

**EFFECT OF DIFFERENT LEVELS OF
CHOLECALCIFEROL (VITAMIN D₃) ON
PERFORMANCE AND SKELETAL BONE FORMATION
OF LAYING JAPANESE QUAIL REARED UNDER HOT
CLIMATE REGION**

By

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Abstract: *This experiment was conducted to determine whether production traits, egg quality (shell quality), bone mineralization and some metabolic functions of calcium and phosphorus could be improved by adding different levels of cholecalciferol (vitamin D₃) 2000, 2500 and 3000 ICU/Kg of diets to laying hen of Japanese quail reared under hot climate region. The experiment lasted 16 weeks during summer season. A total number of 480 laying Japanese quail were used at the start of laying (7th weeks of age) and randomly divided into four experimental groups. Birds were received diets designed to satisfy the recommendation of the NRC (1994). Fertility and hatchability experiment was conducted at the end of experiment (at 23 weeks of age) after the calculation of egg production and feed consumption. Diet and water were provided *ad libitum*. Birds received 16 h of light along the experimental period. Temperature degree (C°) and relative humidity (%) were recorded daily, since, it was 35C° and 70% as average.*

Results indicated that all production traits and egg quality characteristics improved (P<0.05) as the level of cholecalciferol (vitamin D₃) increased in tested diets. Also, the composition, measurements of tibia and plasma parameters increased (P<0.05) by the increase level of cholecalciferol in the diets. Results of the studied traits revealed that the best fertility and hatchability percentages for group fed 3000 ICU/Kg of vitamin D₃ as compared with other dietary treatments. Furthermore, dead embryos decreased with the increases of vitamin D₃ in the diet. Shell calcium and phosphorus contents increased (P<0.05) as the level of vitamin D₃ increase in the diet. In general conclusion, these results indicated that the addition of vitamin D₃ in diet of laying Japanese quail under hot climate conditions improved bone formation and shell thickness.

INTRODUCTION

It is well known that high environmental temperature is one of the most serious factors affecting the production performance of laying hens in both tropical and subtropical countries Mahmoud *et al.*, (1996). In addition to affect on feed intake and any associated indirect affect on egg production performance, high temperature also adversely affect bone weight, ash, strength and shell thickness (Miller and Sunde (1975) and Scott and Balnave (1988).

The disorder of calcium and phosphorus absorptions in birds especially under heat stress is related to the reduction in biosynthesis in 1,25 dihydroxycholecalciferol (1,25-(OH)₂). Calcium absorption and depositions are controlled by the active metabolite of Cholecalciferol (vitamin D₃) undergoes to sequential hydroxylation in liver and kidney to form 25 hydroxycholecalciferol and secondarily to 1,25 dihydroxycholecalciferol Abe *et al.*, (1982) and Soares (1984). However, Soares *et al.* (1995) found that egg shell quality improved a little over 50% of the time due to use of 25 hydroxycholecalciferol (25-OH-D₃) in the diet.

The results obtained by Keshavarz (1996) indicated that egg shell quality and bone mineralization increased by increasing of dietary Cholecalciferol above the requirement levels. In recent studies reported by Hansen *et al.*, (2004) showed that the addition of vitamin D₃ to diet significantly improving calcium absorption. They reported that vitamin D₃ is necessary for the production of Calbindin (28Dk) in the intestine, thus increasing calcium absorption at the intestine.

The aim of the present study was to investigate the effect of addition different levels of vitamin D₃ on performance, bone formation, shell quality and some metabolic function of calcium and phosphorus of laying hen of Japanese quail reared under hot climate region. Additionally, because inferior shell quality is a major problem during the high environmental temperature of summer months, the current study was conducted under these conditions to known the sensitivity of responses to dietary treatments.

MATERIALS AND METHODS

The current study was carried out at the Poultry Research Station, Faculty of Agriculture, Nasr City, Al-Azhar University during the summer season. A total number of 480 birds 7th weeks of age were used in this study. Birds were equally divided into four experimental groups each contained 3 replicates of 40 birds each. Birds were housed in wire cage (25.5X20.0X22.5Cm, two females per cage) of laying batteries provided

with waterer and feeders. Natural light was supplemented with artificial light (fluorescent) to furnish 16 h of continuous light each day. Three levels of cholecalciferol (vitamin D₃) 2000, 2500 and 3000 ICU/Kg of diet were added to basal diet. A basal diet was formulated to meet the nutrient requirements of laying Japanese quail according to NRC (1994). The composition and chemical analysis of the experimental diets are given in Table (1). Diets and water were freely available at all times. All birds were kept under the same managerial, hygienic and environmental conditions. The temperature and humidity degrees were daily recorded and maintained at 35C° and 70% as average respectively. Egg production was calculated by dividing the number of eggs produced per day by the number of hens and multiply 100. Also, feed consumption was recorded weekly. Feed conversion was calculated as (g feed /g eggs).

