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Effects of parenteral gibberellic acid and dietary supplementaion of vitamin D_3 on egg quality and physiological characteristics in aged laying hens

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ABSTRACT

The aim of this study was to determine the effect of parenteral gibberellic acid (GA_3) and/or vitamin D_3 supplementation in diet on egg quality and blood physiological characteristics in aged laying hens. A total of 270 Lohmann Brown Classic laying hens aging 73-week were randomly assigned to equal three treatment groups (T1, T2 and T3) with equal 3 replicas in each group. The birds of group T1 (control group) were injected subcutaneously (SC) with sesame oil at 0.2 mL/kg body weight. The birds of group T2 were given with GA_3 at 400 µg/kg b.wt., SC, whereas group T3 had diet containing vitamin D_3 at 500 IU/kg feed. Relative weight of albumen and egg shell, Haugh unit, shell thickness, serum glucose, serum calcium, serum phosphorous, serum estradiol, and bone calcium absorption were significantly increased in the birds of group T2 and T3. On the other hand, relative weight of yolk, yolk cholesterol, and serum cholesterol were significantly decreased in group T2 and T3 as compared to group T1. However, serum protein and albumen were unaffected in the treatments. In conclusion, the parenteral GA_3 and vitamin D_3 supplementation in diet could improve egg quality traits and serum blood biochemical perperties in agend laying hens.

Keywords

Age, Blood parameters, Egg quality, Gibberellic acid, Layer, Vitamin D3

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INTRODUCTION

Huge economic loss can be occurred in egg industry due to decrease in egg shell quality, and bone quality of hen. Aged hens lay larger egg as compared to younger hens. Therefore, occurrence of poor egg shell quality and tibia dysfunction may be found in aged laying hens (Hansen et al., 2003). Zeidler (2001) noticed that about 10% eggs are cracked or broken between laying farm and retail sales. However, this problem might be expanded with aged hens where the egg shell quality, especially strength (Rodriguez-Navarro et al., 2002) or thickness (Onderci et al., 2006) and proportion of albumen are decreased.

Aged hens have less ability to maintain calcium homeostasis which causes increasing incidence of soft and broken shell eggs. The reasons of this problem might be due to change in hormone profiles, decreased sensitivity of tissues to hormone action, and diminished ability of the hen to transport calcium at the duodenum (Al-Batshan et al., 1994; Hansen, 1998, 2000).

The decreasement of estrogen level with age caused reduction in concentration of circulating 1, 25(OH) $2D_3$ (an essential component to maintain calcium homeostasis). Dietary calcium, phosphorus and vitamin D_3 are often used to improve egg quality and bone structure (Watkins and Seifert, 1997). The complex interactions between calcium and estrogen also include estrogen-activation of vitamin D, and enhancement of calcium transport from the gut.

Hansen et al. (2003) reported that circulating estrogen and its receptor in the tissue could be declined with age leading to breakdown of calcium regulating mechanism. Al-Batshan et al. (1994) mentioned that bone mineral content was decreased in aged hen as compared to the birds at sexual maturity stage.

Use of exogenous estrogen or natural hormones as gibberellic acid (GA_3), or feeding high levels of vitamin D_3 could be the alternate solutions of the problems arised from decreased estrogen levels in hens. Several studies showed beneficial effecets of using GA_3 showing androgenic and estrogenic like actions in mammals and poultry (El-Komy, 2003; El-Sebai et al., 2003; Abdel-Fattah et al., 2007; El-Komy et al., 2008). GA_3 is one of at least 52 known gibberellins that enhance production and egg shell quality. Previously, we showed significant improvement in egg production by using injection of GA_3 and vitamin D_3 supplementation to laying diets (Ali et al., 2010). However, few data are available on use of GA_3 in aged laying hens. Thus, the objective of this study was to determine the influences of treatment with GA_3 alone or fortified by vitamin D_3 on egg quality and blood biochemical characteristics in aged laying hens.

