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ORIGINAL RESEARCH ARTICLE

Influence of supplemental selenium and α–tocopherol on performance and blood profile of laying chickens

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ABSTRACT

Effect of dietary supplement of selenium and α–tocopherol on performance and blood profile of laying hen was assessed in a trial lasting 20 weeks. **ISA brown pullets (n=192) at week 55 of life were randomly assigned to six experimental diets:** ***Basal diet (T1) was without any supplemental selenium or α–tocopherol, T2: Basal + 0.5 mg/kg selenium, T3: Basal diet + 1.0 mg/kg selenium, T4: Basal diet + 1.5 mg/kg selenium, T5: Basal diet + 20 mg/kg α–tocopherol, T6: Basal diet + 40 mg/kg α–tocopherol.*** **Each treatment was replicated four times and a replicate comprised eight hens in a completely randomised design. P*****erformance parameters were not significantly affected (P>0.05) by supplemental selenium and α–tocopherol. The heterophil:lymphocyte ratio was significantly (p<0.05) higher in hens on T6 (0.143) and least in hens on T1 (0.128). Hens on T6 had significantly higher (P<0.05) packed cell volume (34.95%), red blood cells (2.79x10^6 IU/L), haemoglobin (11.81 g/100mL), white blood cells (21.41x10^6), lymphocytes (79.75%), and heterophil (11.41%) than those on Ti which recorded 30.95% PCV, 2.11 x 10^6 IU/L RBC, 10.35 g/100mL Hb, 19.55 x10^6 mm WBC, 78.51% lymphocyte and 10.05% heterophil. Similarly, hens on T6 had significantly higher (P<0.05) serum total cholesterol (90.51 mg/dL), high density lipoprotein (23.31 mg/dL), low density lipoprotein (35.54 mg/dL), calcium (14.57%) and phosphorus (4.39%) but lower triglyceride (31.66 mg/dL) than hens on other dietary supplements. Serum total protein, globulin, creatinine, urea, alkaline phosphatase, aspartate amino transferase and alanine amino transferase for hens on T6 was significantly higher (P<0.05) compared with those on T1. Supplemental α–tocopherol did not influence the performance of hens in this study. However, supplementation up to 40 mg/kg α–tocopherol elevated the blood profile of laying hen.***

Keywords: laying hens, minerals, haematology, serum biochemical indices

INTRODUCTION

Reduced performance of commercial hens is commonly traced to environmental stress. Inclement environmental conditions easily results to oxidative stress at the cellular level (Surai, 2015). Oxidative stress is known to be an important contributing factor of many chronic diseases (Mahima *et al*., 2013). Dietary supplement of vitamin and mineral are the commonly used techniques in alleviating the damaging effects of heat stress (Ismail, 2013). Ipek and Dikmen (2014) reported that the negative effects of the environment on laying hens could be mitigated by the use of vitamin and mineral.

Vitamin E (α-tocopherol) is an important lipid-soluble antioxidant. It performs its functions as antioxidant in the glutathione peroxidase pathway (Wefers and Sies, 1988) and protect cell membranes from oxidation by reacting with lipid radicals produced in the peroxidation chain reaction (Herrera and Barbas, 2001; Traber and Atkinson, 2007). Vitamin E is effective in ensuring adequate stability of the diet and in protecting the bird's immune system thereby improving performance (Bou *et al*., 2004). Selenium is an important natural antioxidant which is essential in many metabolic processes in living organisms. Selenium has beneficial effects on both the innate and adaptive (cell-mediated and antibody-mediated) immune responses (Brown and Arthur, 2001). Sahin *et al*. (2002) reported that higher dietary vitamin E resulted in decreased serum cholesterol concentration of Japanese quails. Selenium and vitamin E as antioxidant play important roles in maintaining birds health, productivity and reproductive characteristics (Surai, 2000).

