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SOME NUTRITIONAL INTERRELATIONSHIPS OF CALCIUM AND VITAMIN D_3 ON LAYING JAPANESE QUAIL

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SUMMARY

Total number of one hundred twenty Japanese quail hens of 6 weeks old were used to study some nutritional interrelationships of calcium (Ca) and vitamin D_3 (Vit D_3) during laying. Hens were divided into 60 productive units, of 2 hens each. The productive units were divided into 6 treatment groups, of 20 hens each. The experimental period started at 6 and terminated at 27 weeks of age. The experimental period was divided to three phases (6 to 13, 13 to 20 and 20 to 27 weeks). A factorial arrangement (2×3) of six treatments was formulated to contain two levels of dietary calcium 2.5 and 3.7 % and three levels of Vit.D_3 injection 0, 200, and 400 IU/bird/2week. The main source of calcium in the diets was calcium carbonate. The diets contained adequate Vit.D_3 requirements. Feed intake and egg production (number & weight) were recorded and feed conversion (egg / feed) was calculated during the experimental period. Blood samples were collected within each treatment at 13, 20, and 27 weeks of age, to study some parameters of the metabolic functions.

Results showed that, the greatest ($P<0.05$) values of egg production (number & weight), feed conversion and the concentrations of calcium, glucose, inorganic phosphorus, total protein, albumin, and globulin in blood plasma were noticed when birds were fed high Ca diet 3.7% and injected with either 200 or 400 IU Vit D_3 compared with other treatments. Increasing either Ca level from 2.5 to 3.7% or Vit.D_3 levels from 0 to 200 or 400 IU, enhanced ($P<0.05$) egg production, feed conversion and the concentrations of the previous blood metabolic parameters. No significant effect was observed on feed intake among treatments.

Keywords: Calcium, vitamin D_3, egg production, blood constituents, Japanese quail

INTRODUCTION

The wide variation of calcium requirements for laying hens is mainly related to the interrelationship of calcium with other nutrients, especially with phosphorus and vitamin D_3. The calcium requirements also, depends on the interrelationship of calcium carbonate and palatability of feed (Roland, 1986).

Increasing calcium level in laying hen diets improved egg weight, egg production, and shell quality (Kunchinski and Harms, 1997 and Scheideler and Robeson, 1997). Vohra, *et al.* (1979) reported that, dietary deprivation of supplementary calcium and Vit.D_3 did not affect the body weight of female or male Japanese quails, even though feed consumption was significantly reduced. The egg production was reduced from about 74% to 10% or 20% in the deprivation of calcium or Vit.D_3 for Japanese quail hens, respectively. The deficiencies resulted in reduced egg weight, shell thickness, and female tibia ash, while not influencing ovarian and oviductal weights or testis weights and tibia ash of males.

Vitamin D_3 at level higher than the requirement was beneficial in improving growth, blood and bone parameters (Stevens and Blair, 1983). Egg production was positively correlated to Vit D_3 level in a quadratic manner (Randoph *et al.*, 1997). Frost *et al.*(1990) found that eggs shell quality was improved when layers were fed on a diet supplemented wit Vit D_3. On the other hand (El- Gindi, *et al.*,1999) reported that Vit D_3 supplementations (800, 1200, or 1600 IU) did not significantly affect on egg weight, egg production or egg mass. They concluded that the rate of calcium at a level of 3.5% and vitamin D_3 at level of 800 IU / kg ration is adequate to achieve the best results and recommended from the economic point view.

The present study was conducted to evaluate some productive and metabolic functions of laying Japanese quail as affected by dietary Ca at levels of 2.5 and 3.7% or Vit D_3 injection at levels of 0, 200 or 400 IU/hen/2weeks.

MATERIALS AND METHODS

Birds and management

One hundred twenty of Japanese quail hens of 6 weeks old were chosen for similar body weight. Hens were divided into 6 treatment groups, each of 20 females. Within each group the hens were subdivided into 10 replicates, each of 2 females. Each replicate was housed in a separate cage. Hens were fed *ad libitum* and the fresh water was available all the time during the experimental period. Moreover, hens were reared under the standard managerial regime of light. The experiment was conducted up to 27 weeks of age.

