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The effects of different levels of vitamin-E and organic selenium on performance and immune response of laying hens

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The aim of this study was to determine the effects of different vitamin-E and organic selenium (selenomethionine) levels on performance and immune response of laying hens. A total of 270 laying hens (65-weeks old Lohman-LSL Lite) were assigned to nine experimental diets consisting of nine replicates (cage) and six hens per cage. A 3×3 factorial arrangement including three levels of vitamin E (0, 125 and 250 mg/kg diet from α-tocopherol acetate) and three levels selenium (0.0, 0.50 and 0.75 mg/kg diet from selenomethionine) was employed for six weeks trial period. The hens performance including hen-day egg production%, feed intake, egg mass (g/hen/day) and feed conversion ratio (FCR, g feed: g egg) were measured. Antibody production against sheep red blood cells (SRBC) also was measured. The general linear model procedure of SAS software was used for data analysis and differences among treatment means was determined using the Duncan's multiple-range test. The results show that the inclusion of vitamin E and selenium had a significant effect on production performance of laying hens (P<0.05). In addition, vitamin E and selenium supplements improved immune response of laying hens and a more positive effect was observed when 0.75 mg/kg selenium and 250 mg/kg vitamin E was added to the diet. From the results of the present study, it could be concluded that utilization of organic selenium plus vitamin E in diets was effective for improving the performance and immune system of laying hens.

Key words: Vitamin-E, selenomethionine, laying hen, performance, immune system.

INTRODUCTION

Vitamins and minerals are vital nutrients that are involved in both metabolic and physiological processes, which are critical for human and animal health and animal feed production. It has been well-documented that in formulating feed, nutritionists have to take into account several factors including stress management and immunity enhancement (Linge, 2005; Moradi Kor et al., 2012). In birds, free radical generation and lipid peroxidation are responsible for the development of various diseases as well as for a decrease in bird's productivity and product quality (Mcdowell, 2000; Surai and Dvorska, 2001). Vitamin E, a fat soluble vitamin, functions as a chain breaking antioxidant which prevents free radical induced oxidative damage by trapping reactive oxyradicals in

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biological membranes (Packer, 1991; Salman et al., 2007). Vitamin E is essential for growth performance, health and maintenance of tissue integrity in poultry. Vitamin E, a fat-soluble vitamin, is an intracellular antioxidant for all cells. It prevents oxidation of unsaturated lipids within cells and protects cell membrane from oxidative damages (Halliwell and Gutteridge, 1999).

Vitamin E is a metabolic nutrient that has received a lot of attention with respect to its importance to the immune response in poultry. However, poultry cannot synthesize vitamin E, therefore, vitamin E requirements must be given from dietary sources (Puthpongsiriporn et al., 2001). In a study done by Canan et al. (2007), egg production in laying hens in a heat stressed group and a non-heat stressed group both increased significantly with the supplementation of dietary vitamin E. Puthpongsiriporn et al. (2001) and Aljamal (2011) showed that supplementation of vitamin E significantly increased egg production in laying hens exposed to heat stress. Vitamin E functions in biological systems primarily as an agent that protects against free radicals (Kaneko, 1989). Apart from its protective effect on lipid peroxidation, the immunoregulatory effects of dietary vitamin E on humoral and cell-mediated immunity are well known (Gu et al., 1999).

Vitamin E improves the action of phagocytic cells, the responsiveness of antibodies to mitogens, and passive immune transfer (Tizzard, 1987). Selenium is an essential component of selenium dependent glutathione peroxidese enzymes, which are antioxidant enzymes that destroy free radicals produced during normal metabolic activity (Payne and Southern, 2005). Selenium has a profound impact on immune function, health and productivity and is associated with protein in animal tissues (Wang and Bao-Hua, 2008). On the other hand, Se deficiency is a global problem related to an increasing susceptibility of animals and humans to various diseases (Heindl et al., 2010). Generally, a deficiency of selenium and/or vitamin E has little effect on the magnitude of the total or the specific antibody responses of domestic species (Finch and Turner, 1996). Due to the fact that selenium is a trace element and add very little to ration, and in powder form not mixes well, in result may some animals use more that is poisoning and some other use lower that causing deficiency.

