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Annals of Biological Research, 2012, 3 (2):842-849
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Influence of dietary sodium selenite and vitamin E (E-Selenovit) supplementation on performance and immune response of laying hens during high environmental temperature

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ABSTRACT

A completely randomized design experiment consisting 5 treatments with 5 replicates (6 birds per replicate) was carried out by allotting 150 white Lohman laying hens at 18 weeks old. The experimental diets were prepared by addition of different levels of E-selenovit supplement (0.0, 35, 65, 80 and 100 mg / kg of feed) to the diets. The diets were isonitrogenous and isocaloric and of fered ad libitum for 12 weeks. The hens performance including hen-day egg production % , feed intake, egg mass (g/hen/day) and feed conversion ratio (FCR, g feed: g egg) was measured. Antibody production against Sheep Red Blood Cells (SRBC) and Newcastle Disease Virus (NDV) also was measured. The results indicated that inclusion of E-selenovit had a significant effect on performance (P<0.05). Supplementation of diets with E-selenovit significantly increased feed intake and improved feed efficiency (P<0.05). Egg production was higher in treatment five than that of control, (94.3 vs 88.1 respectively). Egg mass also significantly affected by inclusion of E-selenovit (P<0.05). Higher egg mass was observed in treatment five than treatment one (54.9 vs 50.7). Addition of E-selenovit significantly increased (P<0.05) antibody titrations against SRBC and Newcastle disease and it was higher in treatment five compared with control group,(8.03 vs 6.33 and 8.93 vs 6.31). These results showed that E-selenovit supplements can improve immune response of laying hens under heat stress condition and a more positive effect was observed when 100 mg/kg E-selenovit added to the diet.

Key words : E-Selenovit, Heat stress, Laying hen, Immune system, Performance.

INTRODUCTION

In many countries, high ambient temperatures induce a large economic losses because of mortality and decreased production. Heat stress in laying hens reduces feed intake, feed efficiency, production and quality of eggs. In hot environment, hens exert an effort to maintain their body temperature within a normal range. This challenge is associated with behavioral, physiological, hormonal and molecular reactions to heat stress. In addition, heat stress stimulates the release of corticosterone and catecholamins and initiates lipid peroxidation in cell membranes Puthpongsiriporn *et al*., [23] including membrane of T and B lymphocytes and there by suppresses antibody production and cell-mediated immunity and also increases heat-stressed dependent mortality. Corticosterone and cat-ecolamines are the most important hormones that are released in response to stress and that have negative effects on performance and immunity of hens [17]. Heat stress leads to generation of free radicals and there by inducing oxidative degeneration of polyunsaturated fatty acids (PUFA) in cell membranes phospholipids [9]. Hens in stressful environments produce lower antibody response to a variety of antigens [17]. Stress increases mineral and vitamin mobilization from tissues and their excretion, thus may exacerbate a marginal vitamin and mineral deficiency or an increased mineral and vitamin requirement [16]. 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 food production. Efficient poultry production is based on the feeding of well balanced diets to highly productive line of birds. The immune system benefits greatly from proper nutrition of the bird. Thus in many instances, proper nutrition decreases the immune suppression associated with the stress response in the bird. It was reported that when formulating feed, nutritionists have to take into account several factors including stress management and immunity enhancement [15]. In this respect, antioxidants play an important role in maintaining bird health, productive and reproductive performance [28]. Selenium (Se) is an essential micronutrient in the diet of many life forms including animals and humans. The biochemical role of Se was demonstrated in 1973 by Rotruck et al. when it was discovered as part of the enzyme glutathione peroxidase (GSH-Px). Glutathione peroxidase acts as an antioxidant to prevent cellular damage by free radicals produced as natural by-products of oxygen metabolism in the body. 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 can not synthesis Vit. E, therefore, Vit. E requirements must be given from dietary sources [23]. In a study done by Canan *et al*., [5], 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*., [23], and Alia Aljamal., [1] showed that supplementation of vitamin E significantly increased egg production in laying hens exposed to heat stress. Supplement used in this expriment was E-selenovit in powder form and 1kg added in 1000kg diet. This supplement was contain 5500 IU vitamin E in form of α-Tocopheryl acetate and 300 mg selenium in form sodium selenite (in organic). Therefore, the present study was aimed to examine the effects of different levels E-Selenovit supplements on performance and immune response of laying hens during high environmental temperature.