Egg quality characteristics were done after 8 weeks from the start of experiment (15 weeks of age) and at the end of experiment (23 weeks of age). The eggs were weighed individually and its specific gravity was measured following the procedure of Hamilton (1982). Thirty eggs were taken from each group broken and its contents (albumin, yolk and shell) were measured. Egg shell with membranes were cleaned and dried. Egg shell weight and its percentage of whole eggs were calculated. Shell thickness (mm) were measured by using Micrometer device, No 433-25M.M . Tibia bones were removed from carcass after slaughter, cleaned from surrounding meat and weighed. The outer dimensions of the tibia bones were determine at two points of midshaft using the Vernier Calipers. Tibia were dried firstly at lower temperature degree 70C° for 24 h to maintain the mineral content from loss. After, that muffle furnace degree was increased to reach 600C° for 3 h and bone ash content was weighed and prepared for calcium and phosphorus determination according to A.O.A.C (1980). Also, egg shell were taken and dried as the same method described above, and samples were taken for calcium and phosphorus analysis

Blood samples were collected within 5 minutes following oviposition from 6 hens from each group that had laid an egg the previous day. This selection eliminated fluctuation in plasma phosphorus due to differences in production status of individual hens according to method described by Miller *et al.*, (1978) and Taylor and Kirkley (1967). Because plasma phosphorus follows a cyclic patt in the laying hen during 24h period as discussed by Mongin and Sauveur (1979). Oviposition is the most suitable physiological reference point in plasma phosphorus determination. Blood samples were centrifuged at 3000 rpm for 15 minutes then the calcium, phosphorus and alkaline phosphates were determined

spectrophotometrically by using commercial Kits manufactured by Diamand Egyptian Company.

At the end of experiment (23 weeks of age) tow females were housed with one male in the same batteries in separate experiment for one week. Fertility and hatchability were done by set 100 eggs from each group in incubator. Fertility was calculated as the percentage of fertile eggs from the total number of eggs were set. While, hatchability was calculated as percentage of hatched chicks from the fertile eggs. Also, dead embryos were tested at the end of incubation period.

All data obtained were statistically analyzed by using the procedure of SAS institute (1996). Significant differences ($P<0.05$) among means were separated by using Duncan's multiple range procedure (Duncan 1955).

RESULTS AND DISCUSSION

Results of laying performance and egg grads as affected by dietary vitamin D₃ are presented in Table (2). Data revealed that egg production; egg weight and egg mass improved ($P<0.05$) as the levels of cholecalciferol increased. However, feed conversion ratio significantly ($P<0.05$) decreased (best) when levels of vitamin D₃ increased in tested diets. While, feed intake was not significantly affected by treatments. Also, it is evident from these results that adding vitamin D₃ in various levels has beneficial effect for decreasing mortality rate of layer hens during heat stress. Egg grads improved when inclusion levels of vitamin D₃ increased. It can be noticed that feeding diet containing higher levels of vitamin D₃ has beneficial effect for laying hen, especially when reared under heat stress conditions. These results are in agreement with the results obtained by Hamilton (1980) found that adding vitamin D₃ in diets of White Leghorn chicken had no significant ($P<0.05$) effect on feed intake. However, Shen *et al.*, (1981) reported that egg production decreased in birds received a diet devoid of synthetic vitamin D₃ as compared to those fed diet have 500 ICU of vitamin D₃/Kg diet. Frost and Roland (1990) they found that adding vitamin D₃ (1,25-(OH)₂) in diet of layer chickens resulted in an increase of egg production and egg weight. On the other hand, Roland and Harms (1976) indicated that addition of 25-OH-D₃ in diet of laying hen had no significant effect on egg production and egg weight. Also, Soares *et al.* (1988) reported that adding vitamin D₃ to laying hen diet for 22 weeks resulted insignificant differences in egg weight, egg production rate and egg mass. Furthermore, Keshavarz (1996) reported that production performance was not influenced by dietary vitamin D₃ of laying hens, while number of cracked eggs reduced. In recent studies reported by Keshavarz (2003) observed that substitution of vitamin

D₃ with 25-OH-D₃ did not provide any beneficial effect on egg production, egg weight, egg mass and feed conversion ratio.