Six treatments were examined in a factorial arrangement (2×3) to contain two levels of dietary Ca 2.5 and 3.7% or three levels of Vit D_3 injection 0, 200, and 400 IU/hen/2weeks. The main source of calcium in the diets was calcium carbonate. Diets were formulated to meet or exceed NRC (1994) recommendation. All diets were iso caloric and iso nitrogenous. Formula of the experimental diets was recorded in Table 1. The injected birds were given Vit.D_3 doses over the requirements of this vitamin which covered in the diets.

Table 1. Composition and chemical analysis of experimental diets

Ingredients	Calcium levels (%) 2.5	3.7
Ground corn, yellow	68.75	62.75
Layer concentrates(51%CP)	10.00	10.00
Soybean meal (44%CP)	16.50	18.00
Poultry fat	0.00	1.50
Limestone	4.50	7.50
Vitamins & Minerals mixture*	0.25	0.25
Total	100.00	100.00
Chemical analysis (calculated)		
Crude protein, %	18.39	18.51
Metabolizable energy, Kcal / kg	3009	3007
Calcium, %	2.508	3.706
Av. Phosphorus, %	0.45	0.45
Methionine + cystine, %	0.77	0.77
Lysine, %	1.03	1.04

* Each 2.5 kg of vitamins and minerals mixture contain:
12000,000 IU vitamin A acetate; 2000,000 IU vitamin D3; 10.000 mg vitamin E acetate; 2000 mg vitamin K3; 100 mg vitamin B; 4000 mg vitamin B2; 1500 mg vitamin B6; 10 mg vitamin B12; 10.000 mg Pantothenic acid; 20.000 mg Nicotenic acid; 1000 mg Folic acid; 50 mg Biotin; 500,000 mg Choline; 10.000 mg Cupper; 1000 mg Iodin; 30,00 mg Iron; 55,000 mg Manganese; 55,000 mg Zinc, and 100 mg Selenium.

Treatments

The six treatments were as following:- 2.5% Ca without Vit D_3, 2.5% Ca with 200 IU Vit D_3, 2.5%Ca with 400 IU Vit D_3, 3.7% Ca without Vit D_3, 3.7% Ca with 200 IU Vit D_3, and 3.7% Ca with 400 IU Vit D_3 for treatments 1, 2, 3, 4 , 5 and 6, respectively. Each bird from Vit D_3 treatments was injected by Vit D_3 dose every 2 weeks.

Measurements and determinations

The experimental period (6 to 27 weeks) was divided into three production phases. These phases presented the productive performance of laying curve. They were: from 6 to 13, 13 to 20, and 20 to 27 weeks of age, each phase presented data of 7 weeks. While the entire period (6 to 27 weeks) showed data of 21 weeks.

For each replicate, egg number and egg weight were recorded daily. Feed intake per replicate was measured weekly. Feed conversion (kg feed/ kg egg) was calculated.

Blood samples were collected in heparinized tubes from wing vein of 5 hens within each treatment at 13, 20, and 27 weeks of age. Whole blood was centrifuged (3000rpm/15minutes). The plasma was obtained and immediately stored at -20° C till analysis. Calcium, glucose, inorganic phosphate, alkaline phosphatase, plasma total protein, and albumin, were determined according to Hawks (1965), Trinder (1969), Goldenberg and Fernandez (1966), Hurwitz and Grimminger (1961), Weischelbaun(1946), and Doumas (1971), respectively.

Data were subjected to ANOVA (SAS, 1985). Comparison between treatment means followed Duncan's multiple range test (Duncan, 1955)

RESULTS AND DISCUSSION

1- Productive performance

Egg production

Egg production data (egg number and egg weight), during the experimental periods are presented in Tables 2 and 3. Hens fed high Ca diet 3.7% and treated with 200 or 400 IU Vit D_3 recorded the best ($P<0.05$) values of egg number and egg weight Table 2. In the same way, the egg production (number and weight) improved ($P<0.05$) with increasing Ca level in the diets from 2.5 to 3.7% and/or increasing Vit D_3 dose from 0 to 400 IU Table 3. This observation could explain the important role of Ca in female reproductive function (Roland *et al.*, 1996), and thereafter the addition of Vit D_3 showed numerical advantage effects. Randoph *et al.* (1997) reported that there is positive correlation between egg production and Vit D_3 levels. On the other hand El-Gindi *et al.*,(1999) found that diets containing low levels of Vit D_3(800IU/kg) improved ($P<0.01$) egg production than higher levels.

