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INFLUENCE OF VITAMIN-E AND SELENIUM SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND ANTIBODY RESPONSES OF LAYER CHICKS

G. KANCHANA [*1] AND G.P. JEYANTHI [2]

[1]Department of Biochemistry, Muthayammal College of Arts & Science, Rasipuram, Tamilnadu, India.
[2]Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam University for Women, Coimbatore, Tamilnadu, India

***Corresponding Author** **kanchmoorthi@yahoo.co.in**

ABSTRACT

An experiment was conducted to study the effect of vitamin E and selenium yeast supplementation on the growth performance and some immune parameters with two hundred and ten commercial straight run day-older layer chicks. The chicks were randomly allotted into seven treatment groups with three replicates of ten chicks each. The chicks were fed basal diet (T_1), basal diet with 100 mg/kg vitamin E (T_2), basal diet with 200 mg/kg vitamin E (T_3), basal diet with 0.2 mg/kg selenium (T_4), basal diet with 0.4 mg/kg selenium (T_5), basal diet with 100 mg/kg vitamin E plus 0.2 mg/kg selenium (T_6), and basal diet with 200 mg/kg vitamin E plus 0.4 mg/kg selenium (T_7) for eight weeks period. No significant difference was found in the body weight of layer chicks for the first four weeks among the treatment groups but the body weight of groups (T_6 and T_7) receiving both vitamin E and selenium was significantly increased ($P<0.05$) at the end of 8 weeks period. The feed intake of layer chicks did not vary significantly between treatment groups. Chicks receiving supplements of 100 mg vitamin E/kg and 0·2 mg selenium/kg produced significantly higher HA titre against SRBC ($P<0.01$), HI titre against NDV ($P<0.01$) and QAGPT titre against IBDV ($P<0.01$). The results suggested that vitamin E and selenium might provide higher level of protection against natural immune challenges.

KEY WORDS

Trace elements, antibody, immunity, antioxidant, selenium

INTRODUCTION

Poultry is one of the fastest growing segments of the agricultural sector in developing countries today. In context of poultry industry, the birds encounter numerous stressors during their lives. These stressors cause hormone changes, reduces feed intake, alter nutrient metabolism and suppresses immune function. Therefore, it is highly essential to find ways and means for enhancement of immune response by nutritional manipulation. The immune system benefits greatly from proper nutrition of the bird and it also prepare the bird for periods of stress, reducing the adverse effects of stress and enhancing recovery from stressful periods. Therefore, it is an increasing challenge to formulate diets that allow highly productive, intensively-reared modern livestock and poultry to reach genetic potential. In recent years, there has been a growing understanding that marginal vitamin and trace element status is a factor limiting health and

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productivity. It was reported that, nutritionists have to take into account several factors including stress management and immunity enhancement while formulating feed [1].

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 [2, 3]. In this respect, antioxidants play an important role in maintaining bird health, productive and reproductive performance [4]. Of the known antioxidants within animal cells, vitamin E, and selenium are considered the most important. Vitamin E functions as a chain breaking antioxidant which prevents free radical induced oxidative damage by trapping reactive oxyradicals in biological membranes [5]. Selenium, an essential component of the enzyme glutathione peroxidase (GSH-Px) destroys peroxides before they can damage body tissues. GSH-Px concentration and activity are directly related to the selenium status of the animal [6]. Selenium and vitamin E are inter-related; hence complete protection of living cells requires both vitamin E and selenium in the diet. Traditionally, selenium has been added to poultry diet in the form of inorganic sources either as sodium selenite or sodium selenate [7]. Selenium yeast has been reported to be an excellent source of organic selenium [8]. Results of current studies provided evidence that organic form of selenium is generally safer and better absorbed [9].