The present results of laying performance traits suggest that economically the inclusion diet with high level of vitamin D₃ gave the best results under hot climate conditions

The data presented in Table (3) show the effect of dietary vitamin D₃ on egg quality traits of layer quail at 15 and 23 weeks of age fed different level of vitamin D₃. Results indicated that egg quality traits increased (P<0.05) as the levels of vitamin D₃ increased in the diet and shell quality was maximized when 3000 ICU of vitamin D₃ was added to the laying diets. It is well known that egg shell quality deteriorates when the laying hen is exposed to high environmental temperature. The addition of vitamin D₃ in diets transported to the liver and hydroxylated to form 25-hydroxyvitamin D₃. This metabolite is transported to the kidney and further hydroxylation to form 1,25 dehydroxy vitamin D₃ as the active metabolite. This hormone, a secosteroid (Norman 1987) play a major role in calcium and phosphorus homeostasis. It functions to stimulate bone resorption of calcium and phosphorus, and stimulate the absorption of calcium and phosphorus in small intestine. In addition 1,25 (OH)₂ D₃ stimulate the production of enzyme, binding protein, and membrane components involved in the transport of calcium and phosphorus (Norman 1978). However, egg shell thickness is the important factor for hatchability specially under heat stress conditions. To overcome the decrease deposition of calcium in shell during summer season and problems related with shell thickness, the addition of vitamin D₃ in diet acting to obtain good shell thickness for incubation or market purpose. The present results are agree with the finding of Soares *et al.*, (1988) who found that adding vitamin D₃ to diet of laying hen for 22weeks resulted improvement in egg shell quality. Furthermore, Forst and Roland (1990) indicated that adding 1,25 (OH)₂ D₃ in layer diet increased egg shell percentage, egg breaking strength and shell weight. Tsang *et al.*, (1990) observed that feeding layer chicken on diet containing 5ug vitamin D₃ per Kg of ration resulted in an improvement in egg shell quality than control group diet, due to the role of vitamin D₃ in egg shell deposition.

Table (4) show the composition of tibia ash, measurement and plasma parameters of laying quail fed different levels of vitamin D₃. The statistical analysis indicated that tibia ash; tibia calcium; and tibia phosphorus percentages and its measurements (i.e., length and width per Cm) increased (P<0.05) as the level of vitamin D₃ increased. Also, plasma calcium, phosphorus and alkaline phosphatase increased (P<0.05) when the inclusion of vitamin D₃ increased. These results are in accordance with those

obtained by Stevens and Blair (1983) found that tibia ash was greater ($P < 0.05$) in poult when fed diet containing 2400 ICU of vitamin D_3 /Kg as compared with diet containing 900 ICU. Also, Randolph *et al.*, (1997) who found that feeding chickens on diet containing 5 μ g of vitamin D_3 /Kg increased plasma phosphorus, plasma calcium and bone ash. Aburto *et al.*, (1998) indicated that adding 25-(OH) D_3 to the diet of young broiler chickens increased ($P < 0.001$) bone ash and plasma calcium and decrease rickets.

The data presented in Table (5) showed that both fertility and hatchability percentages improved ($P < 0.05$) when the level of vitamin D_3 increased in the diet. Data revealed that the highest values ($P < 0.05$) were observed for group fed high level of vitamin D_3 compared with other treatments or control group. The improvement in hatchability percentages attributed to the improvement in egg shell quality, since the poor hatchability in hot climate may be partially due to thin shell eggs (Daghir 1995). However, chick weight improved for group fed high vitamin D_3 as compared with control group, the increase of chick weight attributed to the increase of egg weight for group fed 3000 ICU D_3 / Kg. Concerning to the embryonic mortality, data indicated that the most of embryonic mortality decreased ($P < 0.05$) when levels of vitamin D_3 increased. This means that vitamin D_3 was able to decrease problems of dead embryo related to decrease calcium deposition in shell resulted from increasing high ambient temperature. The results are agree with the results obtained by Sunde *et al.*, (1978) found that vitamin D_3 and its metabolites are necessary for normal chicks embryo developments and improved hatchability. Also, Feltwell and Fox (1978) indicated that vitamin D_3 is required for reproduction. However, Abdulrahim and McGinnis (1979) found that pullets fed on different levels of vitamin D_3 and 25-OH- D_3 gave the highest hatchability percentage with no significant differences among them compared with control group. Tona *et al.*, (2001) who indicated that embryonic mortality varying through the incubation period.

