كالآتي:

- تفوقت البدارى المغذاة على فيتامين د٣ وفيتامين ج بالمستوى (٣٠٠ وحده دولية و ٢٥٠ ملليجرام /كجم ، على الترتيب) معنويا في الوزن النسبي للقشرة وسمك القشرة (١٣,٥١% و٣٤، ملم ، على الترتيب) عند مقارنتها بدجاجات المجموعة المقارنة (١١,٨٥% و ٣١، ملم على الترتيب).
- أبدت دجاجات المجموعة المغذاة على فيتامين د٣ وفيتامين ج بالمستوى (٦٠٠ وحده دولية و ٥٠٠ ملليجرام /كجم ، على الترتيب) تفوقا معنويا في كفاءة تحويل الغذاء (٣،٠٧ جم بيض /جم عليقة) و معدل إنتاج البيض (٦٧،٨ %) /دجاجة/يوم و كتلة البيض (٣٣،٠٣جم/دجاجة/يوم) ونسبة الخصوبة (٩٤,٧٩%) ومعدل اتزان الكالسيوم (٦٨،٥٣%) وكمية الكالسيوم الممتصة الكلية والممتص لكل سم من طول الأمعاء والممتص لكل جرام مادة جافة /ساعة خلال أجزاء الأمعاء الدقيقة المختلفة (٢٢،٨٩ ملجم/ساعة و ٠،٤٦٧ ملجم/سم طول و٨،٣٣ ملجم/جم مادة جافة ، على الترتيب) ومحتوى بلازما الدم من الكالسيوم والفوسفور الغير عضوي (٢١،٧٥و٥،٨٠ ملجم/ديسيلتر ، على الترتيب) مقارنة بباقي المعاملات والمجموعة المقارنة (١٨،٧٤ و ٥،٦٥ ملجم/ديسيلتر ، على الترتيب).
- تفوق بيض دجاجات المجموعة المغذاة على فيتامين د٣ وفيتامين ج بالمستوى (٣٠٠ وحده دولية و ٥٠٠ ملليجرام /كجم ، على الترتيب) تفوقا معنويا في نسبة الفقس(٩٤،٥٩%) مقارنة ببيض دجاج المجموعة المقارنة (٨٨،٩٠%).
- كان لعمر الطيور تأثيرا معنويا على معدل استهلاك الغذاء وكفاءة التحويل الغذائي ومعدل إنتاج البيض/دجاجة/يوم ووزن البيضة وكتلتها والوزن النسبي وسمك القشرة ومحتوى بلازما الدم من الكالسيوم والفوسفور الغير العضوي.
- من وجهة النظر الاقتصادية يمكن التوصية بإضافة فيتامين د٣ وفيتامين ج معا بمعدل ٦٠٠ وحده دولية و ٥٠٠ ملليجرام ، على الترتيب لكل كيلوجرام من علائق الدجاج البياض.