Table 2. Effects of calcium and vitamin D_3 on some productive parameters of laying Japanese quail

Items.	Age in Wks	Treatments					
		Calcium levels(%)					
		2.5			3.7		
		Vitamin D3 levels(IU)					
		0	200	400	0	200	400
Egg number Hen/ house	6 to 13	20.2 ±1.4^{c}	30.4 ±1.9^{b}	32.3 ±2.2^{a}	28.8 ±1.3^{b}	34.6 ±2.1^{a}	34.2 ±2.0^{a}
	13 to 20	30.5 ±3.2^{d}	31.7 ±2.4^{c}	40.8 ±2.7^{b}	33.7 ±2.8^{c}	46.8 ±2.0^{a}	45.8 ±2.1^{a}
	20 to 27	26.4 ±1.6^{d}	29.8 ±2.9^{c}	38.7 ±2.4^{b}	30.4 ±1.7^{c}	40.4 ±2.4^{a}	42.9 ±2.3^{a}
	6 to 27	77.1 ±2.1^{d}	91.9 ±2.4^{c}	111.8 ±2.4^{b}	92.9 ±1.4^{c}	122.8 ±2.2^{a}	123.9 ±1.4^{a}
Egg weight (gm)	6 to 13	8.4 ±1.1^{c}	10.9 ±1.4^{b}	10.9 ±1.7^{b}	10.8 ±1.2^{c}	11.4 ±1.6^{a}	12.3 ±1.4^{a}
	13 to 20	10.2 ±1.3^{c}	11.6 ±1.6^{b}	11.5 ±1.7^{b}	11.4 ±1.3^{b}	12.8 ±1.7^{a}	12.8 ±1.6^{a}
	20 to 27	10.9 ±1.2^{b}	12.2 ±1.5^{a}	12.4 ±1.6^{a}	11.8 ±1.4^{ab}	12.8 ±1.7^{a}	12.7 ±1.7^{a}
	6 to 27	9.8 ±1.2^{c}	11.6 ±1.5^{b}	11.6 ±1.7^{b}	11.3 ±1.3^{b}	12.3 ±1.7^{a}	12.6 ±1.6^{a}
Feed intake, Gm/2hens/day	6 to 13	30.7 ±4	34.4 ±5	32.5 ±7	32.6 ±5	30.6 ±6	32.0 ±5
	13 to 20	48.2 ±5	49.6 ±7	43.4 ±5	42.7 ±6	44.5 ±4	46.3 ±3
	20 to 27	46.4 ±9	43.8 ±9	50.2 ±11	48.3 ±8	51.4 ±10	48.6 ±9
	6 to 27	41.8 ±6	42.6 ±7	42.1 ±8	41.2 ±6.3	42.2 ±7	42.3 ±6
Feed Conversion (feed/egg)	6 to 13	3.5 ±.01^{a}	3.2 ±.02^{b}	2.9 ±.02^{b}	3.6 ±.02^{a}	2.5 ±.03^{c}	2.4 ±.02^{c}
	13 to 20	2.6^{a} ±.02	2.6 ±.03^{a}	2.8 ±04^{b}	2.8 ±.03^{a}	2.2 ±.0^{b}	2.1 ±.05^{b}
	20 to 27	3.4 ±.02^{a}	3.4 ±.06^{a}	3.4 ±.05^{b}	3.6 ±.04^{a}	2.3 ±.04^{b}	2.4 ±.06^{b}
	6 to 27	3.2 ±.02^{a}	3.1 ±.04^{a}	3.0 ±.01^{b}	3.3 ±.03^{a}	2.3 ±.04^{b}	2.3 ±.04^{b}

a, b and c data in the same row followed by unlike letters differ significantly ($P<0.05$). ± S.E