On the other hand, Stevens and Blair (1985) showed that hatchability of the white egg strains was not affected ($P < 0.05$) by adding vitamin D_3 in the diet. However, Charles and Allen (1990) indicated that hatchability reduced when laying hens receiving the diet without cholecalciferol supplementation.

Results of Table (6) indicated that the percentages of egg shell; shell calcium and phosphorus contents increased ($P < 0.05$) at 15 and 23 weeks of age as addition of vitamin D_3 increase in the diet of quail hen. The increases of egg shell components may be due to vitamin D_3 acting to

increase the deposition of calcium and phosphorus in shell, because calcium makes up about 40% of the egg shell Keshavarz (2003).

In general conclusion, these results indicated that although performance, egg shell quality and tibia condition improved as the dietary of vitamin D₃ level increased. The study suggested that vitamin D₃ has a beneficial effect for layer hen of Japanese quail reared under heat stress.

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

Ingredients*	Percentages
Ground yellow corn (8.5%)	60.265
Soybean meal (44%)	20.00
Wheat bran (15.7%)	02.10
Laying concentrate (50.0%)	11.70
Sunflower oil	01.60
Calcium carbonate (Caco3)	04.00
Sodium chloride (NaCl)	00.20
Vitamin and mineral premix**	00.135
Total (Kg)	100.00 kg
<u>Calculated diet composition:</u>	
C.P %.	20.01
ME Kcal / kg.	2907.52
Ca %.	02.50
Available phosphorus %.	00.38
Lysine %.	01.00
Methionine + Cystine %.	•0.70
Vit.D3 ICU.	900
<u>Analyzed</u>	
C.P %	19.90
Ca %	02.48
Ava. phosphorus %	•0.35

* Based on Tables of feed composition of NRC (1994).

** Each 3kg of mixture contains: Vit A 12000.000IU; Vit. D₃ 2000000 ICU; Vit. E 10000mg, Vit. K₃ 1000mg, Vit.B1 1000mg, Vit.B₂ 5000 mg, Vit.B₆ 1500 mg, Vit.B₁₂ 10mg, Banto.Acid 10000mg; Fol. Acid 1000mg, Biotin 50mg, Niacin 20000mg, F₂ 30000mg, Mn 60000mg, Zn. 30000mg; Cob 100mg and Sel. 100mg.

Table (2): Effect of different dietary levels of cholecalciferol (vitamin D₃) on performance of laying Japanese quail.

Parameters	Control group	Cholecalciferol levels (ICU/Kg diet)		
		2000	2500	3000
Egg production, (%).	69.00±3.86 ^d	78.30±2.71 ^c	83.50±1.94 ^b	85.60±2.20 ^a
Egg weight, (g).	8.30±0.74 ^c	10.76±0.90 ^b	10.79±1.03 ^b	12.25±0.72 ^a
Egg mass, (kg).	5.727 ^d	8.425 ^c	9.009 ^b	10.486 ^a
Feed intake, (g/hen/day).	25.40±1.67 ^a	24.90±2.59 ^a	24.80±2.2 ^a	24.50±3.58 ^a
Feed conversion, (g feed/g egg).	3.06±0.10 ^a	2.31±0.12 ^b	2.24±0.15 ^b	2.00±0.15 ^c
Mortality rate, (%).	6.50	4.00	3.50	2.20
Egg grads:				
Large and above (%).	51.60	62.30	71.50	73.60
Medium and below (%).	48.40	37.70	28.90	26.40

a-dMeans the same row have the different superscript are significantly different (P<0.05).

Table (3): Effect of different dietary levels of cholecalciferol (vitamin D₃) on egg quality traits of laying Japanese quail at 15 and 23 weeks of age.