MATERIALS AND METHODS

Chicken, housing and treatments: Two hundred and seventy Lohmann Brown classic laying hens aging 73-week at approximately 55% production were used in this study. The hens randomized in 9 floor pens (30 hens/pen) were bedded with wood shaving provided with one hanging feeder and waterer. Diet for the hens contained- 2,800 kcal of metabolizable energy (ME) per kg of feed, 3.7% calcium, 0.27% available phosphorus, and 15.0% crude protein. The hens were provided with 130 g/bird/day; this exceeded the recommended level (120 g/bird/day) as the experiment was conducted in winter season when temperature was decreased and the facilities could not control the temperature. The water was provided *ad libitum*. The photoperiod was 16L : 8D.

The study was conducted at Poultry Research Station of Office of Agricultural Research under Ministry of Agriculture (30 km west of Baghdad) during the period from 4/01/2009 to 28/03/2009. Treatments were considered as: T1, control groups were injected subcutaneously with 0.2 mL of the injection solution (1 : 11 ethanol : sesame oil, with addition 1 mg $NaHCO_3$/0.1 mL injection solution), T2 same as T1 but the mixture contained 400 µg of GA_3/kg b.wt., and T3, as in T2 but basal diet containing 500 IU of vitamin D_3/kg of feed.

Egg quality measurements: To measure the egg quality, 9 eggs per treatment (3 eggs/replicate) were collected based on the average egg weight and were stored for 24 h at 4°C prior testing. Parameters included for egg quality measurements were egg shell thickness, relative weights of shell, yolk, albumen and Haugh unit ($100* \log(H+7.57-1.7*W^{0.37}$; H, albumen height in mm; W, egg weight in g). Albumen height was determined when eggs broken onto smooth level surface and then measured at the two heights points on opposite sides of yolk using Ames micrometers containing three bases. Shell thickness was determined by measuring the mean of two locations on the egg (air cell and the sharp end) using digital micrometer. Yolk weight was calculated by separating from albumen gently and then putting on smooth paper to leave adhered albumen that was attached with the yolk. Albumen weight was calculated by subtracting the yolk plus shell from total egg weight.

Laboratory analysis for yolk cholesterol, serum biochemical and tibia calcium: Egg yolk cholesterol was measured by using 3 eggs per replicate at the end of the treatment period (8 weeks, from 73 to 80 weeks of age). The analysis was conducted according to the Chen et al. (2005). Total serum protein, albumin, glucose, cholesterol, calcium and phosphorus contents were measured by using commercial kits (Spanish Linear Company). At 80 weeks of age, 6 blood samples were collected per treatment in an unheparinized test tubes via brachial vein at 0900h am (1 h after lighting) and allowed to clot. Serum was obtained by centrifugation at 3000xg for 15 min and then frozen at -20°C until analysis. Calcium in tibia was measured in 6 hens per treatment. Tibia were obtained after slaughtering the hens, and all adhesive soft tissue surrounded the tibia were removed from the cut tibia. Tibia samples were dried at 65°C for 24h to maintain mineral to be stable without losing, and ashed in a muffle furnace at 600°C for 3h. Tibia calcium percentage was measured by using atomic absorption flame spectrophotometers and the values were compared with standard curve as described in AOAC (1980). Calcium retention in body was measured after collecting the excreta from hens at 80 weeks of age according to the treatment. The collected excreta were weighed and kept in plastic bags and dried at 65°C for 24h, and then ashed in a muffle furnace at 600°C for 3h. Calcium in excreta was also measured according to the guidelines of AOAC (1980), and the calcium retention in body were calculated {(calcium intake - calcium in

excreta)/calcium intake *100} where the calcium intake was calculated (calcium intake = calcium in diet *feed intake).