However, poultry cannot synthesize α-tocopherol and selenium, their requirements must be supplied from dietary sources (Puthpongsiriporn *et al.*, 2001). Metabolic roles of selenium are similar to

vitamin E, synergy exits between selenium and vitamin E, they both act as the primer antioxidant by suppressing oxidative damages Marsh *et al*. (1981). A report of decreased body weights in chicks fed vitamin E and selenium deficient diets was documented by Marsh *et al.* (1981). Cantor *et al.* (2000) suggested a positive effect of selenium in diet of laying chicken on egg production, while the positive influence of dietary selenium on blood profile was reported by Jiakui and Xiaolong (2004). Dietary vitamin E supplementation was reported to improve growth performance and boost immunity in animal nutritional and physiological research (Gatlin, 2002). However, another study revealed no significant changes in plasma lipoprotein concentrations after antioxidant supplementation (Brown *et al*., 1994). There is dearth of information on the effect of dietary supplementation of α-tocopherol and selenium on blood profile of laying hens at the late laying phase. Therefore, this study was aimed at evaluating the effect of dietary supplemental selenium and vitamin E on egg production and blood profile of laying hens at the late laying phase.

MATERIALS AND METHODS

Experimental location and animal allotment

The experiment was carried out at the Poultry unit, Teaching and Research Farm, University of Ibadan, Ibadan. ISA brown hens (n=192) at week 55 of life were initially raised on a basal diets for two weeks and subsequently assigned to six experimental diets in a completely randomized design. Each treatment was replicated four times with eight hens per replicate. The experiment lasted 20 weeks.

Data collection and analysis

Pen temperature-humidity index: During 57th and 72nd weeks of the trial, pen temperature-humidity index were monitored three times daily, between the hours of 7:00-8:00, 12:00-13:00 and 17:00-18:00 using digital thermo-hygrotherms placed strategically at different locations within the pen house. Temperature Humidity index (THI) was calculated from the average ambient temperature and relative humidity obtained using the formula: $THI = 0.6\ T_{db} + 0.4\ T_{wb}$, where, T_{db} = dry bulb temperature and T_{wb}=wet bulb temperature.

Experimental diets

Details of the basal experimental diet fed to pullets is shown in Table 1 and has been previously documented (Jemiseye, 2018; Jemiseye and Ogunwole, 2018). Basal diet was supplemented with selenium and α–tocopherol as follows: Basal diet (T1) was without any supplemental selenium or α–tocopherol, T2: Basal + 0.5 mg/kg selenium, T3: Basal diet + 1.0 mg/kg selenium, T4: Basal diet + 1.5 mg/kg selenium, T5: Basal diet + 20 mg/kg α–tocopherol, T6: Basal diet + 40 mg/kg α–tocopherol. Feed and water were supplied *ad libitum* to the laying hens. The composition of the basal diet is shown in Table 1.

Performance parameters

Records of daily egg production were taken, hen day egg production was calculated by number of eggs laid divided by number of chicken at the period of data collection. Feed intake was calculated by subtracting left over from feed offered to the hens. Egg were weighed with sensitive scale to obtain egg weight and egg mass was calculated from the percent hen day egg production and average egg weight. Feed conversion ratio was obtained with the formula:

$$\text{FCR (per g egg mass)} = \frac{\text{Weight (g) of feed consumed}}{\text{Weight (g) of egg produced}}$$

Blood collection

At week 58, blood was collected from two birds per replicate via jugular vein into heparinized test tubes for haematology and others without EDTA for the biochemical indices.

Haematological parameters: Packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC) and differential counts were determined (Schalm *et al.,* 1975).