Table 3. Effects of calcium or vitamin D_3 on some productive parameters of laying Japanese quail

Items	Age in Wks.	Treatments: Calcium levels (%) 2.5	Calcium levels (%) 3.7	Vitamin D3 levels (IU) 0	Vitamin D3 levels (IU) 200	Vitamin D3 levels (IU) 400
Egg number Hen/house	6 to 13	27.63 ±3.8^{b}	31.87 ±1.6^{a}	24.50 ±1.3^{b}	32.5 ±2.1^{a}	33.25 ±1.0^{a}
	13 to 20	34.33 ±3.3^{b}	42.10 ±4.2^{a}	32.10 ±2.3^{b}	39.25 ±5.4^{a}	43.30 ±2.5^{a}
	20 to 27	31.63 ±3.7^{b}	37.90 ±2.8^{a}	28.40 ±2.8^{b}	35.10 ±5.6^{ab}	40.80 ±2.1^{a}
	6 to 27	93.60 ±4.8^{b}	112.6 ±7.3^{a}	85.00 ±7.9^{b}	107.8 ±16^{a}	117.8 ±7.05^{a}
Egg weight (gm)	6 to 13	10.70 ±0.83	11.17 ±0.2	9.60 ±.2	11.15 ±0.3	11.10 ±0.4
	13 to 20	11.10 ±0.4^{b}	12.33 ±0.5^{a}	10.80 ±0.7	12.20 ±0.6	12.15 ±0.7
	20 to 27	11.83 ±0.8^{b}	12.47 ±0.3^{a}	11.35 ±0.5^{b}	12.50 ±0.4^{ab}	12.55 ±0.3^{a}
	6 to 27	11.00 ±1.7^{b}	11.98 ±0.9^{a}	10.85 ±2.3^{b}	11.95 ±1.2^{a}	12.1 ±1.5^{a}
Feed intake, Gm/2hens/day	6 to 13	32.53 ±2	31.43 ±1	31.65 ±6	32.50 ±4	32.25 ±5
	13 to 20	47.07 ±4	44.50 ±3	45.45 ±8	47.05 ±5	44.85 ±7
	20 to 27	46.08 ±2	49.80 ±4	47.85 ±9	47.60 ±6	49.40 ±5
	6 to 27	42.13 ±3	41.90 ±5	41.47 ±12	42.41 ±13	42.17 ±11
Feed Conversion (feed/egg)	6 to 13	3.20 0.03^{a}	2.83 ±0.04^{b}	3.55 ±0.05^{a}	2.85 ±0.04^{b}	2.65 ±0.04^{b}
	13 to 20	2.66 ±0.06	2.36 ±0.02	2.70 ±0.01^{a}	2.40 ±0.02^{b}	2.45 ±0.05^{ab}
	20 to 27	3.40 ±0.01^{a}	2.76 ±0.02^{b}	3.00 ±0.06	2.85 ±0.03	2.90 ±0.09
	6 to 27	3.10 ±0.05^{a}	2.63 ±0.03^{b}	3.25 ±0.05^{a}	2.70 ±0.05^{b}	2.65 ±0.04^{b}

a, b and c data in the same row under each treatment followed by unlike letters differ significantly ($P<0.05$). ± S.E

Feed intake and feed conversion

No significant ($P>0.05$) effect was observed on the amount of feed intake throughout the experimental period (Tables 2 and 3). However, there was a little adverse affect in feed intake during the periods from 13 to 20 and 6 to 27 weeks of age with increasing Ca levels in the diet (Table 3). The adverse effect of excess calcium, may be due to the high levels of other minerals (example, magnesium) present in the calcium carbonate (Roland, 1986) which may reduce feed palatability and could adversely affect on feed intake. El Gindi *et al.* (1999) reported that dietary Vit D_3 levels (800. 1200, or 1600 IU) had no significant effect on the feed consumption.