Even though most nutrients required by the immune system are present in the diet in sufficient concentrations, there is evidence that increased dietary supplementation of certain nutrients especially antioxidants, above that needed for maximum growth and feed efficiency, is of benefit to the immune response. Thus, the aim of the present investigation was to assess the effect of dietary supplementation of different combinations of vitamin E and selenium in excess of the recommended level either independently or simultaneously on growth and immune response of layer chicks.

MATERIALS AND METHODS

Two hundred and ten commercial straight run day-older layer chicks belonging to a single hatch were purchased from Venkateswara Hatcheries, Namakkal. The birds were wing banded, weighed and randomly allotted into seven treatment groups with three replicates of ten chicks each. The chicks were fed basal diet (T_1), basal diet with 100 mg/kg vitamin E (T_2), basal diet with 200 mg/kg vitamin E (T_3), basal diet with 0.2 mg/kg selenium (T_4), basal diet with 0.4 mg/kg selenium (T_5), basal diet with 100 mg/kg vitamin E plus 0.2 mg/kg selenium (T_6), and basal diet with 200 mg/kg vitamin E plus 0.4 mg/kg selenium (T_7).

The chicks of all treatment groups were reared in cages under standard managemental condition throughout the study period of 8 weeks. The experimental diet was formulated according to the standard prescribed in Bureau of India Standards [10]; expect the vitamin E and selenium level in basal diet. Vitamin E in the form of dl-α-tocopheryl acetate (Promix E) and selenium in the form of Sel-plex containing mainly as selenomethionine were incorporated into the basal diet either independently or simultaneously in the basal diet. The chicks were fed with weighed quantity of feed ad libitum and have free access to whole some water throughout the investigation period.

(i) Growth rate study

Individual body weight of the chicks in each replicate in all the treatment groups was recorded at the end of every 28 days period up to 8 weeks of age. Feed consumption of all the treatment groups was recorded once in 28 days period and the mean total feed consumption per bird was calculated.

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(ii) Immunity against Sheep Red Blood Cells

Whole blood was collected from Mecheri breed of sheep in Alsever's solution (5.125 g of dextrose, 2 g of sodium citrate and 1.05 g of sodium chloride dissolved in 250 ml of distilled water). The sheep red blood cells (SRBC) were washed thrice in phosphate buffered saline (PBS, pH 7.4) and resuspended in PBS to prepare 25 %(v/v) SRBC suspension. On the twenty eighth day of age, six chicks per dietary treatment were randomly picked up and immunized with 0.5ml of 25% SRBC on each thigh muscle [11]. Blood samples were collected fifteen days after immunization and haemagglutination (HA) titre was assessed. HA titre was expressed as the $\log_2$ of the reciprocal of the highest dilution showing 100% agglutination [12].

(iii) Immunity against Newcastle Disease Virus

Blood samples were collected randomly from six birds in each treatment group fifteen days after immunization with Newcastle disease vaccine (Ventri Biologicals, Pune, India). The serum samples were separated and utilized for the haemagglutination inhibition (HI) test to find out the immunity developed against the Newcastle disease virus (NDV) [13].

(iv) Immunity against Infectious Bursal Disease Virus

Blood samples were collected randomly from six birds in each treatment group fifteen days after immunization with IBD vaccine (Ventri Biologicals, Pune, India). The serum samples were separated and utilized for analyzing the antibody level. Quantitative agar gel precipitation test (QAGPT) was used to assess the antibody level against Infectious Bursal Disease Virus (IBDV) as per the method described by Wood et al., with slight modification [14].

About 1.2g of agar was dissolved in 100 ml hypertonic saline (8% sodium chloride) and boiled. To the above boiled solution, 0.02% sodium azide was added as a preservative to prevent bacterial contamination. The solution was cooled to 50-55^{o} C and then cast approximately 5 ml on the clean glass slide and allowed for solidification. After solidification, with the aid of gel puncher two sets of wells were made on the agar plates, each set was made of a central well surrounded by six equidistant satellite peripheral wells of about 5 mm diameter with an inter-space of 0.3 cm. The peripheral wells were numbered from 1 to 12. The two central wells were loaded with infectious bursal disease virus antigen. The wells 6 and 7 were kept as known negative and positive control respectively. The first peripheral well was filled with test serum and in the remaining wells, serial two fold dilutions of test serum were added. The slides were then incubated at 37^{o} C for 24 to 28 h in a humid chamber. The reciprocal of the highest dilution of serum showing precipitin line was taken as the QAGPT titre of the serum.