Serum biochemical analysis: Total protein was determined by Biuret method as described by Kohn and Allen (1995), albumin was determined using Bromocresol green method as described by Peters *et al.* (1982). The globulin concentration was obtained by subtracting albumin value from total protein. Urea and creatinine were determined by the method of Harr (2006). Alanine amino transferase (ALT),

Table 1: Composition of experimental basal diet

Ingredients	%
Corn	50.00
Wheat offal	11.00
Soya bean meal	22.00
Palm kernel cake	11.25
Dicalcium phosphate	1.30
Salt	0.30
Methionine	0.15
Lysine	0.15
Vitamin/mineral premix	0.30
Mycofix ®	0.15
Biotronine	0.30
Oyster shell	3.00
Total	100.00
Calculated Nutrients	
ME (Kcal/Kg)	2736.01
Protein (%)	17.23
Calcium (%)	4.18
Phosphorus (%)	0.17
Methionine (%)	0.51
Lysine (%)	0.87

Premix- Vitamin A–10,000,000IU, Vitamin D3–500IU, Vitamin E–40,000mg, Vitamin K–2,000mg, Vitamin B1–1,500mg, Vitamin B2–4,000mg, Vitamin B6–40,000mg, Vitamin B12–200mg, Niacin–40,000mg, Panthothenic–10,000mg, Folic– 1,000mg, Biotin–100mg, Choline Chloride– 300,000mg, Manganese–80,000mg, Zinc–60,000mg, Iron–40,000mg, Copper–80,000mg, Iodine–800mg, Selenium–200mg, Cobalt–300mg, Antioxidant–100,000mg.*

aspartate amino transferase (AST) and alkaline phosphatase (ALP) activities were determined using spectrophotometric method as described by Rej and Hoder (1983) Calcium and phosphorus were analysed according to AOAC (2000). Total cholesterol, high density lipoprotein, low density lipoprotein and triglyceride were determined according to the methods of Gowenlock *et al*. (1988), Dacia and Lewis (1991), Friedewal *et al.* (1972) and Jacobs and VanDermark (1960), respectively.

Statistical analyses

Data were subjected to analyses of variance (SAS, 2003) and means were separated using Duncan multiple range test option of the same software at $\alpha_{0.05}$.