β 17 estradiol hormone: Three periods of estimation (0800 am, 1,000 am and 1,300 pm) were used. The methodology for determination of β 17 estradiol hormones was done by using radioimmunoassay (RIA) as outlined in kit provided from French Immunotech Company. The procedure that imposed to obtain β 17 estradiol hormones from hen's serum involved by adding 100 µL of neutral serum, control serum and sample serum to test tube containing antibody buffer. Also, 500 µL from radio receptor solution was added to the three models (samples) and agitated by hand until proper mixing. The tubes of samples were incubated at 18-25°C for 3h, and agitated by automated shaker at 350 rpm. After complete shaking, the RIA activity was counted by reading the radio activity of iodine isotopes associated with estradiol conjugated with antibodies. The radio assay activity was then read by using gamma counters at level of I^{125} isotopes. Concentration of serum estradiol was obtained by using standard curve provided with kit.

Statistical analysis: The data were analyzed by using GLM procedure of SAS (SAS Institute, 1996). Duncan's multiple range was used to detect differences among groups at level $p<0.05$.

RESULTS AND DISCUSSION

Relative weight of albumen, Haugh unit, relative weight of yolk and shell, and shell thickness for different period of measurement are present in **Table 1**. The subcutaneous injection of GA_3 to aged hens fed basal diets either unsupplemented (T2) or supplemented with vitamin D_3 (T3) increased relative weight of albumen and Haugh unit during overall period from 74 to 80 weeks of age ($p<0.05$). The relative weight of yolk was decreased ($p<0.05$) after 1-2 weeks of treatment in T2 or T3 as compared to T1. Improvement of albumen in eggs of hens injected with GA_3 might be due to GA_3 having an estrogenic effect. **Figure 5** revealed that increases in estradiol in serum in the hens that were injected with GA_3 and fed diet containing vitamin D_3. When level of estrogen increase could stimulate magnum to secrete albumen the oviduct size increased. El-Komy (2003) and El-Sebai et al. (2003) noticed that albumen weight was increased due to injection of GA_3 to the laying Japanese quail. In contrast, El-Sheikh and Hanafy (2006) and Abdel-Fattah et al. (2007) found that neither GA_3 nor vitamin D_3 could significantly improve internal egg quality.

Aged hens tend to decrease egg production and egg shell quality due to the reduction of circulating estrogen and its receptors in the tissue which caused reduction in calcium regulation mechanism (AL-Batshan et al., 1994; Hansen et al., 2003). In the present study, the relative weight of shell and shell thickness were increased in T3 and T2 as compared to control group (T1). Therefore, hens producing egg with superior egg shell quality found in T2 and T3 treatments, and had higher serum calcium and phosphorus, as shown in **Figure 1.**

The higher level of estradiol in the serum in the hens injected with GA_3 or GA_3 and dietary supplementation of vitamin D_3 caused increase in parathyroid hormone receptors leading to increased 1*a*-hydroxylase activity, vitamin D_3 synthesis, and increased Ca-binding protein calbindin D28K (CaBP D28K) in the intestine leading to increase calcium transport and subsequently increase egg shell quality (Klandorf et al., 1992; Elaroussi et al., 1993). El-Sebai et al. (2003) and Abdel-Fattah et al. (2007) showed improvement in egg shell quality of Japanese quail fed diet supplmented with GA_3.

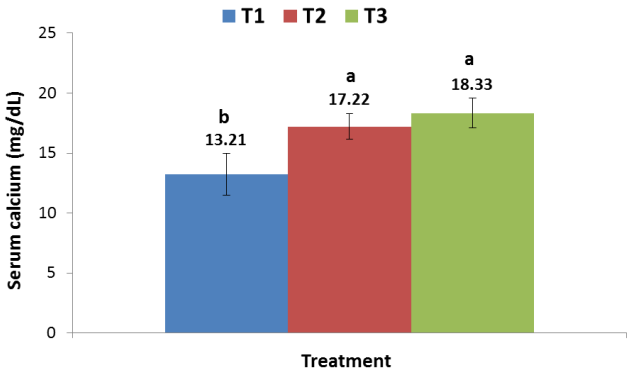

Figure 1: The effect of gibberellic acid (GA_3) and/or vitamin D_3 supplementation to diet on blood serum of calcium levels in aged laying hens. Bars with different superscripts differred significantly ($p<0.05$). T1, control birds were injected subcutaneously with 0.2 mL of injection solution/kg of body weight, T2 as in T1 but mixture containing 400 µg of GA_3/kg, and T3, as in T2 but fed basal diet containing 500 IU of vitamin D_3/kg of feed. Data presented are means±Standard deviation.