Selenium is an essential trace mineral in poultry for body development, maintenance of glutathione peroxidase activity (Whitracre et al., 1986) and avoidance of exudative diathesis (Noguchi et al., 1973). Selenium necessity for proper glutathione peroxidase enzyme function, which is an antioxidant enzyme destroying free radicals is produced during normal metabolic activity in the organism (Moradi Kor et al., 2012). Selenium is the only trace element that is necessary for the growth and efficiency of animals. It is an important essential mineral for the health of people and animals and one of the antioxidants, which improve the ability of the organism to protect itself. In addition, it protects some ingredients of food, primarily lipids and vitamins, from undesirable oxidation. Along with vitamin E, it has a positive influence on the technological characteristics of meat thanks to its anti-oxidation properties.

Selenium plays an important role in the antioxidant defense system due to its requirement by the selenium dependent GSHPx, which is involved in cellular antioxidant protection. It has been suggested that there is a synergistic relationship between selenium and vitamin E, because GSHPx continues the work of vitamin E by detoxifying hydroperoxides. Recent understanding of antioxidant system functions and new discoveries regarding the GSHPx enzyme family are the basis for further development in the selenium nutrition of poultry (Surai, 2002). Selenium is a constituent of the cytosolic enzyme glutathione peroxidase and facilitates the action of vitamin E in reducing peroxy radicals. In chickens, absorption of vitamin E is impaired by severe selenium deficiency and selenium alleviates vitamin E deficiencies by permitting higher levels of vitamin E to be absorbed (Machlin, 1991). There are reports of beneficial effects from selenium supplementation on the weight gain of lymphoid organs (Kukreja and Khan, 1997).

Metabolic functions of selenium are similar to vitamin E. A well known synergy exits between selenium and vitamin E. Selenium and vitamin E, both act as the primer antioxidant by suppressing oxidative damages. Marsh et al. (1981) reported that body weights decreased in chicks fed with vitamin E and selenium deficient diets. Selenium deficiency, especially together with the low level of vitamin E in chickens, is responsible for the arising of diseases such as exudative diathesis (Noguchi et al., 1973) and pancreatic atrophy (Thompson and Scott, 1970). Devore et al. (1983) demonstrated that dietary supplementation of 0.25 ppm selenium substantially increased GSH-Px activity in breast and leg muscles. Lipid accumulation leads to oxidative stress which may contribute to peroxidation of LDL. These peroxidative fatty acids and reactive oxygen species induce hepatic damage. Some researchers have reported that antioxidant supplementation causes significant improvement in blood lipid parameters of humans (Jain et al., 1996; Kacmaz et al., 1997; Miller et al., 1997). However, another study has reported that there were no changes in plasma lipoprotein concentrations after antioxidant supplementation (Brown et al., 1994). Organic selenium had an advantage in reducing oxidative stress in birds (Mahmoud and Edens, 2003).

Organic selenium is an effective antioxidant and hypolipidemic agent in normal hamsters (Vinson et al., 1998). Several animal studies have also shown that vitamin E supplementation affects lipoprotein metabolism by reducing serum triacylglycerols (Oriani et al., 1997) and total cholesterol, and increasing HDL-cholesterol levels. Selenium and vitamin E are inter-related; hence complete protection of living cells requires both vitamin E and selenium in the diet. The present study was conducted to evaluate the effect of different levels of Vitamin-E (α-

Table 1. Amounts of vitamin E and selenium added to the diet (mg/ kg diet).

Treatment	Selenium	Vitamin E
S0E0	0	0
S1E0	0. 5	0
S2E0	0.75	0
S0E1	0	125
S1E1	0. 5	125
S2E1	0.75	125
S0E2	0	250
S1E2	0. 5	250
S2E2	0.75	250

Table 2. Ingredients and chemical composition of the diets.