(v) Statistical analysis of data

The collected data on various parameters were subjected to analysis of variance for statistical significance as per the methods of Snedecor and Cochran [15].

RESULTS

1. ***Growth performance***

The influence of vitamin E and selenium supplementation independently and simultaneously on the body weight gain of layer chicks from 1 to 8 weeks of age is presented in Figure1. The mean body weight of layer chicks during the first 4 week growth period did not differ significantly among the treatment groups. The body weight was significantly (P<0.05) higher in T_6 and T_7 at the end of eighth week, as compared to control and other treatment groups. However, no significant difference was observed between the experimental groups T_2, T_3, T_4 and T_5.

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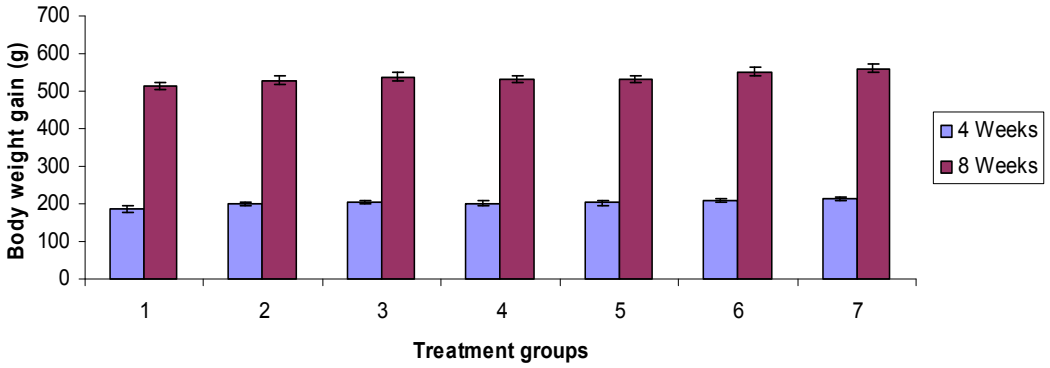
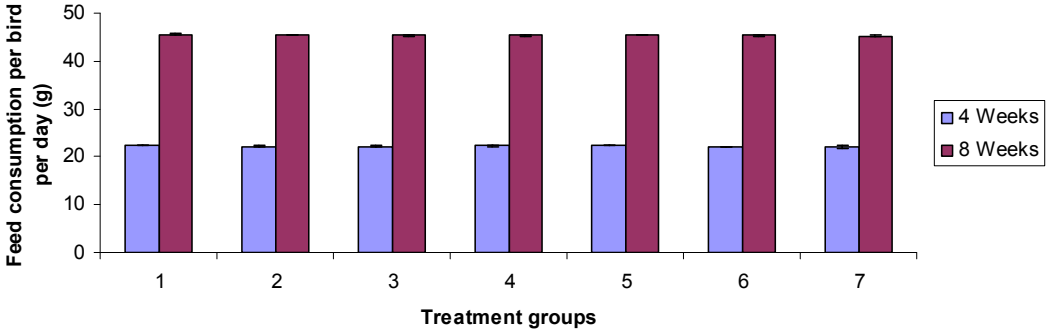
Figure 1 Effect of vitamin E and selenium supplementation on body weight gain of layer chicks



2. Feed consumption

The mean daily feed consumption of layer chicks from 1 to 8 weeks of age as influenced by supplementation of vitamin E and selenium independently and simultaneously is presented in Figure 2. The feed intake of layer chicks did not differ significantly among the treatment groups throughout the investigation period. However, the treatments groups (T_6 and T_7) that received combination of vitamin E and selenium had lower feed consumption.