The enhanced egg production without significant effect on feed intake for birds fed high dietary Ca 3.6% and injected with 200 or 400 IU Vit D_3, resulted in improvement ($P<0.05$) of feed conversion (Table 2). Generally, hens fed diets containing high Ca (3.7%) level or treated with high Vit D_3 levels (200 or 400IU) presented enhancement ($P<0.05$) in feed conversion (Table 4). This result agree with the findings of Aburto and Britton (1996) who stated that feed conversion improved as the level of Vit D_3 increased. The production shape paralleled with the feed conversion improvement for all experimental treatments. Such improvement could be attributed to the biological function of Vit D_3. Vitamin D_3 is necessary for the bird to absorb, transport and utilize calcium and phosphorus through the intestinal wall. Dietary Vit D_3 is transported to the liver and metabolized producing 25-hydroxy Vit D_3 metabolite which is producing hormonal like form 1,25 di-hydroxy vitamin D_3 [(1.25-(OH)2D3] which is considered the active metabolite needed for active transport of calcium ions through the cellular membrane in small intestine (Holick and Deluca, 1974). The secosteroid hormone plays a major role in calcium and phosphorus homeostasis. It functions to stimulate bone reabsorption of calcium and phosphorus and stimulates the absorption of calcium and phosphorus in the small intestine (Norman, 1987). This may explain the biological function of Vit D_3.

The consistency of egg production and feed conversion trends and plasma parameters showed a beneficial effects of dietary calcium and Vit D_3 injection. Therefore, it is probably advisable to use both dietary Ca and Vit D_3 injection for Japanese quail layers. In general, increasing Ca or Vit D_3 levels improved ($P<0.05$) all plasma metabolic parameters which were measured in this study (Table 5).

2 - Blood plasma metabolic changes

Data of blood plasma metabolies i.e, calcium, glucose, inorganic phosphate, alkaline phosphatase, total protein, albumin, and globulin are listed in Tables 4 and 5. Obtained data revealed that hens fed 3.7% Ca and injected with 200 or 400 IU Vit D_3 had the highest ($P<0.05$) values of calcium, glucose, inorganic phosphate, total protein, albumin and globulin compared with other treatments. While, the highest ($P<0.05$) values of alkaline phosphatase were for hens fed calcium levels (2.5 or 3.7%) without Vit D_3 injection (Table 4).

Results of plasma constituents indicated that both Ca or Vit D_3 raised the metabolic activity. The positive association between dietary calcium and plasma glucose suggests that calcium enhanced insulin secretion and consequently influenced liver lipogensis, Thereby tending to affect on the growth (Shafey *el al.*, 1990). El-Gendi *et al.* (1999) found that increasing dietary Vit D_3 improved ($P<0.01$) plasma inorganic phosphorus and activity of alkaline phosphatase. In present study the increase in total protein reflect an increase in plasma globulin, while, plasma albumin was not affected. Moreover plasma alkaline phosphatase is apparently of a somatic cell origin and is thought to arise from active anabolic tissue (Bell,1971). McGilvery and Goldstein (1979); explained that vit D_3 transported in the blood in combination with a specific transport globulin, and it is taken up and stored in the liver. A 25-hydroxylase in the endoplasmic reticulum forms 25-hydroxycholecalciferol. This enzyme is a typical monooxygenase, utilizing NADPH as the second electron doner.

Table 4. Effects of calcium and vitamin D_3 on some plasma parameters of laying Japanese quail.

