Results and Discussion
The production of reactive free radicals as by-products of metabolism that have the potential to damage or destroy cellular structures is in a dynamic equilibrium under normal conditions in living organisms. This dynamic equilibrium is provided by a balance between antioxidants and pro-oxidants. However, stress factors such as nutritional (low digestible feed, feeds rich in polyunsaturated fatty acids, mycotoxins and oxidized oil, vitamin E and selenium deficiency, vitamin A excess, the presence of heavy metals and other toxicants), environmental (high or low ambient temperatures, transportation) and pathogenesis of numerous diseases including parasitic infections, have a negative impact on this antioxidant/pro-oxidant balance (Koinarski et al., 2005).

The imbalance between an antioxidant and pro-oxidant system is named oxidative stress. In commercial poultry production, oxidative stress has been associated with the deterioration of many physiological functions including health, growth, reproduction and immunity. In this respect, dietary antioxidants such as vitamin E and selenium are beneficial in preventing these detrimental effects (Fellenberg and Speisky, 2006). Vitamin E and selenium are key components of the antioxidant system, reducing lipid peroxidation.

The present study was aimed to assess the effect of supplementation of vitamin E and selenium and their combinations on the growth, immune response, production performance and biochemical profile of layer chickens.

The results of the present study are discussed under the following headings:

PHASE I

4.1 EFFECT OF VITAMIN E AND SELENIUM SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND IMMUNOLOGICAL RESPONSE OF LAYER CHICKENS
4.1.1 Effect of vitamin E and selenium supplementation on the body weight gain of layer chickens from 0-16 weeks of age

4.1.2 Effect of vitamin E and selenium supplementation on cumulative feed consumption of layer chickens from 0-16 weeks of age

4.1.3 Effect of vitamin E and selenium supplementation on feed efficiency of layer chickens from 0-16 weeks of age

4.1.4 Effect of vitamin E and selenium supplementation on livability percentage of layer chickens from 0-16 weeks of age

4.1.5 Effect of vitamin E and selenium supplementation on immunological response of layer chickens

PHASE II

4.2 EFFECT OF VITAMIN E AND SELENIUM SUPPLEMENTATION ON PRODUCTION PERFORMANCE, EGG QUALITY CHARACTERISTICS AND BIOCHEMICAL PROFILE OF LAYERS

4.2.1 Effect of vitamin E and selenium supplementation on the body weight of layers from 20-40 weeks of age

4.2.2 Effect of vitamin E and selenium supplementation on livability percentage of layers from 21-40 weeks of age

4.2.3 Effect of vitamin E and selenium supplementation on egg production of layers from 21-40 weeks of age

4.2.4 Effect of vitamin E and selenium supplementation on cumulative feed consumption and feed efficiency of layers from 21-40 weeks of age

4.2.5 Effect of vitamin E and selenium supplementation on egg quality characteristics of layers from 21-40 weeks of age

4.2.6 Effect of vitamin E and selenium supplementation on α-tocopherol content in the egg yolk of layers

4.2.7 Effect of vitamin E and selenium supplementation on selenium content in the egg albumen and egg yolk of layers

4.2.8 Effect of vitamin E and selenium supplementation on lipid peroxidation in the plasma and liver of layers

4.2.9 Effect of vitamin E and selenium supplementation on the enzymic and non-enzymic antioxidant status in the liver of layers
PHASE I

4.1 EFFECT OF VITAMIN E AND SELENIUM SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND IMMUNOLOGICAL RESPONSE OF LAYER CHICKENS

Vitamin E is one of the antioxidants widely used in poultry diets and has been proposed as a major antioxidant in plasma membranes of all cells and sub-cellular organs, functioning as a chain-breaker and free radical scavenger. Selenium plays an important role in the antioxidant defense system due to its requirement by the selenium dependent glutathione peroxidase, which is involved in cellular antioxidant protection. It has been suggested that there is a synergistic relationship between selenium and vitamin E, because glutathione peroxidase continues the work of vitamin E by detoxifying hydroperoxides (Malayoglu et al., 2009).

Vitamin E and selenium supplementation in animal diets might enhance the immune status and the ability of the immune system to respond to disease
challenges. The combination of high levels of vitamin E and selenium might play complementary roles in the cellular and humoral immune response of poultry.

4.1.1 Effect of vitamin E and selenium supplementation on the body weight gain of layer chickens from 0-16 weeks of age

The influence of vitamin E and selenium supplementation independently and in combination on the body weight gain of layer chickens from 0 to 16 weeks of age are presented in Figure 4.

