SELECTED BLOOD INDICES OF LAYING CHICKENS AS AFFECTED BY SUPPLEMENTAL SELENIUM AND VITAMIN E

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ABSTRACT
Effect of supplemental inorganic selenium and vitamin E on haematological and selected serum biochemical indices of laying chickens was assessed in this study. In a completely randomized design, ISA brown hens (n=192) at week 24 of laying, were assigned to six experimental diets: T1 (Basal diet), T2 (Basal diet + 0.5mg selenium/kg), T3: Basal diet + 1.0mg selenium/kg, T4 (Basal diet + 1.5mg selenium/kg), T5 (Basal diet + 20mg vitamin E/kg), T6 (Basal diet + 40mg vitamin E/kg). Each treatment was replicated four times with eight birds per replicate. At week 24 (early laying phase), blood was sampled from hens and analysed. Increased supplemental selenium and vitamin E did not affect (P>0.05) serum superoxide dismutase, aspartate amino transferase and phosphorus. Also, packed cell volume (PCV), red blood cells (RBC), haemoglobin (HB) and blood differential counts were not affected (P>0.05). However, serum calcium (mg/dL) was lowered from 11.60 in T1 to 6.83 in T4, increased vitamin E inclusion did not alter serum calcium level. Also, treatments had significant effects on alkaline phosphatase (ALP), catalase and alanine amino transferase (ALT). In this study, increased supplemental selenium and vitamin E affected blood indices in irregular trend.
Keywords: Blood cellular concentrations, supplemental selenium and vitamin E, Haematology, Serum biochemical indices.

INTRODUCTION
Vitamins and minerals are vital nutrients that are involved in both metabolic and physiological processes, critical for animal health and animal food production.Their specifications in animal feed by various international feed organisations (Agriculture Research Council and National Research Council) were with objective of preventing nutritional deficiencies. McDowell and Ward (2009), recommended optimal vitamin nutrition for good poultry performance in terms of weight gain, immune responses and disease resistance. Cunha (1985) and Nockels *et al.* (1996) reported that nutrient levels that are adequate for growth, feed efficiency and gestation may not be adequate for normal immunity for maximizing the animal's resistance to disease. It was reported that when formulating feed, nutritionists have to take into account several factors including stress management and immunity enhancement (Linge, 2005). In this respect, antioxidants nutrients play an important role in maintaining bird health, productive and reproductive performance (Surai, 2002).Vitamin E and Selenium are antioxidants that are related to immune function in domestic animals (Finch and Turner, 1996).Vitamin E, a fat-soluble vitamin, is an intracellular antioxidant for all cells. It functions as a chain breaking antioxidant which prevents free radical induced oxidative damage by trapping reactive oxyradicals in biological membranes (Packer, 1991). While selenium has sparing effects on vitamin E great attention has been focused on the role of vitamin E and selenium in protecting leukocytes and macrophages during phagocytosis, the mechanism whereby mammals immunologically kill invading bacteria. Both vitamin E and GSH-Px are antioxidants that protect phagocytic cells and surrounding tissues from oxidative attack by free radicals produced by the respiratory burst of neutrophils and macrophages during phagocytosis (Baker and Cohen, 1983; Baboir, 1984).
Therefore, the objective of this study was to evaluate the effect of dietary selenium and

vitamin E supplementation on blood parameters, serum and liver enzymes of laying chickens.

MATERIALS AND METHODS

Experimental Location and Animals Allotment

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan. A total of 192 ISA brown hens at the point of lay were assigned to six experimental diets in a completely randomized design experiment, each treatment was replicated four times with eight birds per replicate.

Experimental Diets

Basal diet was supplemented with selenium and vitamin E as follows: T1=Basal diet, T2=Basal diet + 0.5mg selenium/kg, T3= Basal diet + 1.0mg selenium/kg, T4= Basal diet + 1.5mg selenium/kg, T5=Basal diet + 20mg vitamin E/kg, T6=Basal diet + 40mg vitamin E/kg) Feed and water were supplied *ad libitum*.

Data Collection and Analysis

At week 24 week of laying, blood was collected from two birds per replicate viajugular vein into heparinized test tubes for haematology and another without EDTA for serum analyses. Haematological parameters; packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC) and differential counts were determined using standard procedures (Schalm *et al.,* 1975). Data were subjected to analysis of variance using the SAS (1999) package. Means were separated using Duncan's multiple range test of the same package.