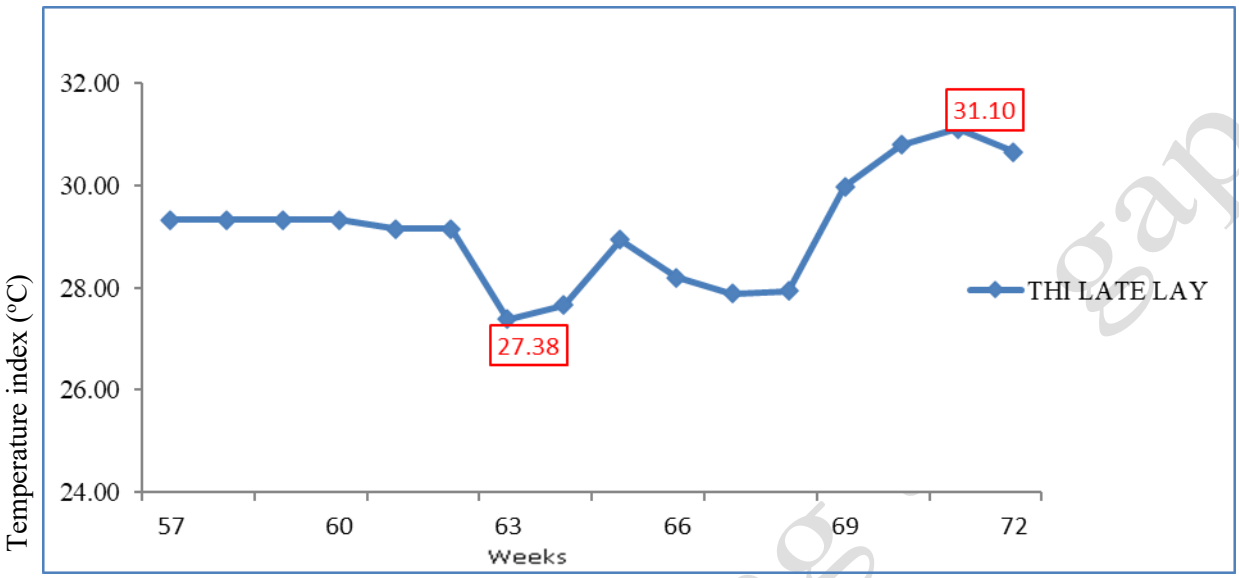
RESULTS AND DISCUSSION

Temperature-Humidity index of the pen house is shown in Figure 1. The maximum and minimum values were 31.10 and 27.38, respectively. The reported temperature and relative humidity were higher than thermoneutral temperature range (18-22 ^{o}C) of laying chicken reported by Charles (2002) which is an indication that the hens were perpetually stressed. Higher ambient temperature and relative humidity have direct implications on feed consumption. Hens tend to reduce feed consumption during heat stress thereby reducing nutrients needed in egg production (Arima *et al.,* 1976: Etches *et al*., 1995).

When hens are stressed in hot environment, corticosterone and catecolamines are produced so as to keep body temperature within the normal physiological range. The hormonal reactions that are released in response to stress have a negative effect on performance and laying hens' immunity (Freeman and Crapo, 1982).

Performance of laying hens fed supplemental selenium and α–tocopherol at the late laying phase is presented in Table 2. Varied dietary inclusion of supplemental selenium and α–tocopherol had no significant effects (P>0.05) on all performance parameters examined. Mohiti-Asli *et al.* (2010) reported no significant effect of vitamin E and selenium supplementation in the reduction of negative effects of high environmental temperature on performance of laying hens. Meanwhile, Sahin *et al.* (2001) reported that higher levels of α–tocopherol and higher dietary selenium inclusions led to improved feed intake.

The haematological parameters of laying hens fed supplemental selenium and α–tocopherol at the late laying phase is shown in Table 3. Haematological parameters are positive indicators of the physiological response of farm animals. Hens on 40 mg/kg α-tocopherol (T6) had significantly higher (P<0.05) PCV (34.95 %), RBC (2.79 x10^{6} IU/L), Hb (11.81 g/100mL), and WBC (21.41 x10^{6} mm), lymphoytes (79.75 %), heterophils (11.41 %) and heterophil/lymphocyte ratio (0.148) indices compared with those on the control diet (T1) and other treatments.



THI=Temperature Humidity Index

Figure 1: Temperature-Humidity Index of the experimental pen from August - December 2016

Table 2. Performance of laying hens fed diets supplemented with selenium and α – tocopherol at the late laying phase

Parameters	T1	T2	T3	T4	T5	T6	SEM
Hen day egg production (%)	65.03	69.49	67.04	60.96	72.59	58.34	2.31
Feed intake (g)	109.67	108.19	109.50	109.51	109.65	108.76	0.19
Egg weight (g)	62.28	62.58	58.29	58.25	59.81	60.54	0.67
Egg mass	40.63	43.15	38.97	35.60	43.59	35.55	1.50
Feed Conversion Ratio	2.70	2.51	2.81	3.10	2.52	3.10	0.28

SEM-Standard error of mean, T1- Basal diet, T2- Basal + 0.5 mg/kg selenium, T3- Basal diet + 1.0 mg/kg selenium, T4- Basal diet + 1.5 mg/kg selenium, T5- Basal diet + 20 mg/kg α–tocopherol, T6- Basal diet + 40 mg/kg α–tocopherol.

The observed higher WBC in hens on T6 could be a reflection of adequate nutrient content of the diet which suggest improved immunity status of the hens and an indication that hens were resistant to disease. The PCV is involved in nutrient absorption and oxygen transportation, increased PCV show a better nutrient transportation and increased primary and secondary polycythemia (NseAbasi, 2014). Haemoglobin has the physiological function of oxygen transportation to animal tissue for oxidation of ingested food so as to release energy for other body parts to function as well as carbon dioxide transportation out of animal body, therefore, increased haemoglobin level recorded for hens on T6 could suggest an enhanced oxygen carrying capacity of the hens. The heterophil lymphocyte ratio has been used as a dependable pointer of stress in chickens, increases in this ratio are an indicator of stress (Mariana *et al.,* 2018). The values obtained for heterophil lymphocyte ratio in this study were below the reference values suggested by Gross and Siegel (1993), this could be an indication that the hens were less stressed and able to withstand damaging effects of heat stress.