**FIGURE 4**

**MEAN BODY WEIGHT GAIN (g) OF LAYER CHICKENS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 0-16 WEEKS OF AGE**

| T1 | Basal diet |
| T2 | Basal diet+100 mg vitamin E/kg feed |
| T3 | Basal diet+200 mg vitamin E/kg feed |
| T4 | Basal diet+0.2 mg selenium/kg feed |
| T5 | Basal diet+0.4 mg selenium/kg feed |
| T6 | Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed |
| T7 | Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed |
The mean body weight gain of layer chicks during the first 4 week growth period did not differ significantly among the treatment groups. The body weight gain of layer chickens from fifth week to sixteenth week was significantly (p<0.05) higher in T_6 and T_7 groups that received both vitamin E and selenium as compared to the control and other treatment groups. Although the body weight of layer birds supplemented with either vitamin E or selenium was increased markedly, no significant difference was observed between the vitamin E and selenium alone fed groups (T_2, T_3, T_4 and T_5) and the control group fed basal diet.

Similar findings were observed by Swain et al. (2000) who reported that broiler chicks supplemented with vitamin E at 150 and 300 IU per kg basal diet had higher body weight gain at 42 days of age when compared to control group and with no difference found between the two levels of vitamin E supplementation. The increased gain in body weight was also supported by El-Sebai (2000), Swain and Johri (2000), Aravind et al. (2001) and Salman et al. (2007) in broilers supplemented with vitamin E and selenium.

Bonomi (2001) reported that dietary selenium supplementation improved the weight gain of broilers. It was observed that separately or as a combination, supplemental vitamin E and vitamin A increased weight gain of broilers (Sahin et al. 2001b). Mlodkowski et al. (2003) found that supplementation of 10 percent rapeseed oil with vitamin E (5, 20 and 50mg/kg) in the basal diet significantly increased the weight gain in all the treatment groups of broilers.

Sahin et al. (2003b) reported that Japanese quail fed basal diet supplemented with vitamin C and vitamin E singly or in combination had significant increase in the body weight. Supplementation of vitamin E at 100 mg per kg feed significantly increased the growth rate of broilers from one to three weeks of age (Guo et al., 2003).

Mahmoud and Edens (2005) evaluated the effect of organic selenium on the performance and different physiological parameters of broilers either in
pathogenic *Escherichia coli*-challenged or under heat-stressed conditions. They found that broilers supplemented with 0.2 mg organic selenium/kg feed, improved the body weight.

Surai (2006) noted that the improved growth rate of broilers fed on organic selenium supplemented diet could be related to the increased concentrations of the active form of thyroid hormone in the serum of chickens supplemented with organic selenium as well as to the immunomodulating properties of selenium. Similar increase in the body weight of broiler chickens supplemented with dietary vitamin E and selenium was also confirmed by Nameghi *et al.* (2007) and Sevcikova *et al.* (2006).

Peric *et al.* (2007) and Upton *et al.* (2008) reported that body weight of sel-plex fed broilers was found to be increased compared to the control group. Skrivan *et al.* (2008) showed that the basal diet supplemented with 0.3 mg/kg selenomethionine increased the body weight of broiler chickens by about 3% compared to the control and sodium selenite supplemented group. Significant improvement in the body weight and body weight gain of broilers supplemented with vitamin E or organic selenium was observed by Malayoglu *et al.* (2009).


In the present study, the increase in the body weight gain of layer chickens from the fifth to sixteenth week of age might be due to the protective effects of antioxidants vitamin E and selenium on cell membranes and sub-cellular organelles. Vitamin E functions biologically as a membrane-specific scavenger of free radicals and selenium, a component of glutathione peroxidase functions as a destroyer of peroxides.
The reason for the contradiction of the result might be due to species, dosage and duration of supplementation of vitamin E and selenium in the feed of birds.

4.1.2 Effect of vitamin E and selenium supplementation on cumulative feed consumption of layer chickens from 0-16 weeks of age

The mean cumulative feed consumption of layer chickens supplemented with vitamin E and selenium independently and simultaneously from 0 to 16 weeks of age is presented in Figure 5.

FIGURE 5
MEAN CUMULATIVE FEED CONSUMPTION (g/ bird) OF LAYER CHICKENS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 0-16 WEEKS OF AGE

T₁ - Basal diet
T₂ - Basal diet+100 mg vitamin E/kg feed
T₃ - Basal diet+200 mg vitamin E/kg feed
T₄ - Basal diet+0.2 mg selenium/kg feed
T₅ - Basal diet+0.4 mg selenium/kg feed
T₆ - Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T₇ - Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed
The feed intake of layer chickens by supplementing vitamin E and selenium in the feed did not differ significantly among the treatment groups throughout the growing period.