Ingredient (%)	S0E0	S1E0	S2E0	S0E1	S1E1	S2E1	S0E2	S1E2	S2E2
Vitamin E (mg/kg)	**0**	**0**	**0**	**125**	**125**	**125**	**250**	**250**	**250**
Selenium (mg/kg)	**0**	**0. 5**	**0.75**	**0**	**0.5**	**0.75**	**0**	**0. 5**	**0.75**
Corn	61.73	61.73	61.73	61.73	61.53	61.49	61.38	61.27	61.04
Soybean meal	21.12	21.12	21.12	21.12	21.12	21.12	21.12	21.12	21.12
Oil	2.23	2.23	2.23	2.23	2.23	2.23	2.23	2.23	2.23
Dicalcium phosphate	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Oyster shell	9.10	9.10	9.10	9.10	9.10	9.10	9.10	9.10	9.10
Common salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vit & Min premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Sand	3.97	3.91	3.47	3.97	3.00	2.26	2.63	2.63	2.63
Calculated analysis									
Crude protein (%)	13.27	13.27	13.27	13.27	13.27	13.27	13.27	13.27	13.27
Ether extract (%)	4.38	4.38	4.38	4.38	4.38	4.38	4.38	4.38	4.38
Crud fiber (%)	2.63	2.63	2.63	2.63	2.63	2.63	2.63	2.63	2.63
Calcium (%)	3.82	3.82	3.82	3.82	3.82	3.82	3.82	3.82	3.82
Available phosphorus (%)	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
ME (Kcal/kg)	2752	2752	2752	2752	2752	2752	2752	2752	2752

tocopheryl acetate) and organic selenium (seleno-methionine) supplementation on performance and immune response of laying hens.

MATERIALS AND METHODS

Location and experimental design

This study was conducted in the Rezvan junior college aviculture farm in Kerman province (latitude 25° 55'N, longitude 53° 26/ E, altitude 1755 m) from April to June 2012. A total of 270 laying hens (65-weeks old Lohman-LSL Lite) were assigned to nine experimental diets with five replicates (cage) and nine hens per cage. A 3×3 factorial arrangement including three levels of vitamin E (0, 125, 250 mg/kg diet from α-tocopherol acetate) and three levels of selenium (0, 0.5 and 0.75 mg/kg diet from seleno-methionine, Javaneh Khorasan company, Iran) was employed for six weeks trial period (Table 1). Each replicate consisted of two adjoining cages with three hens per individual cage for a total of three hens per replicate. Before the start of experiment, all hens were fed basal diet for two weeks and were similar in body size and production. Layers were fed experimental diets for 42 days. The laying hen received a diet with three concentrations of supplemental vitamin E and selenium in a corn-soyabean basal diet (Table 2). These levels of supplementation were selected based on the optimum recommendation level in some researches. The composition of basal diet is shown in Table 2. During the experiment, hens fed approximately 120 (g/day) and water was offered *ad-libitum*. The hens performance including hen-day egg production %, feed intake and egg mass (g/hen/day) was measured. Feed conversion (FCR, g feed: g egg) was also calculated as the ratio of gram of feed consumed per gram of egg weight produced. At the 4th week of the experiment, 5 hens were randomly selected from each group (1 from each replicate) and injected with 0.2 ml of 9% suspension of sheep erythrocytes (SRBC) in phosphate buffer saline. One week after SRBC injection, 3 mL blood was taken from selected hens using jugular venipuncture, and serum was separated and evaluated for antibody titer. Haemagglutination inhibition (HI) test was used for determining antibody titer sera (Salk, 1944).

Table 3. Effects of dietary Vitamin E and Selenium supplementation on egg production (EP) and egg mass (EM) of laying hens.