MATERIALS AND METHODS

A total of 150 laying hens, 18 week old, Single Comb White Lohman laying hens were divided into five groups. Experiment was conducted by using as a completely randomized design and five dietary treatments were utilized. The treatment involved: control (T_1), basal diet plus 35 mg/Kg of diet (T_2), basal diet plus 65 mg/Kg (T_3), basal diet plus 80 mg/Kg (T_4) and basal diet plus 100 mg/Kg (T_5) E-selenovit Supplement. These levels of supplementation selected base on optimum recommended level in some researches. The composition of basal diet is shown in Table 1.

Table 1. Ingredients and chemical composition of the diets

Feed ingredients %	Treatments				
	T1	T2	T3	T4	T5
Corn	65.06	65.06	65.06	65.06	65.06
Soybean meal	17.55	17.55	17.55	17.55	17.55
Wheat bran	4.98	4.98	4.98	4.98	4.98
Oil sunflower	0.3	0.3	0.3	0.3	0.3
Fish meal	2	2	2	2	2
Limestone	7.87	7.87	7.87	7.87	7.87
Di calcium phosphat	1.29	1.29	1.29	1.29	1.29
Salt	0.26	0.26	0.26	0.26	0.26
Mineral supplement [1]	0.25	0.25	0.25	0.25	0.25
Vitaminised supplement [2]	0.25	0.25	0.25	0.25	0.25
D-L metyonin	0.15	0.15	0.15	0.15	0.15
L- Lysin	0.03	0.03	0.03	0.03	0.03
E-selenovit supplement (mg)	0	35	65	80	100
	Chemical composition(Analysed)				
Metabolizable energy, (Kcal /kg)	2720	2720	2720	2720	2720
Crude protein %	15.42	15.42	15.42	15.42	15.42
Ether extract %	3.13	3.13	3.13	3.13	3.13
Crude fiber %	3.23	3.23	3.23	3.23	3.23
Calcium %	3.42	3.42	3.42	3.42	3.42
Av.phosphor %	0.35	0.35	0.35	0.35	0.35
Metyonin	0.47	0.47	0.47	0.47	0.47
Lysin	0.77	0.77	0.77	0.77	0.77

[1]per kg mineral supplement include 74400 mg Mg, 75000 mg Fe, 64.675 mg Zn, 6000 mg Cu, 876 mg iodine, 200 mg selenium, [2]- per kg vitamin supplement include 8500000 IU vitamin A, 2500000 IU Vitamin D 3, 11000 IU Vitamin E, 2200 mg Vitamin K3, 1477 mg Vitamin B 1, 4000 mg Vitamin B 2, 7840 mg Vitamin B 3, 34650 mg Vitamin B5, 2464 mg Vitamin B6, 110 mg Vitamin B9, 10 mg Vitamin B12, 400000 mg choline chloride.