The results of the present study were in agreement with those of Nageshwara et al. (2003) who reported that broilers supplemented with E-Care-Se-Herbal (one ml of E-Care-Se-Herbal containing 100 mg vitamin E, 0.5 mg selenium and Ocimum extract) at 0.5 ml per litre of drinking water during the first, third and fifth week of age did not influence the feed intake. Payne and Southern (2005a) compared inorganic and organic selenium sources among broilers and found that daily feed intake was not affected by selenium source or level of supplementation in any period of growth.

Sakamoto et al. (2006), Abdukalykova and Ruiz-Feria (2006), Nameghi et al. (2007) and Niu et al. (2009) found no significant effect on feed intake in broilers fed supplemental vitamin E in the feed. Biswas et al. (2006) also found no significant effect on feed intake in Japanese quails fed higher levels of dietary selenium.

Salman et al. (2007) found that inclusion of vitamin E in combination with either organic or inorganic selenium in the diet of broilers had no effect on feed intake. However, Yoon et al. (2007) observed that broiler chicks fed supplemental selenium from organic (selenium yeast A and B) or inorganic (sodium selenite) sources had lower feed intake during the first 3 weeks, but not during the following 3 weeks.

Sahin et al. (2009) observed that feed intake of Japanese quail was not affected by vitamin C and vitamin E supplementation under thermo-neutral conditions. However, feed intake increased with the vitamin C or vitamin E supplementation either singly or in combination in heat-stressed quail. Dietary vitamin E and organic selenium supplementation enriched with n-3 poly unsaturated fatty acid in broilers had no effect on feed intake (Malayoglu et al., 2009).
In contrast to the results of the present study, Naylor et al. (2000) observed that broilers fed basal diet supplemented with selenium as sel-plex or sodium selenite (0.1 and 0.25 ppm) for 38 days had lower feed intake. Swain et al. (2000) concluded that supplementation of vitamin E (150 and 300 IU/kg) in the basal diet resulted in significantly lesser feed consumption in broilers up to 42 days of age than those fed control diet. However, Sahin et al. (2002a) noted increased feed intake by vitamin E supplementation in Japanese quails under heat stress.

The variation in the feed intake if any, could 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 prevailing during the experimental period.

4.1.3 Effect of vitamin E and selenium supplementation on feed efficiency of layer chickens from 0-16 weeks of age

The mean feed efficiency of layer chickens from 0 to 16 weeks of age supplemented with vitamin E and selenium independently and simultaneously in the basal diet is presented in Table 7.

Supplementation of vitamin E and selenium in the basal diet did not cause significant improvement in feed efficiency of layer chickens during the first 4 weeks period of age. During five to sixteen weeks period of age, better feed efficiency was observed in T7 group that was supplemented with vitamin E at a level of 200 mg/kg and selenium at a level of 0.4 mg/kg in the diet. The feed efficiency values of T6 (vitamin E 100 mg/kg and selenium 0.2 mg/kg), T3 (vitamin E 200 mg/kg) and T5 selenium 0.4 mg/kg) were comparable to that of T7. However, the control group (T1) that was fed basal diet without antioxidant supplementation had lower feed efficiency compared to other treatment groups.
### TABLE 7
MEAN FEED EFFICIENCY OF LAYER CHICKENS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 0-16 WEEKS OF AGE

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>3.32±0.08</td>
<td>3.52±0.02</td>
<td>3.94±0.04</td>
<td>4.65±0.02</td>
</tr>
<tr>
<td>T2</td>
<td>3.05±0.07</td>
<td>3.40±0.02</td>
<td>3.84±0.05</td>
<td>4.60±0.02</td>
</tr>
<tr>
<td>T3</td>
<td>2.94±0.15</td>
<td>3.34±0.03</td>
<td>3.81±0.05</td>
<td>4.56±0.05</td>
</tr>
<tr>
<td>T4</td>
<td>3.04±0.12</td>
<td>3.40±0.02</td>
<td>3.83±0.04</td>
<td>4.59±0.03</td>
</tr>
<tr>
<td>T5</td>
<td>3.01±0.22</td>
<td>3.37±0.04</td>
<td>3.81±0.03</td>
<td>4.57±0.03</td>
</tr>
<tr>
<td>T6</td>
<td>2.85±0.03</td>
<td>3.25±0.07</td>
<td>3.72±0.06</td>
<td>4.47±0.05</td>
</tr>
<tr>
<td>T7</td>
<td>2.80±0.12</td>
<td>3.19±0.07</td>
<td>3.68±0.02</td>
<td>4.43±0.01</td>
</tr>
</tbody>
</table>

CD (0.01)          0.18        0.13                  0.14

CD (0.05)          -          -                     -

T1. Basal diet
T2. Basal diet+100 mg vitamin E/kg feed
T3. Basal diet+200 mg vitamin E/kg feed
T4. Basal diet+0.2 mg selenium/kg feed
T5. Basal diet+0.4 mg selenium/kg feed
T6. Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T7. Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of five birds in three replicates.