Treatment	Trait					
	Egg production (%)			Egg mass (g egg/hen/day)		
	1-3 weeks	4-6 weeks	Overall	1-3 weeks	4-6 weeks	Overall
S0E0	76.1±1.03^{b}	77.2±1.32^{c}	76.7±1.17^{c}	50.3±0.89	50.5±1.22^{b}	50.4±1.05^{b}
S1E0	75.0±1.23^{b}	77.6±0.98^{c}	76.3±1.10^{c}	50.7±1.07	51.8±1.27^{b}	51.2±1.17^{b}
S2E0	76.9±0.79^{b}	77.9±0.75^{c}	77.4±0.77^{c}	50.6±1.22	51.8±1.09^{b}	51.2±1.15^{b}
S0E1	78.4±1.25^{b}	81.6±1.23^{b}	80.0±1.24^{b}	50.8±1.0	51.9±1.48^{b}	51.3±1.28^{b}
S1E1	80.2±0.98^{b}	84.2±1.62^{b}	82.2±1.30^{b}	52.4±1.0	53.4±0.97^{b}	52.9±0.99^{ab}
S2E1	85.9±0.83^{a}	85.4±0.93^{b}	85.6±0.88^{ab}	51.4±0.85	55.2±0.81^{ab}	53.3±0.83^{ab}
S0E2	85.7±1.43^{a}	88.3±1.22^{ab}	87.0±1.32^{a}	52.4±1.12	57.2±1.72^{a}	54.8±1.42^{ab}
S1E2	87.5±1.68^{a}	89.1±1.38^{ab}	88.3±1.53^{a}	53.4±1.58	58.6±1.38^{a}	56.0±1.48^{a}
S2E2	87.6±1.05^{a}	91.6±1.41^{a}	89.6±1.23^{a}	53.7±1.71	59.3±1.01^{a}	56.5±1.36^{a}
SEM	0.343	0.453	0.362	0.438	0.629	0.578
Sources			P Values			
Vitamin E	0.438	0.619	0.428	0.238	0.275	0.312
Selenium	0.381	0.738	0.624	0.384	0.281	0.352
Interaction	0.034	0.041	0.038	0.528	0.027	0.047

Statistical analysis

The general linear model procedure of SAS software was used for data analysis and differences among treatment means determined using the Duncan's multiple-range test. As all two-way interactions of the study were significant, analyses of simple main effects were performed using the LSMEANS/ SLICE feature in the PROC MIXED statement. Significant differences at each slice level were further analyzed using LSMEANS/ DIFF of the PROC MIXED procedure.

RESULTS

The effect of different levels of supplemental vitamin E and selenium on egg production and egg mass of laying hens during the entire six weeks period of the study are presented in Table 3. The results indicate that inclusion of vitamin E and selenium had a significant effect on egg production and egg mass (P<0.05). Percentage of egg production was higher during the second three weeks compared to that in first three weeks. Egg production was higher in S2E2 treatment compared with other treatments. Egg mass (g egg/hen/day) was higher during the second three weeks compared to that in first three weeks. Egg mass was higher in S2E2 treatment compared with that of other treatments. Egg production and Egg mass increased with increasing the levels of vitamin E and selenium.

Values of the same column with no common superscripts are significantly different (P < 0.05). S0E0 0 mg/kg vitamin E and 0 mg/kg selenium, S1E0 0.5 mg/kg selenium and 0 mg/kg vitamin E, S2E0 0.75 mg/kg selenium and 0 mg/kg vitamin E, S0E1 0 mg/kg selenium and 125 mg/kg vitamin E, S1E1 0. 5 mg/kg selenium and 125 mg/kg vitamin E, S2E1 0.75 mg/kg selenium and 125 mg/kg vitamin E, S0E2 0 mg/kg selenium and 250 mg/kg vitamin E, S1E2 0. 5 mg/kg selenium and 250 mg/kg vitamin E, S2E2 0.75 mg/kg selenium and 250 mg/kg vitamin E, SEM standard error of the mean.

The effects of different levels of supplemental vitamin E and selenium on feed intake and feed conversion ratio of laying hens during the entire six weeks period of the study is shown in Table 4. The results indicate that inclusion of vitamin E and selenium had a significant effect on feed conversion ratio (P<0.05). Results from this table showed that inclusion of vitamin E and selenium had not a significant effect on feed intake (P>0.05). Supplementation of diets with vitamin E and selenium decreased feed intake in second three weeks but this difference was not significant (P>0.05, Table 4). Feed intake was lower in S2E2 treatment compared with other treatments of second 3 weeks. In addition supplementation of diets with vitamin E and selenium significantly decreased feed conversion ratio. Feed conversion ratio was lower in the second three weeks compared to that of the first 3 weeks. Feed conversion ratio decreased with increasing the levels of vitamin E and selenium.

The effects of different levels of supplemental vitamin E and selenium on egg weight and antibody titer against SRBC of laying hens during the entire six-weeks period of the study is shown in Table 5. Results from this table indicated that inclusion of vitamin E and selenium had a significant effect on egg weight and antibody titer against SRBC (P<0.05). Egg weigh was higher during the second 3 weeks when compared with egg weight in first 3 weeks. In addition egg weight was higher in S2E2 treatment compared with that of other treatments. Egg weight increased with increasing dietary vitamin E and selenium. The hens receiving the diet containing 0.75 mg/kg selenium and 250 mg/kg vitamin E had significantly higher titers of total, IgM and IgG antibodies than that of those

Table 4. Effects of dietary Vitamin E and Selenium supplementation on feed intake (FI) and Feed conversion ratio (FCR) of laying hens.