Table 3: Haematological parameters of laying hens fed diets supplemented with selenium and α – tocopherol at the late laying phase

Parameters	T1	T2	T3	T4	T5	T6	SEM	
PCV (%)	30.95^{f}	31.35^{e}	33.99^{d}	34.51^{b}	34.09^{c}	34.95^{a}	0.38
RBC (x100^{6} IU/L)	2.11^{f}	2.21^{e}	2.33^{d}	2.55^{b}	2.45^{c}	2.79^{a}	0.05
Hb (g/100mL)	10.35^{e}	11.55^{b}	10.99^{d}	11.59^{b}	11.05^{c}	11.81^{a}	0.12
WBC (x10^{6}mm)	19.55^{e}	20.75^{d}	21.07^{c}	21.25^{b}	21.21^{b}	21.41^{a}	0.15
LYMP (%)	78.51^{e}	78.71^{d}	78.95^{c}	79.39^{b}	78.95^{c}	79.75^{a}	0.10
HET (%)	10.05^{f}	10.39^{e}	10.88^{d}	11.19^{b}	11.06^{c}	11.41^{a}	0.11
HET:LYMP	0.128^{f}	0.131^{e}	0.137^{d}	0.141^{b}	0.140^{c}	0.143^{a}	0.07

^{a,b,c,d,e,f}Mean in the same row with different superscript are significantly different (P<0.05). SEM-Standard error of mean, T1- Basal diet, T2- Basal + 0.5 mg/kg selenium, T3- Basal diet + 1.0 mg/kg selenium, T4- Basal diet + 1.5 mg/kg selenium, T5- Basal diet + 20 mg/kg α–tocopherol, T6- Basal diet + 40 mg/kg α–tocopherol. Packed cell volume (PCV) Haemoglobin, (RBC) Red blood cells, (Hb), mean cell haemoglobin, (WBC) White blood cells, (LYMP) Lymphocyte, (HET) Heterophils

The serum biochemical parameters of hens fed supplemental selenium and vitamin E at the late laying phase is shown in Table 4. Increased high density lipoprotein (HDL) has been attributed to better health condition (Gordon *et al*., 1989). Cholesterol (mg/dL), high density lipoprotein (mg/dL) and low density lipoprotein (mg/dL) (90.51, 23.31 and 35.54) in hens on 40 mg/kg α-tocopherol (T6) were significantly higher than 88.89, 22.06 and 33.05 respectively, in hens on the control diet (T1). The reduced low density lipoprotein level observed in T1 (33.05 mg/dL) however may be attributed to the decreased total cholesterol in the serum. Kanchana and Jeyanthi (2010) observed reduced low density lipoprotein when laying hens diet was supplemented with vitamin E. Increased triglyceride (33.78 mg/dL) was recorded in hens on control diet compared with other dietary treatments. Rashidi *et al.* (2010) reported that increase in environmental temperature reduced feed consumption and hens tends to compensate their energy need through lipolysis of body lipids which results in increment in blood cholesterol and triglycerides. Significantly higher (P<0.05) glucose (mg/dL) level was recorded in hens fed T4 (92.69) and T6 (92.71), while lower value was recorded in those on control (90.05). Conversely, Sahin *et al*. (2002) reported that vitamin E supplementation markedly decreased blood glucose and cholesterol concentrations in Japanese quails under heat stress. Phosphorus (%) was found to be higher in hens on T6 (4.39) than in control (3.71) and other treatments. Sahin *et al*. (2002) reported increased plasma calcium and phosphorus concentrations in heat-stressed Japanese quails fed diet supplemented with vitamin E which conforms to observations in this study.