RESULTS AND DISCUSSION

Serum biochemical indices: Supplementation of selenium and vitamin E did not have any significant effects (P>0.05) on AST.This confirmed result of Assar *et al.* (2016) on non-significant values when different levels of alpha tocopherol were fed to Japanese quail under low ambient temperature.No significant effect was also observed for phosphorus. However, therewere significant differences in ALP, ALT and Ca, in this study ALP values for birds on T2, T3, T4, T5 and T6 were similar (P>0.05), while that of T1 (control) was significantly different (P<0.05) from all other treatments.In this study, ALP (IU/L) was higher (P<0.05) in control (37.47) diet compared with those on T2 (28.75), T3 (30.75), T4 (30.66), T5 (31.25) and T6 (29.33) which was contrary to the observation of Ajakaiye *et al.* (2010) of no differences in the ALP between the control of the vitamin E treated birds.The reduction in activities of ALP relative to the control suggests that supplementation was able to induce some degree of toxicity. Supplemental selenium and vitamin E groups had relatively higher levels of ALT that were not significantly different from each other. However, increased selenium supplementation increased the values of ALT which could be attributed to liver damage, while increased vitamin E levels reduced the values of ALT. During absorption, metabolism and excretion, (calcium) Ca and (phosphorus) P interact, establishing a ratio of approximately 2:1, which seldom varies (Scott *et al.*, 1982). When there is excessive Ca, the availability of other minerals, such as P, magnesium, manganese, and zinc, may be affected, causing secondary deficiencies. High Ca intake may impact P utilization due to Ca:P ratio changes (Anderson *et al.*, 1995). On the other hand, high P levels may also cause calcium deficiency. The established adequate calcium and phosphorus levels for layers have been challenged due to the continuous advances in genetic improvement, nutrition, environment, and management (Pelicia *et al.,* 2009).Ca values in birds on T1 was not significantly different fromthose obtained in T2, T3, T4, and T5. Low Ca level observed might be an indication that high amount of calcium have been deposited for strong egg shell production.

Haematology parameters of laying chickens: There were no significant different (P>0.05) in all the haematological parameters measured across the treatment except for monocytes and eosinophils. Birds on T4, T5 and T6 had elevated monocytes, values which were similar to the values obtained in T2 and T3 when compared to T2 (control). The eosinophils, of birds on T1, T2, T3, T4 and T5 were similar while birds on T6 had significantly lowered eosinophils.

CONCLUSION

Increased selenium supplementation increased the values of ALT and increased vitamin E levels reduced the values of ALT while its increase leads to an increase in ALP values, while supplementation of both selenium and vitamin E reduced serum calcium levels. Whereas, supplemental selenium and vitamin E affected blood indices in irregular trend.

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Table 1: Composition of experimental basal diet

Ingredient	%
Corn	50.00
Wheat offal	11.00
Soya bean meal	22.00
PKC	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
Total	100
Calculated nutrients	
ME (Kcal/Kg)	2736.01
Protein (%)	17.23
Calcium (%)	4.18
Phosphorus (%)	0.17
Methionine (%)	0.51
Lysine (%)	0.87

Table 2: Selected serum biochemical indices of layers fed graded supplemental selenium and vitamin E

Parameters	T1	T2	T3	T4	T5	T6	SEM
ALP(IU/L)	37.47^{a}	25.53^{b}	30.87^{ab}	25.04^{b}	23.43^{b}	27.59^{b}	1.45
AST(IU/L)	92.08	100.24	85.93	91.28	105.58	117.98	4.59
ALT(IU/L)	6.75^{b}	9.29^{a}	11.15^{a}	11.82^{a}	9.56^{a}	9.30^{a}	0.44
Ca(mg/dL)	11.60^{a}	8.35^{b}	6.31^{b}	6.83^{b}	7.74^{b}	9.39^{ab}	0.50
P(mg/dL)	10.53	13.42	14.85	13.48	10.83	14.19	0.64

T1-Basal diet,T2-Basal diet+0.5mg selenium/kg ,T3-Basal diet+1.0mg selenium/kg ,T4-Basal diet+1.5mg selenium/kg,T5-Basal diet+20mg vitamin E/kg,T6-Basal diet+40mg vitamin E/kg. ALT-Alanine amino transferase AST-Aspartate amino transferase , ALP-Alkaline phosphatase, Ca-Calcium, P-Phosphorus.
Mean in the same row with different superscript are significantly different ($P<0.05$).

Table 3: Haematological parameters of laying chickens fed levels of dietary selenium and vitamin E supplementation

	T1	T2	T3	T4	T5	T6	SEM
PCV(%)	28.50	28.75	30.75	30.66	31.25	29.33	0.73
RBC($x100^{6}$ IU/L)	2.91	3.09	3.30	2.87	2.87	3.37	0.10
WBC($X10^{3}$ mm)	17737.50	18062.50	19775.00	18950.00	19800.00	18450.00	337.70
Hb(g/100mL)	9.30	9.45	10.22	10.03	10.40	9.03	0.28
LYMP(%)	64.00	64.25	58.50	65.66	63.25	57.00	1.47
HET(%)	29.75	30.25	35.75	25.33	31.50	35.66	1.56
HET:LYMP	0.48	0.48	0.63	0.40	0.50	0.63	0.03
PLAT($x100^{5}$)	1.62	1.72	2.41	2.31	2.28	2.06	1.34
MON(%)	2.00^{b}	3.25^{ab}	3.25^{ab}	4.00^{a}	3.75^{a}	4.00^{a}	0.22
EOS(%)	4.25^{a}	4.00^{a}	3.50^{a}	4.33^{a}	3.75^{a}	1.33^{b}	0.32
BAS(%)	0.00	0.25	0.25	0.33	0.00	0.00	0.00

T1-Basal diet,T2-Basal diet+0.5mg selenium/kg ,T3-Basal diet+1.0mg selenium/kg ,T4-Basal diet+1.5mg selenium/kg,T5-Basal diet+20mg vitamin E/kg,T6-Basal diet+40mg vitamin E/kg. PCV-Packed cell volume, Hb- Haemoglobin, LYMP-Lymphocytes, HET-Heterophyl, PLAT-platelets, MON- Monocytes, EOS-Eosinophils, BAS-Basophil
Mean in the same row with different superscript are significantly different ($P<0.05$).