Table 2 showed cholesterol level in egg yolk and serum. The injected with GA_3 either alone (T2) or combined with diet containing supplemental vitamin D_3 with level of 500 IU/kg of feed (T3) caused significant reduction in yolk and serum cholesterol as compared to control group (T1). On the other hand, the level of reduction in yolk cholesterol in T2 and T3 was about 22% and 14%, respectively. Furthermore, The level of reduction in serum cholesterol in T2 and T3 was about 27% and 33%, respectively. The possible reasons of this result might be due to increase in egg

Table 1: The effect of injected with gibberellic acid (GA_3) and /or vitamin D3 supplementation to diet on internal egg quality and egg shell weight quality (mean± SEM) of aged laying hens.

Parameters	Treatments[1]			*p*-value
	T1	T2	T3	
74 WK				
Relative albumen weight (%)	63.6±0.34	63.9±1.38	64.1±1.17	0.6574
Haugh unit	86.6±2.07	90.3±3.32	87.9±0.83	0.1144
Relative yolk weight (%)	26.4±0.33	25.9±1.15	25.9±1.22	0.4231
Relative shell weight (%)	10.0±0.15	10.1±0.29	10.1±0.24	0.7654
Shell thickness (mm)	0.351±0.006	0.353±0.003	0.352±0.001	0.3328
76 WK				
Relative albumen weight (%)	63.3±1.46	64.6±0.63	64.5±0.54	0.7690
Haugh unit	84.2±4.24	91.3±0.97	90.8±2.61	0.1543
Relative yolk weight (%)	26.9±1.17	25.2±0.53	25.2±0.42	0.5876
Relative shell weight (%)	9.8±0.35	10.2±0.10	10.4±0.14	0.4152
Shell thickness (mm)	0.345±0.002^{b}	0.354±0.002^{a}	0.353±0.001^{a}	0.0342
78 WK				
Relative albumen weight (%)	62.4±0.50	65.2±0.49	65.3±0.86	0.0501
Haugh unit	82.1±2.34^{b}	89.3±1.15^{a}	86.9±1.24^{a}	0.0221
Relative yolk weight (%)	27.9±0.39^{a}	24.6±0.36^{b}	25.4±0.87^{b}	0.0178
Relative shell weight (%)	9.8±0.35^{b}	10.2±0.10^{a}	10.4±0.14^{a}	0.0442
Shell thickness (mm)	0.345±0.002^{b}	0.352±0.001^{a}	0.354±0.001^{a}	0.0221
80 WK				
Relative albumen weight (%)	62.3±1.15^{b}	64.6±0.29^{a}	64.0±0.53^{a}	0.0393
Haugh unit	77.6±4.35^{b}	86.6±0.86^{a}	87.2±1.20^{a}	0.0256
Relative yolk weight (%)	28.1±1.14^{a}	25.0±0.17^{b}	25.7±0.40^{b}	0.0467
Relative shell weight (%)	9.7±0.20	10.2±0.12	10.3±0.17	0.0500
Shell thickness (mm)	0.343±0.003	0.350±0.001	0.352±0.003	0.5643
Overall mean (74-80 WK)				
Relative albumen weight (%)	62.9±0.43^{b}	64.6±0.35^{a}	64.2±0.54^{a}	0.0491
Haugh unit	82.6±1.90^{b}	89.4±1.22^{a}	88.7±0.79^{a}	0.0245
Relative yolk weight (%)	27.3±0.50^{a}	25.2±0.26^{b}	25.5±0.56^{b}	0.0465
Relative shell weight (%)	9.8±0.08^{b}	10.2±0.10^{a}	10.2±0.03^{a}	0.0499
Shell thickness (mm)	0.346±0.001^{b}	0.352±0.001^{a}	0.353±0.001^{a}	0.0511

^{abc} means in row with different superscripts differ significantly (p<0.05). [1] T1, control were injected subcutaneously with 0.2 mL of injection solution/kg of body weight of, T2 as in T1 but mixture containing 400 µg of GA_3/kg and T3, as in T2 but fed basal diet containing 500 IU of vitamin D3/kg of feed, WK = weeks

Table 2: The effect of injected with gibberellic acid (GA_3) and /or vitamin D3 supplementation to diet on yolk cholesterol, serum cholesterol, serum protein, serum albumin and serum glucose (mean± SEM) of aged laying hens at 80 week of age.