Table 4: Serum lipid and minerals of laying hens fed diets supplemented with selenium and α–tocopherol at the late laying phase

Parameters	T1	T2	T3	T4	T5	T6	SEM
Total CHO (mg/dL)	88.89^{f}	89.54^{e}	89.92^{d}	90.31^{b}	90.14^{c}	90.51^{a}	0.13
HDL (mg/dL)	22.06^{f}	22.52^{e}	23.04^{d}	23.20^{b}	23.14^{c}	23.31^{a}	0.11
LDL (mg/dL)	33.05^{f}	33.47^{d}	35.03^{c}	33.41^{e}	35.17^{b}	35.54^{a}	0.24
TG (mg/dL)	33.78^{a}	33.55^{c}	31.85^{d}	33.69^{b}	31.84^{d}	31.66^{e}	0.23
Glucose (mg/dL)	90.05^{e}	90.91^{d}	92.05^{c}	92.69^{a}	92.18^{b}	92.71^{a}	0.24
Ca (%)	13.05^{f}	13.31^{e}	14.13^{d}	14.33^{b}	14.22^{c}	14.57^{a}	0.13
P (%)	3.71^{f}	3.86^{e}	4.06^{d}	4.35^{b}	4.25^{c}	4.39^{a}	0.06

^{a,b,c,d,e,f}Mean in the same row with different superscript are significantly different (P<0.05). SEM-Standard error of mean, T1- Basal diet, T2- Basal + 0.5 mg/kg selenium, T3- Basal diet + 1.0 mg/kg selenium, T4- Basal diet + 1.5 mg/kg selenium, T5- Basal diet + 20 mg/kg α–tocopherol, T6- Basal diet + 40 mg/kg α–tocopherol. (CHO)Cholesterol, (HDL) High density lipoprotein, (LDL) Low density lipoprotein, (TG) Triglyceride, (Ca) Calcium, (P) Phosphorus

The serum biochemical parameters of laying hens fed supplemental selenium and α-tocopherol at the late laying phase are presented in Table 5. Total protein, globulin, albumin, creatinine and urea differed significantly (P<0.05) with dietary supplementation of selenium and α-tocopherol. Serum total protein is an indicator of protein digestion, absorption and utilization. Significantly higher (P<0.05) total protein was observed in the serum of hens on T4 (6.61 g/dL) and T6 (6.58 g/dL) than those on T1 (6.15 g/dL). Total proteins are used to access the avian body condition, it play a significant role in maintaining homeostasis and as a rapid substitute for indispensable amino acids, it also aid in transporting mineral and hormones (Yaman *et al.,* 2000). Uric acid is known as the major avian nitrogenous waste product and an important antioxidative agent (Dawson *et al*., 1991; Harr, 2002). Changes in protein catabolism are mainly revealed in serum uric acid concentrations. Creatinine is a byproduct of phosphocreatine breakdown in skeletal muscle, it is an important indicator of protein metabolism, concentration of creatinine is directly proportional to muscle mass it can be influenced by diet. (Szabo *et al*., 2005; Rajman *et al*., 2006).

This was contrary to the findings of El-Mallah (2011) who recorded a range values of 4.01-5.37 for total protein when selenium and vitamin E was fed solely or in combination to laying hens. An increased value obtained could be an indication that higher levels of both dietary selenium and α-tocopherol did not impair protein synthesis. Hens on T6 (3.39) had higher (P<0.05) level of globulin which suggests enhanced immunological potential of α-tocopherol on the hens (Nayak *et al*., 2004). The synthesis of albumin is reported to be in the liver and its main function is the transportation of hormones, vitamins and important minerals in the body (Melilo, 2007).