This study was conducted in the Rezvan junior college aviculture farm in Kerman province (latitude 25^0 55 / N, longitude 53^0 26/ E , altitude 1755m) from July to September 2010. Hens were randomly assigned to cages so that there were five replications. Each replicate consisted of 2 adjoining cages with 3 hens per individual cage for a total of 6 hens per replicate. Before the start of the experiment, all hens fed basal diet for 2 weeks and were similar in body size and production. Layers were fed with experimental diets for 84 days. Feed (in mash form) and water were provided *ad-libitum* throughout the experiment. The experiment was conducted in the summer season and were similar to guidelines set in the white Lohman laying hens. The constant temperature and relative humidity of hen house was 24±2°C and 50±10%, respectively. The hens performance including hen-day egg production % , feed intake was measured and egg mass (g/hen/day) as well as feed conversion ratio (FCR, g feed: g egg) also was calculated. Yolk and plasma cholesterol were determined during the last week of the trial. These measurements were made by spectrophotometer (UV-visible S2100, Scinco, Korea) using commercial kits by method of Mohiti Asli et al. [19]. At the 9th 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. All titers were expressed as the $\log_2$ of the reciprocal of the serum dilution [20]. Haemagglutination inhibition (HI) test was used for determining Newcastle viruses antibody titer sera.

Statistical Analysis

Data were analyzed by ANOVA using General Linear Models procedure of SAS software [27]. Means were compared using Duncan's multiple range test. Level of significance used in all results was 0.05. Least square treatment means were compared if a significant F statistic (5% level of P) was detected by analysis of variance. Linear and quadratic polynomial contrasts were used to evaluate the effect of different levels of E-selenovit sources.

RESULTS AND DISCUSSION

The effects of supplemental E-selenovit on performance are shown in Table 2. The results indicated that inclusion of E-selenovit had a significant effect on performance ($P<0.05$, Table 2). Supplementation of diets with E-selenovit significantly increased feed intake and improved feed efficiency ($P<0.05$, Table 2). Egg production was higher in treatment five than that of control, (94.3 vs 88.1 respectively). Egg mass also significantly affected by inclusion of E-selenovit ($P<0.05$). Higher egg mass was observed in treatment five than treatment one (54.9 vs 50.7). Addition of E-selenovit significantly increased ($P<0.05$) antibody titrations against Newcastle disease and it was higher in treatment five compared with control group,(8.9 vs 6.3). Also, egg mass, feed consumption and feed conversion ratio was effected by treatments. E- Selenovit inclusion did not influence the egg weight significantly, which has already been reported by Mohan *et al*., [18], Haddadin *et al*., [10] and Chen and Chen [7].

Table 2. Effect of dietary supplementation of E-Selenovit during heat stress on performance of laying hens

Parameters	Different levels of E-Selenovit supelement (mg/kg)				
	0	35 mg	65 mg	80 mg	100 mg
Feed Consumption (gr/hen/day)	108.7^{c}	109.2^{bc}	109.4^{bc}	109.9^{b}	110.8^{a}
Hen-day Egg Production (%)	88.1^{b}	89.4^{bc}	90.5^{bc}	92.5^{ab}	94.3^{a}
Egg Mass (gr/hen/day)	50.7^{d}	51.8^{cd}	53.6^{bc}	53.8^{bc}	54.9^{a}
Egg Weight (gr)	62.4	62.8	62.6	62.7	62.9
Feed Conversion Ratio (gr feed/gr egg)	2.2^{a}	2.06^{ab}	2.03^{ab}	2.04^{ab}	1.98^{b}

^{a,b} : means within a row followed by the same superscript are not significantly different (P >0.05).

The effects of supplemental E-selenovit on Serum and egg cholesterol, Antibody titrations against SRBC and NDV are shown in Table 3. There was a significant difference in serum and yolk cholesterol concentrations between experimental groups ($p<0.05$). Egg cholesterol was significantly lower in hens received 80 and 100 mg E-selenovit than other groups. Serum cholesterol in groups 80 and 100 mg E-selenovit was significantly lower than other treatment groups. Antibody production against Sheep Red Blood Cells (SRBC) and Newcastle Disease Virus (NDV) in laying hens that fed E-selenovit supplementation was greater than control group ($p<0.05$). Antibody titrations against Newcastle Disease Virus in treatment 3, 4 and 5 was significantly higher than treatment 2 and control groups, 8.27, 8.52, 8.93, 7.75 and 6.31

respectively. However, dietary E-selenovit supplementation increased immune response ($p<0.05$).