Treatment	Trait					
	Feed intake (g Feed/hen/day)			Feed conversion ratio (FCR)		
	1-3 Weeks	4-6 Weeks	Overall	1-3 Weeks	4-6 Weeks	Overall
S0E0	117.6±1.02	116.0±1.22	116.8±1.12	2.0±0.75^{b}	2.3±1.22^{b}	2.2±0.98^{b}
S1E0	117.2±1.61	116.1±0.85	116.7±1.23	2.2±0.81^{b}	2.2±1.02^{b}	2.2±0.91^{b}
S2E0	117.4±1.09	117.1±1.23	117.3±1.16	2.1±0.92^{b}	2.2±0.91^{b}	2.1±0.91^{b}
S0E1	118.4±1.08	117.4±1.06	117.9±1.07	2.4±1.08^{b}	2.2±1.33^{b}	2.3±1.20^{b}
S1E1	117.3±0.99	116.9±1.51	117.1±1.25	2.3±0.81^{b}	2.2±0.79^{b}	2.2±0.81^{b}
S2E1	119.5±1.23	115.4±0.84	117.5±1.03	2.1±1.24^{b}	2.0±1.75^{b}	2.1±1.49^{b}
S0E2	117.9±1.31	115.9±1.13	116.9±1.22	2.4±1.61^{b}	1.2±1.40^{a}	2.2±1.50^{b}
S1E2	118.1±1.55	114. 2±1.27	116.1±1.41	2.1±1.17^{b}	1.9±1.58^{a}	2.0±1.37^{b}
S2E2	119.4±1.73	114.7±1.12	117.1±1.42	2.0±1.13^{b}	1.9±1.12^{a}	1.9±1.12^{a}
SEM	0.489	0.365	0.255	0.189	0.082	0.055
Sources			P Values			
Vitamin E	0.547	0.684	0.328	0.234	0.429	0.462
Selenium	0.651	0.722	0.491	0.371	0.432	0.573
Interaction	0.763	0.692	0.624	0.569	0.039	0.047

Means (±SD) within a column showing different superscripts are significantly different (P<0.05).

Table 5. Effects of dietary Vitamin E and Selenium supplementation on egg weight (EW) and antibody titer against SRBC of laying hens.

Treatment	Trait					
	Egg weight (g)			Antibody titer against SRBC		
	1-3 weeks	4-6 weeks	Overall	IgM	IgG	Total
S0E0	59.3±1.06^{b}	59. 7±1.32^{b}	59.5±1.19^{b}	2.0±1.02^{b}	2.1±1.42^{c}	2.3±1.14^{c}
S1E0	60.3±1.36^{b}	59.9±1.21^{b}	60.1±1.28^{b}	2.2±0.88^{b}	2.3±1.07^{c}	2.6±0.78^{c}
S2E0	60.1±0.99^{b}	61.4±0.73^{b}	60.8±0.86^{b}	2.2±1.29^{b}	3.1±0.89^{c}	3.4±1.09^{c}
S0E1	59.2±1.48^{b}	61.5±1.52^{8b}	60.4±1.51^{b}	2.4±1.48^{b}	3.4±1.08^{c}	3.5±1.28^{c}
S1E1	60.4±1.52^{ab}	63.2±1.02^{ab}	61.8±1.27^{b}	2.5±0.81^{b}	4.3±0.85^{b}	5.0±0.83^{b}
S2E1	62.5±0.86^{ab}	63.3±0.68^{ab}	62.9±0.77^{ab}	2.6±0.97^{b}	4.5±1.52^{b}	5.4±0.96^{b}
S0E2	63.4±1.40^{a}	64.4±1.14^{ab}	63.9±1.27^{ab}	2.8±1.19^{b}	5.3±1.34^{a}	6.2±1.56^{a}
S1E2	64.3±1.38^{a}	66.2±1.26^{a}	65.3±1.32^{a}	2.8±1.38^{a}	5.4±1.52^{a}	6.6±1.28^{a}
S2E2	64.4±1.12^{a}	66.6±1.61^{a}	65.5±1.36^{a}	3.0±1.21^{a}	5.7±1.39^{a}	7.7±1.51^{a}
SEM	0.238	0.312	0.245	0.069	0.183	0.285
Sources			P Values			
Vitamin E	0.637	0.356	0.468	0.352	0.447	0.653
Selenium	0.521	0.458	0.382	0.421	0.558	0.345
Interaction	0.046	0.028	0.036	0.002	0.038	0.019