Parameters	Treatments[1]			*p*-value
	T1	T2	T3	
Yolk cholesterol (mg/g yolk)	16.84± 066^{a}	13.02± 0.41^{b}	14.54± 0.02^{b}	0.0389
Serum cholesterol (mg/dL)	137.1± 13.80^{a}	98.8± 11.63^{b}	91.2± 5.85^{b}	0.0498
Serum protein (g/dL)	5.04± 0.46	6.94± 0.77	6.63± 0.41	0.1568
Serum albumin (g/dL)	1.82± 0.11	2.23± 0.13	1.97± 0.13	0.2134
Serum glucose (mg/dL)	190.8± 5.29^{b}	220.5± 7.14^{a}	202.6± 8.64^{ab}	0.0465

^{abc} means in row with different superscripts differ significantly (p<0.05); [1] T1, control were injected subcutaneously with 0.2 mL of injection solution /kg of b.wt., T2 as in T1 but mixture containing 400 µg of GA_3/ kg and T3, as in T2 but fed basal diet containing 500 IU of vitamin D3 / kg of feed.

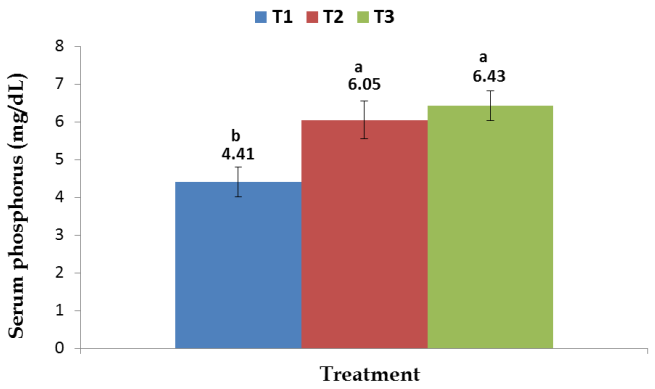

Figure 2: The effect of gibberellic acid (GA_3) injection and/or vitamin D_3 supplementation to diet on blood serum of phosphorus levels in aged laying hens. Bars with different superscripts differred significantly (p<0.05). T1, control birds were injected subcutaneously with 0.2 mL of injection solution/kg of body weight, T2 as in T1 but mixture containing 400 µg of GA_3/kg, and T3, as in T2 but fed basal diet containing 500 IU of vitamin D3/kg of feed. Data presented are means±Standard deviation.

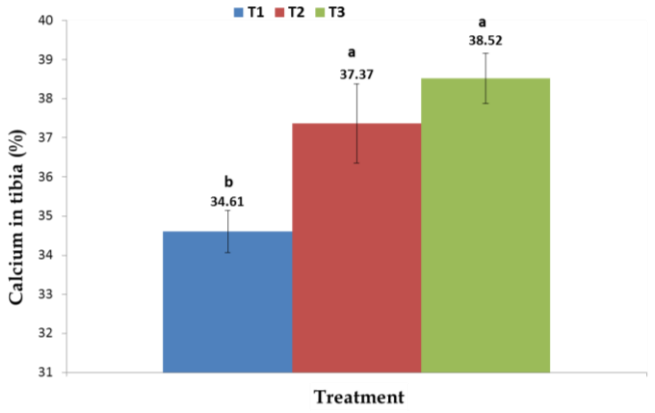

Figure 3: The effect of gibberellic acid (GA_3) and/or vitamin D_3 supplementation to diet on calcium concentration in tibia bone at 80 weeks of age. Bars with different superscripts differred significantly (p<0.05). T1, control birds were injected subcutaneously with 0.2 mL of injection solution/kg of body weight, T2 as in T1 but mixture containing 400 µg of GA_3/kg, and T3, as in T2 but fed basal diet containing 500 IU of vitamin D3/kg of feed. Data presented are means ±Standard deviation.