Table 3. Effect of E-Selenovit supplement on serum and egg yolk cholesterol and immune response

Parameters	Different levels of E-Selenovit supelement (mg/kg)				
	0	35 mg	65 mg	80 mg	100 mg
Serum cholesterol (mg/dl)	152.35^{b}	147.95^{b}	147.34^{b}	137.04^{a}	133.24^{a}
Egg cholesterol (mg/gr yolk)	14.46^{b}	14.33^{b}	13.89^{ab}	13.21^{ab}	12.26^{a}
Antibody titer ($\log_2$) against SRBC[1]	6.33^{b}	8.67^{a}	8.83^{a}	7.83^{ab}	8.03^{ab}
Antibody titrations against NDV [2]	6.31^{d}	7.75^{c}	8.27^{b}	8.52^{b}	8.93^{a}

^{a,b} : Row means with common superscripts do not differ significantly ($p>0.05$), [1]SRBC: Sheep Red Blood Cells, [2] Newcastle Disease Virus

In the present study also effect of different levels E-Selenovit supelement on leukocyte profiles of laying hens was measured (Table 4). Heterophil and lymphocyte content in treatment 4 and 5 (80 and 100 mg of E-Selenovit supelement) was higher than other treatment and control groups, but this difference was not statistically significant. Relation Heterophil to lymphocyte in treatment 5 (100 mg) was lower than other treatment. This reduction indicate that E-Selenovit supelement can increase immune system function.

Table 4. Effect of E-Selenovit supplement on white blood cell counts (percentage of total)

	E-Selenovit supelement (mg/kg)					
	Heterophil	Lymphocyte	Monosyte	Eosinophil	Basophil	H/L
Control	32.27	60.70	0.667	0.900	1.567	0.594
35 mg	34.53	61.40	0.333	0.900	1.533	0.575
65 mg	33.25	62.95	0.500	1.100	1.500	0.539
80 mg	35.15	63.60	0.400	0.750	1.300	0.528
100 mg	32.25	63.65	0.600	0.850	1.850	0.523
SEM	0.078	0.804	0.115	0.118	0.168	0.019
p-value	0.227	0.217	0.174	1.00	0.921	0.251

^{a,b} : Means within a column with no common superscript differ significantly ($p<0.05$), SEM= Standard error of means.

Dietary treatments did significantly affect egg production, Egg mass and feed efficiency of hens (Table 2). These data are consistent with the findings of researches involving laying hens [3, 24]. In the present study hens fed diets with 100 mg E-Selenovit supplement consumed significantly high feed than those of other groups. The inclusion of 35, 65 and 80 mg E-Selenovit in diets had no significant effects on feed intake during the 12 week experimental period. These data are consistent with the study of Puthpongsiriporn *et al*., [23], who in hens found that feed intake was not affected when 45, 65 IU vitamin E/kg was incorporated into the diet. The increase in egg production in our study was most likely due to increase the available nutrients for egg production. No significant changes were observed in egg weight, regardless of E-Selenovit supplementation during heat stress. This is in agreement with puthpongsiriporn *et al*., [23] who reported that vitamin E supplementation did not affect egg weight during short time cyclic heat stress. However, some researchers [2, 3, 13, 8] reported that vitamin E can alleviate the negative effect of heat stress on egg production in laying hens. Some studies showed that, vitamin E had no effect on egg weight [3, 23, 13]. In contrast, ciftci *et al*., [8] reported that egg weight was increased with vitamin E supplementation. The most of above studies