Figure 2 Effect of vitamin E and selenium supplementation on feed consumption of layer chicks



3. Immunity

The HA, HI and QAGPT antibody titres of layer chicks are shown in Table 1. The mean $\log_2$ HA titre against SRBC was significantly ($P<0.01$) higher in T_6 and T_7 with mean values of 4.88 and 4.50 respectively as compared to T_1

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which recorded HA titre of 2.13. The HI titre against NDV was significantly ($P<0.01$) higher in T_6 (5.13) and T_7 (4.63) followed by selenium supplemented groups T_4 (4.25) and T_5 (3.88). The lowest HI titre was recorded in T_1, T_3 and T_2 with mean values of 2.88, 3.50 and 3.63 respectively. The QAGPT titre against IBDV was significantly ($P<0.01$) higher in T_6 (3.50) followed by T_7 (3.25) as compared to the control and other treatment groups.

Table 1

Mean (±S.E.) log_2 HA titre against SRBC, HI titre against NDV and QAGPT titre against IBDV as influenced by supplementing vitamin E and selenium

Treatment groups	HA titer against SRBC	HI titre against NDV	QAGPT titre against IBDV
T_1- Control	2.13 ± 0.13^{A}	2.88 ± 0.23^{A}	2.25 ± 0.16^{A}
T_2- Vitamin E 100 mg/kg	3.38 ± 0.18^{B}	3.63 ± 0.18^{AB}	2.75 ± 0.25^{AB}
T_3- Vitamin E 200 mg/kg	3.13 ± 0.23^{B}	3.50 ± 0.19^{AB}	2.50 ± 0.27^{AB}
T_4- Selenium 0.2 mg/kg	3.75 ± 0.25^{BC}	4.25 ± 0.25^{BC}	3.00 ± 0.19^{AB}
T_5- Selenium 0.4 mg/kg	3.63 ± 0.26^{B}	3.88 ± 0.23^{BC}	2.88 ± 0.23^{AB}
T_6- Vit-E 100 mg/kg + Se-0.2 mg/kg	4.88 ± 0.23^{D}	5.13 ± 0.13^{D}	3.50 ± 0.19^{C}
T_7- Vit-E 200 mg/kg + Se-0.4 mg/kg	4.50 ± 0.19^{CD}	4.63 ± 0.18^{CD}	3.25 ± 0.16^{BC}

Value given in each cell is the mean of six observations.
$^{A-D}$ Means within a column with no common superscript differ significantly ($P<0.01$).

DISCUSSION

The supplementation of vitamin E (100 & 200 mg/kg) and selenium (0.2 & 0.4 mg/kg) independently and simultaneously in the basal diet improved the body weight gain of layers chicks. However, higher body weight gains were recorded in combination of vitamin E and selenium supplemented groups (T_6 & T_7) than vitamin E and selenium alone fed group. Salman et al. [16] showed that body weight of broilers was higher ($P>0.05$) in vitamin E (250 mg/kg) and vitamin E plus organic selenium supplemented groups(250 mg+0.2 mg/kg) than that of control and vitamin E plus inorganic selenium supplemented groups (250

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mg+0.2 mg/kg). It was also reported that body weight of sel-plex (0.2 ppm) fed broilers were increased compared to control and sodium selenite treatment groups (0.2 ppm) and the combination of sodium selenite(0.1 ppm) and sel-plex (0.1 ppm) was no more effective than sel-plex alone [17].