Means (±SD) within a column showing different superscripts are significantly different (P<0.05).

fed the other diets (P<0.05). The titer of total antibody, IgM and IgG significantly increased with increasing dietary vitamin E and selenium.

DISCUSSION

Results from the current study indicate that inclusion of vitamin E and selenium had a significant effect on production performance of laying hens (P<0.05). These results are in agreement with the finding of Nasiroleslami and Torki (2011) who demonstrate that the diet supplementation by vitamin E have beneficial effect on performance of laying hens during the period of 71 to 81 week of age. Moradi Kor et al. (2012) demonstrated that under heat stress vitamin E and selenium supplementation decreased FCR in laying hens. Our results are in contrast with the finding of Osman et al. (2010) who reported that the dietary organic selenium supplementa-

tion did not have significant effect on egg production in laying hens. In addition, Puthpongsiriporn et al. (2000) reported that under environmental stress, feed intake, egg production decreased in birds fed with vitamin E and C. Grobas et al. (1997) using two levels of Vitamin E (13 and 263 mg/kg) observed no difference in egg production, egg weight, feed conversion and shell thickness in ISA brown laying hens.

Mohiti Asli et al. (2007) reported that diet inclusion of vitamin E did not significantly affect on Egg weigh and FCR. Mohiti Asli et al. (2010) found that FCR and egg production were not significantly influenced by vitamin E and organic and inorganic selenium supplementation during heat stress. Scheideler and Froning (1996) supplemented layer (Babcock B-300) with 50 mg/kg Vitamin E in the diet and observed 2% improvement in egg production during peak production. The differences between previous reports and the present study may be partly related to differences in hens' age and environmental condition. The effect of dietary selenium and vitamin E and their different combination on body weight gain, food consumption, food conversion efficiency, leucocyte migration inhibition and antibody production was studied in broilers (Swain and Johri, 2000; Swain et al., 2000a; b). It was reported that the dietary supplementation of selenium significantly increased the egg production and hatchability and decreased the percentage of infertile eggs and early dead embryos. Selenium and vitamin E have been increasingly recognized as an essential element in biology and medicine.

Antibody production against SRBC in laying hens that fed high level of vitamin E and selenium supplementation was greater than other treatment ($p<0.05$). Serological data from the present study showed the effectiveness of vitamin E and selenium supplementation on systemic immunity.

The results of this experiment was similar to the finding of Mohiti Asli et al. (2007) who indicated that vitamin E could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens. Dietary selenium and vitamin E stimulates immune response in poultry against bacterial and viral infections (Morandi et al., 1993), improve reproductive performance (Barreto et al., 1997) of broiler breeders as well as to increase economic returns (Ganpule and Manjunatha, 2003). Selenium and Vitamin E have been found to alter immunocompetence in various species.

The etiology of their stimulatory roles in the immune response is unknown; however, it may be related directly to their antioxidant properties (Ghazi Harsini et al., 2012). Spallholz et al. (1973) demonstrated that high dietary selenium enhanced serum immunoglobulin G (IgG) and immunoglobulin M (IgM) antibody titers in mice challenged with sheep red blood cells. Vitamin E has been implicated in stimulation of serum antibody synthesis, particularly IgG antibodies (Ghazi Harsini et al., 2012).

Conclusion

The present investigation suggested that the combination of vitamin E and selenium at a level of 250 and 0.75 mg/kg diet, respectively can improve production performance and immune responses of laying hens. Thus, supplementation of vitamin E and selenium at levels above recommended as nutritional requirements for improve humoral and cellular immunity. In addition, improving performance and immune responses of laying hens by vitamin E and selenium supplementation is relatively a novel result, so the antioxidative effect of vitamin E and selenium could be the subject of further investigations.

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