production and decrease of relative weight of yolk that received GA_3 and vitamin D_3 (**Table 1**), as describe by Ali et al. (2010). The low level of egg production and greater egg yolk in aged hens could be due to increasing egg yolk cholesterol. Basmacioglu and Ergul (2005) found negative correlations between mg/g yolk cholesterol content and yolk weight and positive correlations between mg/egg cholesterol content and yolk weight for two genotypes of layer chickens (white and brown). Furthermore, it was also noticed negative correlations between mg/g yolk cholesterol content and egg production. The role of GA_3 was investigated by El-Sheikh and Hanafy (2006) who observed that increases in egg yolk cholesterol in quail treated with GA_3 at 53 weeks of age. Rath et al. (1996) showed increased serum cholesterol due to estradiol implant dried pellets with 10 mg/ kg of body weight. Jorde and Grimnes (2011) showed that low level of vitamin D_3 was linked to elevated serum cholesterol levels in human. In contrast, Sloan et al. (1994) showed no significant differences in cholesterol content either in egg yolk or in serum due to cholecalciferol supplementation to non-aged layers. As shown in **Table 2**, there were no significant differences in total serum protein and albumen due to treatment with GA_3 and vitamin D_3 supplementation. This is in agreement with the findings of Abdel-Fattah et al. (2007). However, the total serum glucose was increased significantly in T2 as compared to the control (T1) and no difference were found between the treatment groups (T2 and T3), which might be related to high level of egg production, as because hens need more blood glucose to sustain high level of egg output. El-Komy (2003) noticed significant increase in blood glucose concentration in the birds treated with GA_3.

Serum concentration of calcium and phosphorus are illustrated in **Figure 1** and **2**; treatments were affected significantly on these two traits where the T3 exhibited higher calcium and phosphorus in the serum followed by T2 and T1. These findings supported previous studies of Afifi and Abo-Taleb (2002), El-Sheikh and Hanafy (2006), Mostafa and Abdel-Mageed (2006), and Abdel-Fattah et al. (2007); in which serum calcium and phosphorus levels were increased due to the supplementation of GA_3. Vitamin D_3 and it's derivatives cause elevation of serum calcium and phosphorus levels by regulating calcium absorption (Mostafa and Abdel-Mageed, 2006), and enhance intestinal phytase that stimulate phosphorus absorption (Yan and Waldroup, 2006).

Furthermore, both calcium level in tibia (**Figure 3**) and retention in body (**Figure 4**) were significantly increased in T3 and T2 as compared to control (p<0.05). Administration of 1,25-dihydroxycholecalciferol via diet essentially places the active hormone at the site (the intestine) where it can exert an effect on Ca absorption as intact, activated steroid hormone. Frost and Roland (1991) found that supplementing diet with calcium or vitamin D_3 or both improved bone breaking strength. Newman and Leeson (1999) concluded that possibility of improving bone strength by adding 5 µg/ kg of 1,25(OH)2D3. Frost et al. (1990) indicated that vitamin D_3 could provide enough 1,25(OH)2D3 through hydroxylation to sustain production and shell quality but not tibia weight or strength in aged hens.