On contrary, Yoon et al. [18] reported that broiler chicks fed supplemental selenium from organic (selenium yeast A and B) or inorganic (sodium selenite) sources did not affect ($P > 0.05$) the final body weight and average daily gain. It was also reported that dietary supplementation of *Ocimum sanctum* leaf powder (0.25% and 0.5%) and organic selenium (0.3 ppm) and their combination did not significantly influence the growth rate of broilers [19]. The body weight gain of vitamin E and selenium supplemented groups may be due to the fact that vitamin E is an excellent biological chain breaking antioxidant that protects cells and tissues from lipoperoxidative damage induced by free radicals. Vitamin E interacts with selenium containing enzyme glutathione peroxidase to prevent the oxidative breakdown to tissue membranes and cell integrity and membrane damage [20].

Vitamin E and selenium supplementation either alone or in combination in layer chicks did not differ significantly on feed consumption from 1 to 8 weeks of age. These findings were in accordance with previous studies in which, Payne et al.[21] found that feed intake was not affected ($P > 0.05$) by organic or inorganic selenium source or level of supplementation in any period of growth of broilers. The inclusion of vitamin E (250 mg/kg) in combination with either organic or inorganic selenium (0.2 mg/kg) in the diet of broilers had no effect on feed intake [16]. It was reported that feed intake was not affected by vitamin E supplementation under thermo-neutral conditions. However, feed intake increased with the vitamin E supplementation either singly or in combination with vitamin C in heat-stressed Japanese quail [22]. On the other hand, it was reported that supplementation of vitamin E (150 and 300 IU/Kg) in the basal diet of broilers caused significant reduction in feed take [23]. The variation in feed intake may be due to dosages of vitamin E and selenium supplementation, strain of the birds, season, energy content of the diet, ambient temperature, housing designs, hygienic conditions and rearing environment prevailed at the time of experiment.

The mean HA titre against SRBC, HI titre against NDV, and QAGPT titre against IBDV were significantly higher in chicks fed diets containing vitamin E (100 mg/kg) and selenium (0.2 mg/kg) compared to other treatments and control group. This report is well supported that combination of vitamin E and selenium supplementation significantly improved both humoral and cell mediated immunity in birds [24]. Similar results have been observed in broilers fed different levels of vitamin E (0, 10, 25, 50, 100 and 200 IU/kg diet) and found better immune response against IBDV and SRBC at levels of 25 and 50 IU/kg feed respectively [25]. Significantly higher antibody titres (HI and ELISA) against NDV were found in broilers fed diets receiving 0.06 mg/kg selenium and 150 IU/kg vitamin E [23]. Increased antibody production against SRBC was observed in laying hens supplemented with combination of probiotics, yeast, vitamin E and vitamin C [26].

On the other hand, no significant effect on antibody titres against SRBC was observed in broilers fed vitamin E (10 and 300 IU/kg) and concluded that vitamin E supplementation increases heterophil/lymphocyte ratio, indicating that vitamin E improved the phagocytic capacity of the immune system, promoting the birds against the invasion of pathogenic microorganisms [27]. The reason for the contradiction among the results might be the result of the difference in the species

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used, age of animals and the amount of vitamin E and selenium supplemented to the diet.

The present findings suggest that vitamin E may have an immunomodulator effect, increasing the resistance to diseases. A possible mechanism of this enhanced immune function may be a down regulation of the biosynthesis of prostaglandins, which inhibit several immunity parameters [28]. Vitamin E and GSH-Px are two molecules that help to prevent the oxidative damage. Free radicals are scavenged by vitamin E as a first line of defense and selenium as a constituent of cytosolic enzyme glutathione peroxidase convert free radicals to inert substances rendering them harmless. Thus, vitamin E and selenium have biosynergistic effect and complete protection of living cells require both the nutrients in the diet.

CONCLUSION

The present investigation suggested that combination of vitamin E at a level of 200 mg and selenium at 0.4 mg/kg diet are good for improving growth rate and 100 mg vitamin E and 0.2 mg selenium per kg diet improves the immune responses of layer chickens. Thus, supplementation of vitamin E and selenium at levels above those recommended as nutritional requirements enhances disease resistance which has been attributed to significant stimulation of humoral and cellular immunity and phagocytosis.

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