were conducted on 25–30 week-old hens which were at the early phase of egg production. This means that they produced large quantity of small eggs. When the age of hens increases, egg production rate decreases and egg weight increases. Since a decline occurs in the number of large yellow follicles (LYF), in which very low density lipoproteins (VLDL) are deposited, their competition for VLDL and yolk precursors decreases resulting in larger yolks and eggs. Reduction of egg weight under high environmental temperature was not due to lower yolk weight, as there was no significant difference between yolk weights ($P > 0.05$). This may be caused by aging of hens and fewer LYF for equal amount of VLDL and vetillogenin. In contrast, Bollengier-lee *et al*., [2] demonstrated that heat exposure reduced circulating levels of plasma yolk precursor proteins in hens, and vitamin E, through its ability to protect cell membrane integrity, may help preventing oxidative damage, thereby averting the impairment of hepatic cell synthesis and release of yolk precursor proteins which is necessary for yolk formation. Moreover, Bollengier-lee *et al*., [3] concluded that a dietary supplementation of 250 mg vitamin E/kg is optimum for alleviating the adverse effects of chronic heat stress in laying hens. Therefore, it seems that a reason for the difference between our results and the results of other researchers was the low production rate and higher egg weight due to selecting the late phase of egg production. Another possible explanation for the discrepancies observed in literature could be related to differences in time and severity of high temperature that was subjected to hens. Did not find any study on the effect of E-Selenovit supelement on hen performance during heat stress. However, several researchers observed no difference in daily egg production due to level or source of Vitamin E and selenium supplementation [6, 12, 22]. Other researchers reported no differences in egg weight in regard to selenium supplementation [6, 21,12]. The results of this trial did not show any significant effect of vitamin E and selenium supplementation in laying diets on reducing the negative effects of high environmental temperature on hen performance. The results of this study was support data reported by Ciftci et al [8] that vitamin E supplementation at high levels can improve performance of hens exposed to heat stress. The enhancement of serum cholesterol concentration under hot conditions might be related to the fact that higher plasma cholesterol is needed for stress hormones synthesis like corticosterones which was indicated that the release of glucocorticoids increase in response to stress. Because the liver is main organ of cholesterol synthesis in layer hen and corticosterones synthesized in adrenal glands, the increased release of cholesterol from liver into blood maybe essential for corticosterone synthesis. E-Selenovit supelement was decrease serum cholesterol and yolk cholesterol in this experiment, these finding were in agreement with Hidiroglou *et al*., [11], who showed that supelementation diet with vitamin E and selenium had effect on decrease serum and yolk cholesterol. According to earlier reports, the cholesterol concentration decreases with increasing antioxidants in the diet [4,14]. The mechanism of cholesterol decrease may be the inhibition of sterol biosynthesis by oxysterols [4]. However, Sakuma *et al*., [26] did not observe any changes in the total cholesterol concentrations in the bile and plasma of rats which received Se-deficient diet compared with the control group. Sahin *et al*., [25] reported that serum cholesterol concentrations decreased, when vitamin E was added to the diet of laying hens reared at high ambient temperatures. Antibody production against SRBC and NDV in laying hens that fed E-Selenovit supplementation was greater than control group ($p<0.05$). Serological data from the present study showed the effectiveness of E-Selenovit supplementation on systemic immunity. The results of this experiment was similar to finding of Mohiti Asli *et al*., [19].

They indicated that vitamin E could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens.

CONCLUSION

In conclusion, evidence from this study suggests that dietary supplementation of laying hens with E-Selenovit during heat stress condition can improve the immune response of birds and can leads to improve performance and a more positive effect was observed when 100 mg/kg E-selenovit added to the diet. Decreasing serum and yolk cholesterol concentration by E-Selenovit supplementation is relatively a new result, so the antioxidative effect of vitamin E and selenium on serum and yolk cholesterol could be the subject of further investigations.

Acknowledgement
The researcher would like to thanks Mr. Ebrahim Esfandiar pour and staff of Rezvan junior college aviculture farm for his coordination, supported, assisting data collection and caring the hens and also I would like to have a special thank to my dear sister Mrs, Kolsoom Moradi for criticism reading and editing of the manuscript.

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