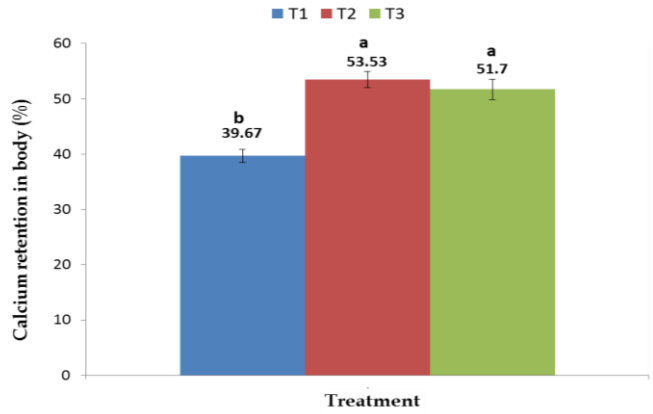

Figure 4: The effect of gibberellic acid (GA_3) and/or vitamin D_3 supplementation to diet on calcium retention in hens body at 80 weeks of age. Bars with different superscripts differed significantly (p<0.05). T1, control birds were injected subcutaneously with 0.2 mL of injection solution/kg of body weight, T2 as in T1 but mixture containing 400 µg of GA_3/kg, and T3, as in T2 but fed basal diet containing 500 IU of vitamin D_3/kg of feed. Data presented are means ±Standard deviation.

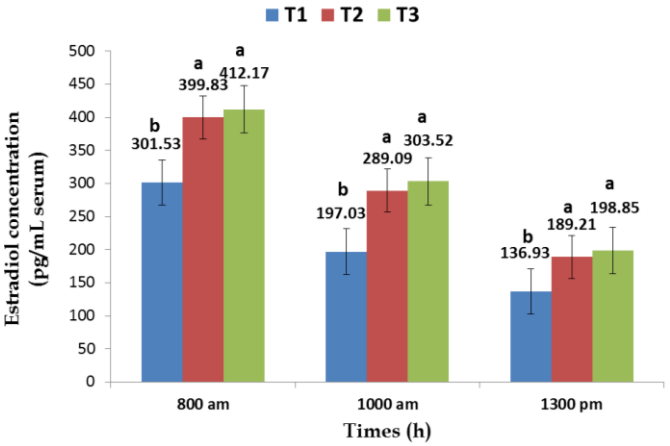

Figure 5: The effect of gibberellic acid (GA_3) and/or vitamin D_3 supplementation to diet on estradiol concentration in serum at 80 weeks of age. Bars with different superscripts differed significantly (p<0.05). T1, control birds were injected subcutaneously with 0.2 mL of injection solution/kg of body weight, T2 as in T1 but mixture containing 400 µg of GA_3/kg, and T3, as in T2 but fed basal diet containing 500 IU of vitamin D_3/kg of feed. Data presented are means ±Standard deviation.

Serum estradiol levels at 80 weeks of age measured at different period in day time (800 am, 1000 am and 1300 pm) were affected by treatments where T2 and T3 exhibited higher levels of estradiol as compared to T1 (**Figure 5**). The level of serum estradiol was higher in the initial time at 0800 am (~400 pg/mL) and gradually reduced at 1000 am (~300 pg/ mL) and 1300 (~200 pg/ mL) pm in T3 as compared with those in T1, in which the values were ~300 pg/mL, ~200 pg/mL and ~130 pg/mL for three different times, respectively. There was also marked variation in the pattern of change in serum estradiol levels during different times of the day. This might be due to lights coming on at 0800 am and nesting hens left their nests to eat feed and water which caused dropping in oviposition around 0900 am and increase onward. Zakaria and Omar (2013) described changing of ovipositions occurred before 1100 am in the mid-age flock as 68%, and the remaining 32% of ovipositions occurred from 1200 to 1700 pm. Qin et al. (2013) found that the plasma estradiol concentration increased during oviposition and rebounded to intermediate levels within 5h due to increasing in the plasma concentration of luteinizing hormone. On the other hand, Williams et al. (2004) found that the plasma estradiol levels in European starling Sturnus vulgaris varied significantly with stage of ovarian development; increasing from low levels (55 pg/mL) in non-breeders to 201 pg/mL in pre-laying birds with 3–4 yolk follicles. El-Komy (2003) and El-Komy et al. (2008) concluded that GA_3 can stimulate estrogen secretion in hens.

CONCLUSION

The results indicated that injection with GA_3 alone or synergism with vitamin D_3 supplementation to basal diet could improve egg quality traits and serum blood biochemical's in aged laying hens.

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