Items	At 15 weeks of age				At the end of experiment			
	Cholecalciferol (ICU/Kg diet)				Cholecalciferol (ICU/Kg diet)			
	Control	2000	2500	3000	Control	2000	2500	3000
Egg weight, (g).	8.76±0.19 ^c	10.30±0.11 ^b	10.60±0.24 ^b	11.96±0.16 ^a	9.18±1.2 ^d	10.34±1.5 ^c	11.12±0.6 ^b	12.60±0.86 ^a
Specific gravity.	1.062±1.03 ^b	1.064±2.4 ^a	1.064±2.8 ^a	1.064±2.3 ^a	1.064±3.5 ^b	1.068±3.0 ^a	1.068±3.1 ^a	1.068±3.2 ^a
Egg shape index.	0.70±0.02 ^c	0.70±0.03 ^c	0.73±0.07 ^b	0.75±0.04 ^a	0.73±0.05 ^b	0.87±0.02 ^a	0.87±0.02 ^a	0.87±0.05 ^a
Yolk weight, (g)	2.40±0.12 ^b	3.60±0.42 ^a	3.70±0.15 ^a	3.60±0.4 ^a	2.56±0.36 ^c	3.00±0.70 ^b	3.80±0.6 ^a	3.90±0.64 ^a
Albumin weight, (g).	4.92±0.22 ^c	5.28±0.18 ^b	5.21±0.20 ^b	6.50±0.21 ^a	5.32±0.18 ^c	5.92±0.22 ^b	5.69±0.16 ^b	6.90±0.23 ^a
Shell weight,(g).	1.18±0.09 ^d	1.42±0.11 ^c	1.69±0.09 ^b	1.86±0.12 ^a	1.30±0.14 ^d	1.42±0.20 ^c	1.63±0.18 ^b	1.80±0.15 ^a
Shell thickness, (mm).	0.36±0.05 ^a	0.42±0.02 ^c	0.47±0.06 ^b	0.59±0.08 ^a	0.38±0.04 ^d	0.43±0.04 ^c	0.51±0.06 ^b	0.59±0.07 ^a
Yolk colour degree.	3.00±0.71 ^b	3.80±0.52 ^b	4.11±0.81 ^a	4.50±1.01 ^a	3.38±0.78 ^b	3.92±1.13 ^b	4.21±1.02 ^a	4.50±1.20 ^a
Creaked eggs, (%).	12.00 ^a	8.00 ^b	6.00 ^c	3.00 ^d	15.00 ^a	10.00 ^b	5.00 ^c	2.00 ^d

a-d Means the same row have the different superscript are significantly different (P<0.05).

Table (4): Effect of different dietary levels of cholecalciferol (vitamin D₃) on composition and measurements of tibia and plasma parameters.

Parameters	Control Group	Cholecalciferol levels (ICU/Kg diet)		
		2000	2500	3000
Tibia ash (%). *	18.36±0.41 ^d	20.15±0.17 ^c	26.49±0.58 ^b	32.43±0.78 ^a
Tibia calcium (% of ash).	9.77±0.60 ^d	13.91±0.63 ^c	16.12±0.15 ^b	22.64±0.51 ^a
Tibia phosphorus (% of ash).	2.68±0.13 ^d	2.92±0.11 ^c	3.50±0.18 ^b	4.00±0.22 ^a
Tibia length (Cm).	4.30±0.15 ^b	5.20±0.17 ^a	5.40±0.18 ^a	5.60±0.16 ^a
Tibia width (Cm).	0.112±0.006 ^d	0.123±0.005 ^c	0.138±0.004 ^b	0.143±0.003 ^a
Plasma calcium (mg/100ml).	7.93±0.01 ^d	9.10±0.08 ^c	10.14±0.08 ^b	11.06±0.03 ^a
Plasma inorganic phosphorus (mg/100ml).	3.09±0.04 ^c	4.22±0.08 ^b	4.47±0.06 ^b	5.07±0.03 ^a
Alkaline phosphatase (IU/L).	743.20±4.9 ^d	765.20±9.7 ^c	873.80±13.4 ^b	931.90±11.6 ^a

* Values represent average of 16 samples.

a-d.... means the same row have the different superscript are significantly different (P<0.05).

Table (5): Effect of different dietary levels of cholecalciferol (vitamin D₃) on fertility, hatchability and dead embryos of laying quail.

Parameters	Control group	Cholecalciferol levels ICU/Kg diet		
		2000	2500	3000
Fertility (%)	62.30	65.00	69.40	86.6
Hatchability (%)	57.50	61.60	65.30	78.80
Chick weight (g)	8.12	8.92	9.12	10.20
Dead embryos (%) at the end of incubation period*				
0 - 18 (days).	22.20	18.00	12.32	10.18

* Dead embryos detected at the end of incubation period by broken unhatched eggs by the visual observation.