Itmes	Age in Wks	Treatments					
		Calcium levels(%)					
		2.5			3.7		
		Vitamin D_3 levels(IU					
		0	200	400	0	200	400
Calcium	13	12.1 ± 0.7^{c}	14.4 ± 1.2^{c}	15.6 ± 1.2^{b}	13.5 ± 1.2^{d}	17.4 ± 1.1^{a}	17.6 ± 1.6^{a}
(M mole/L)	20	12.4 ± 0.8^{c}	15.6 ± 1.3^{c}	16.8 ± 1.0^{b}	14.4 ± 1.5^{d}	17.5 ± 1.4^{a}	17.7 ± 1.7^{a}
	27	12.6 ± 1.2^{d}	15.8 ± 1.5^{c}	$17.4\pm.1.4^{b}$	15.7 ± 1.4^{c}	18.8 ± 1.5^{a}	18.5 ± 1.8^{a}
Glucose	13	218.4 ± 3.7^{b}	280.8 ± 3.8^{a}	288.7 ± 3.1^{a}	248.6 ± 3.2^{a}	309.8 ± 3.7^{a}	284.6 ± 3.4^{a}
(Mg/100ml)	20	211.2 ± 3.8^{c}	312.7 ± 3.9^{b}	329.2 ± 4.8^{b}	321.5 ± 4.1^{b}	352.2 ± 4.1^{a}	343.7 ± 4.2^{a}
	27	315.3 ± 4.2^{c}	330.8 ± 4.4^{b}	321.1 ± 4.5^{b}	332.4 ± 4.3^{b}	364.4 ± 5.6^{a}	373.8 ± 5.8^{a}
Inorganic	13	4.3 ± 1.5^{c}	5.3 ± 1.0^{b}	5.2 ± 1.2^{b}	5.4 ± 0.9^{b}	6.6 ± 1.1^{a}	6.4 ± 1.1^{a}
Phosphate	20	4.4 ± 1.1^{c}	5.8 ± 1.1^{b}	5.5 ± 1.3^{b}	5.7 ± 1.0^{bc}	8.4 ± 1.3^{a}	7.7 ± 1.2^{a}
(Mg/dl)	27	5.8 ± 1.1^{c}	6.2 ± 1.2^{bc}	$6.9\pm.1.5^{b}$	6.2 ± 1.1^{bc}	8.5 ± 1.4^{a}	8.8 ± 1.3^{a}
Alkaline	13	4.8 ± 1.1^{a}	3.2 ± 1.3^{b}	3.0 ± 1.1^{c}	3.5 ± 1.3^{b}	3.2 ± 1.2^{b}	3.1 ± 1.2^{b}
Phosphatase	20	5.6 ± 1.2^{a}	3.7 ± 1.3^{c}	3.2 ± 1.2^{d}	4.7 ± 1.4^{b}	3.4 ± 1.3^{c}	3.4 ± 1.3^{c}
(U/L)	27	5.4 ± 1.4^{a}	4.1 ± 1.4^{b}	3.7 ± 1.3^{b}	4.8 ± 1.4^{a}	3.8 ± 1.5^{b}	3.6 ± 1.4^{b}
Total	13	3.4 ± 0.3^{d}	4.3 ± 0.2^{b}	4.2 ± 0.3^{b}	3.7 ± 0.3^{c}	5.2 ± 0.3^{a}	4.8 ± 0.5^{a}
Protein	20	4.3 ± 0.5^{d}	5.4 ± 0.3^{b}	5.6 ± 0.4^{b}	5.2 ± 0.4^{c}	6.2 ± 0.4^{a}	6.4 ± 0.5^{a}
Gm/100ml	27	5.4 ± 0.6^{c}	6.8 ± 0.5^{b}	6.3 ± 0.5^{b}	6.8 ± 0.5^{b}	7.4 ± 0.6^{a}	7.3 ± 0.6^{a}
Albumin	13	1.3 ± 0.3^{c}	2.1 ± 0.2^{b}	2.2 ± 0.3^{ab}	1.8 ± 0.2^{b}	2.3 ± 0.3^{a}	2.4 ± 0.2^{a}
(Gm/100ml)	20	1.5 ± 0.2^{c}	2.4 ± 0.2^{b}	2.5 ± 0.3^{ab}	2.1 ± 0.2^{b}	2.7 ± 0.3^{a}	2.8 ± 0.3^{a}
	27	1.7 ± 0.2^{c}	2.8 ± 0.2^{b}	2.7 ± 0.2^{b}	2.9 ± 0.1^{b}	3.4 ± 0.3^{a}	3.2 ± 0.3^{a}
Globulin	13	2.1 ± 0.2^{b}	2.2 ± 0.2^{b}	2.1 ± 0.1^{b}	1.9 ± 0.1^{b}	2.9 ± 0.2^{a}	2.4 ± 0.2^{a}
(Gm/100ml)	20	$2.8\pm.03^{c}$	3.0 ± 0.2^{b}	3.1 ± 0.2^{b}	3.1 ± 0.3^{b}	3.5 ± 0.3^{a}	3.6 ± 0.3^{a}
	27	3.7 ± 0.3^{b}	3.9 ± 0.3^{ab}	3.6 ± 0.3^{b}	3.9 ± 0.4^{ab}	4.0 ± 0.4^{a}	4.1 ± 0.4^{a}

a, b and c data in the same row followed by unlike letters differ significantly ($P<0.05$). ± S.E