Table 5: Serum biochemical indices of laying hens fed diets supplemented with selenium and α-tocopherol at the late laying phase

Parameters	T1	T2	T3	T4	T5	T6	SEM
Total Protein (g/dL)	6.15^{c}	6.41^{b}	6.12^{c}	6.61^{a}	6.11^{c}	6.58^{a}	0.05
Globulin (g/dL)	3.06^{e}	2.20^{c}	3.11^{d}	3.31^{b}	3.10^{de}	3.39^{a}	0.03
Albumin (g/dL)	3.09^{c}	3.21^{b}	3.00^{c}	3.30^{a}	3.01^{c}	3.19^{b}	0.03
Creatinine (mg/dL)	1.40^{e}	1.49^{c}	1.45^{d}	1.57^{b}	1.57^{b}	1.71^{a}	0.02
Urea (mmol/dL)	13.49^{f}	13.77^{e}	13.93^{d}	14.25^{b}	14.05^{c}	14.39^{a}	0.07

^{a,b,c,d,e,f}*Means in the same row with different superscript are significantly different (P<0.05), SEM-Standard error of mean. T1- Basal diet, T2- Basal + 0.5 mg/kg selenium, T3- Basal diet + 1.0 mg/kg selenium, T4- Basal diet + 1.5 mg/kg selenium, T5- Basal diet + 20 mg/kg α–tocopherol, T6- Basal diet + 40 mg/kg α–tocopherol.*

Albumin in laying hens on T4 (3.30) was higher (P<0.05) than 3.09 in T1. Serum urea is often used as renal function test, liver functioning, hydration status as well as protein breakdown (Agboola *et al*., 2013). Serum urea level of hens on T6 (14.39) was significantly higher (P<0.05) than 13.49 in hens on T1. Creatinine (1.71) in hens on T6 was higher (P<0.05) than 1.40 (T1), 1.49 (T2), 1.45 (T3), 1.57 (T4) and 1.57 (T5).

Serum enzymes of hens fed diets supplemented with selenium and α-tocopherol at the late laying phase are presented in Table 6. Increased level of serum enzymes were suggestive of hepatic damage (Harr, 2006). The ALP, AST and ALT compositions differed significantly (P<0.05) with dietary supplementation of selenium and α-tocopherol. Hens on T6 had significantly higher (P<0.05) serum ALP (33.49 IU/L), AST (22.51 IU/L) and ALT (17.99 IU/L) than those on T1 which recorded 32.07 IU/L ALP, 21.13 IU/L AST and 17.19 IU/L ALT. The present findings indicated that higher level of supplemental α-tocopherol may not be effective in preventing hepatic damage.

Table 6: Serum enzymes of laying hens fed with diets supplemented with selenium and α-tocopherol at the late laying phase

Parameters	T1	T2	T3	T4	T5	T6	SEM
ALP (IU/L)	32.07[f]	32.30[e]	33.04[d]	33.30[b]	33.21[c]	33.49[a]	0.13
AST (IU/L)	21.13[f]	21.61[e]	22.05[d]	22.44[b]	22.21[c]	22.51[a]	0.12
ALT (IU/L)	17.19[f]	17.45[e]	17.63[d]	17.83[b]	17.76[c]	17.99[a]	0.06

[a,b,c,d,e,f]Means in the same row with different superscript are significantly different (P<0.05), ALT -Alanine amino transferase, AST-Aspartate amino transferase. ALP -Alkaline phosphatase. T1- Basal diet, T2- Basal + 0.5 mg/kg selenium, T3- Basal diet + 1.0 mg/kg selenium, T4- Basal diet + 1.5 mg/kg selenium, T5- Basal diet + 20 mg/kg α–tocopherol, T6- Basal diet + 40 mg/kg α–tocopherol. (ALP) Alkaline phosphatase, (AST) Aspartate amino transferase, (ALT) Alanine amino transferase

CONCLUSION

The present study demonstrated that dietary supplements of selenium and α–tocopherol had no effect on performance of laying hens at the late laying phase. However, supplemental dietary α–tocopherol at 40 mg/kg in the diet of hens had positive influence on the serum enzymes of the tested hens.

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