Table (6): Effect of different dietary levels of cholecalciferol (vitamin D₃) on egg shell calcium and phosphorus contents of laying Japanese quail at 15 and 23 weeks of age.

Parameters	Control group	At 15 weeks of age			Control group	At 23 weeks of age		
		2000	2500	3000		2000	2500	3000
Egg shell (%)	13.88±0.70 ^b	13.79±0.98 ^b	15.94±1.12 ^a	15.55±1.01 ^a	14.16±1.00 ^b	13.73±0.99 ^c	14.66±1.32 ^a	14.29±1.66 ^a
Shell calcium content (%)	10.33±2.11 ^d	11.26±3.10 ^c	11.83±3.50 ^b	13.23±2.10 ^a	9.89±1.92 ^d	10.22±2.00 ^c	11.63±2.50 ^b	13.4±3.10 ^a
Shell phosphorus Content (%)	0.38±0.09 ^d	0.48±0.07 ^c	0.52±0.06 ^b	0.58±0.08 ^a	0.39±0.01 ^d	0.41±0.05 ^c	0.58±0.04 ^b	0.60±0.08 ^a

a- d means in the same row have the different superscript are significantly different (P<0.05).

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الملخص العربي

دراسة عن تأثير مستويات مختلفة من الكولي كالسيوم (فيتامين د₃) على الأداء الإنتاجي وتكوين الهيكل العظمي في السممان الياباني البياض المرعى تحت ظروف المناخ الحار

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أجريت هذه التجربة لتقدير ما إذا كانت الصفات الإنتاجية، صفات جودة البيضة وترسيب الأملاح في العظم وكذلك مختلف الصفات الأخرى تتحسن بإضافة مستويات مختلفة من فيتامين د₃ ، ٢٠٠٠ ، ٢٥٠٠ ، ٣٠٠٠ وحدة دولية / كجم علف في غذاء السممان الياباني البياض والمرعى تحت ظروف الأجواء الحارة. استمرت التجربة فترة ١٦ أسبوع أثناء موسم الصيف. وكان العدد الكلي المستخدم هو ٤٨٠ سمانة عمر ٧ أسابيع. كما تم عمل تجربة منفصلة لدراسة تأثير فيتامين د₃ على كل من الخصوبة والفقس في نهاية التجربة (عند الأسبوع ٢٣ من العمر) بعد الانتهاء من حساب إنتاج البيض والغذاء المستهلك. وتم تكوين غذاء أساسي لفي بالاحتياجات الخاصة في هذه الفترة وفقاً للاحتياجات الغذائية المنشورة في NRC (١٩٩٤)

تم تقديم الغذاء والماء للطيور بحرية ولحد الشبع، وكانت الإضاءة بمعدل ١٦ ساعة يومياً خلال فترة التجربة. أيضاً كان يتم تسجيل درجة الحرارة ودرجة الرطوبة النسبية يومياً. حيث كان المتوسط ٣٥°م ، ٧٠% على التوالي.

وأشارت النتائج المتحصل عليها من هذه الدراسة أن الصفات الإنتاجية وكذلك صفات جودة البيضة تحسنت معنوياً بزيادة مستوى فيتامين د₃ في الغذاء. كذلك فإن تركيب ومقاييس عظمه الساق وكذلك بعض مقاييس الدم مثل الكالسيوم والفوسفور والكالكاين فوسفاتيز تحسنت أيضاً معنوياً بزيادة مستوى فيتامين د₃. أيضاً أشارت النتائج المتحصل عليها أن نسبة الخصوبة والفقس كانت أفضل للمجموعة المغذاة على غذاء يحتوي على مستوى عالي من فيتامين د₃ بالمقارنة بباقي المجموعات أو مجموعة الكنترول. كما أن نفوق الأجنة قل مع زيادة مستوى فيتامين د₃ في الغذاء. أشارت النتائج أيضاً إلى أن محتوى القشرة من الكالسيوم والفوسفور يزيد معنوياً بزيادة مستوى الفيتامين. وعموماً فلن هذه الدراسة أشارت إلى أن إضافة فيتامين د₃ في غذاء السممان الياباني البياض يؤدي إلى تحسن درجة الاستفادة من الكالسيوم والفوسفور تحت ظروف الجو الحار وبالتالي يؤدي إلى التحسن في حالة العظام وكذلك سمك قشرة البيضة.