Table 5. Effect of calcium or vitamin D_3 on some metabolic parameters of laying Japanese quail

Items	Age in Wks	Treatments				
		Calcium levels (%)		Vitamin D3 (IU)		
		2.5	3.7	0	200	400
Calcium	13	14.0±1.2[b]	16.2±1.3[a]	12.8±0.2[b]	15.9±1.5[a]	16.6±1.1[a]
(M mole/L)	20	14.9±1.3[b]	16.5±1.1[a]	13.4±1.0[b]	16.5±0.9[a]	17.2±0.4[a]
	27	15.3±1.4[b]	17.7±0.9[a]	14.2±1.4[b]	17.3±1.4[a]	17.9±0.8[a]
Glucose	13	262.6±7.7[b]	293.7±9.8[a]	251.5±3.1[b]	595.3±4.2[a]	286.6±2.7[a]
(mg/100ml)	20	175.7±9.1[b]	225.7±8.9[a]	266.3±5.5[b]	332.6±9.7[a]	336.4±7.3[a]
	27	322.3±4.5[b]	356.9±6.4[a]	323.9±8.6[b]	347.6±6.8[a]	347.5±6.6[a]
Inorganic	13	4.4±0.4[b]	6.1±0.7[a]	4.9±0.2[b]	6.0±0.1[b]	5.8±0.4[a]
Phosphate	20	5.2±0.1[b]	7.3±0.3[a]	5.1±0.3[b]	7.1±1.3[a]	6.6±1.1[a]
(mg/dl)	27	6.3±0.3[b]	7.8 ±0.8[a]	6.0±0.2[b]	7.4±1.1[a]	7.9 ±0.4[a]
Alkaline	13	3.7±0.6	3.2±1.0	4.2±0.1[a]	3.2±0.3[b]	3.1±0.2[b]
Phosphatase	20	4.2±0.1	3.8±0.9	5.2±0.7[a]	3.6±0.4[b]	3.3±0.9[b]
(U/L)	27	4.4±0.5	4.1±1.4[b]	5.1±0.3[a]	4.0±0.8[b]	3.7±0.5[b]
Total	13	4.0±0.3[b]	4.6±0.2[b]	3.6±0.3[b]	4.8±0.3[a]	4.6±0.4[a]
Protein	20	5.1±0.5[b]	5.9±0.3[b]	4.8±0.4[b]	5.8±0.4[a]	6.0±0.4[a]
(Gm/100ml)	27	6.2±0.4[b]	7.2±0.2[a]	6.1±0.5[b]	7.1±0.5[a]	6.8 ±0.6[ab]
Albumin	13	1.9±0.2[b]	2.2±0.2[a]	1.6±0.3[b]	2.2±0.4[a]	2.3±0.3[a]
(Gm/100ml)	20	2.1±0.2[a]	2.5±0.1[a]	1.8±0.2[b]	2.5±0.2[ab]	2.7±0.3[a]
	27	2.4±0.3[b]	3.2 ±0.2[a]	2.3±0.3[b]	3.1 ±0.3[a]	3.0±0.2[a]
Globulin	13	2.1±0.3	2.4±0.2	2.0±0.1	2.6±0.3	2.3±0.2
(Gm/100ml)	20	3.0±0.1	3.4±0.2	3.0±0.3	3.3±0.3	3.4±0.3
	27	3.7±0.1	4.0 ±0.1	3.8±0.1	4.0 ±0.2	3.9±0.4

a, b and c data in the same row followed by unlike letters differ significantly ($P<0.05$). ± S.E

As is becoming apparent, Ca and Vit D_3 indicated to influence the reproductive performance positively as well as metabolic activity. The best ($P<0.05$) improvement occurred when birds were fed on a diet containing 3.7% calcium and injected by 200 or 400 IU Vit D_3.

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