- Mean values within a column with no common superscript differ significantly (p<0.05).
- Mean values within a column with no common superscript differ significantly (p<0.01).

In agreement with the results of the present study, Edens et al. (2000) indicated that dietary selenium supplementation significantly improved the feed conversion ratio of broilers. Swain et al. (2000) and Guo et al. (2003) reported significant improvement in feed efficiency of broilers due to vitamin E supplementation.

Mahmoud and Edens (2005) while evaluating the effect of organic selenium on the performance and different physiological parameters of broilers either in pathogenic *Escherichia coli*-challenged or under heat-stressed...
conditions found improved feed conversion ratio by supplementing 0.2 mg organic selenium/kg feed.

The feed efficiency was found to be significantly higher in untreated control group while it was lower among all the groups treated with antioxidants in broilers during summer (Maini et al., 2007). It was also reported that feed conversion ratio was improved by all selenium sources with the sel-plex and sel-plex plus sodium selenite group being superior compared to sodium selenite group (Upton et al., 2008). Niu et al. (2009) observed that feed conversion was significantly affected by vitamin E at 100 mg/kg in broilers under heat stress.

On the other hand, Nageshwara et al. (2003) observed no significant difference in feed efficiency of broilers supplemented with E-Care-Se-Herbal. Payne and Southern (2005a) and Biswas et al. (2006) found no significant improvement in feed efficiency by selenium supplementation in broilers and Japanese quails respectively. Vitamin E supplementation did not cause any significant change in feed conversion ratio in broiler chicks as observed by Nameghi et al. (2007).

Improvement in feed efficiency might be due to the proper utilization of the feed by supplementation of vitamin E and selenium in the diet of layer chickens. Fat soluble vitamin E is associated with cell wall membranes. Selenium is located within the cells as an important component of the enzyme glutathione peroxidase. Together they might function to protect the cell membrane and cell content from oxidative damage and might improved the nutrient utilization.

4.1.4 Effect of vitamin E and selenium supplementation on livability percentage of layer chickens from 0-16 weeks of age

The mean livability percentage of layer chickens due to vitamin E and selenium supplementation are presented in Figure 6.
FIGURE 6
MEAN LIVABILITY PERCENTAGE OF LAYER CHICKENS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 0-16 WEEKS OF AGE

Mortality was observed in birds that received only basal diet throughout the growing period. However, no mortality was observed in any of the vitamin E and selenium supplemented groups of layer chickens during the study period from zero to twelve weeks of age. But during thirteen to sixteen weeks of age period, mortality was observed in treatment group (T4) that was supplemented with 0.2 mg/kg selenium.

The results of the present study coincide with that of Aravind et al. (2001) and Ganpule and Manjunatha (2003) who observed 100 percent livability in broiler chickens due to vitamin E and selenium supplementation. 100 percent livability in broiler chickens was also achieved by
Nageshwara et al. (2003) with E-Care Se-Herbal supplementation at 0.5 ml per litre of drinking water.

Improvement in mortality at 42 days of age was observed by Mahmoud and Edens (2005) while evaluating the effect of organic selenium on the performance and different physiological parameters of broilers either in pathogenic *Escherichia coli*-challenged or under heat-stressed conditions. Low mortality percentage was observed during the entire experimental period in broilers fed vitamin E and glutamine (Sakamoto et al., 2006).

However, Abdukalykova and Ruiz-Feria (2006) indicated that mortality was not affected in broiler chickens due to vitamin E supplementation. Japanese quails fed higher levels of dietary selenium had no significant effect on livability (Biswas et al., 2006). Sevcikova et al. (2006) and Wang and Xu (2008) also found no significant change in the survival rate due to dietary selenium supplementation of different sources on broiler chickens. Mortality of broilers remained unaffected by vitamin E and selenium supplementation in broilers as observed by Malayoglu et al. (2009).

In the present investigation, supplementation of vitamin E and selenium in excess of the recommended level did not seem to have any adverse effect on the health of the layer chickens and promoted the survival rate of chickens. Vitamin E and selenium would have worked in concert to accomplish antioxidant functions and improved the survival rate of layer chickens.

4.1.5 Effect of vitamin E and selenium supplementation on immunological response of layer chickens

Nutrients and diets can be used as means to specifically prevent infectious diseases in poultry (Klasing, 2007). Nutrition can be an important determinant in quantitative and qualitative aspects of the immune responses to pathogens. Vitamin E has been reported to protect the cells involved in immune response such as lymphocytes, macrophages and plasma cells against
oxidative damage and enhance the function of these cells (Konjufca et al., 2004). Selenium is an essential trace element for all animals. Selenium enhances immune responses leading to better resistance against diseases (McCartney, 2006).

The hemagglutination (HA), hemagglutination inhibition (HI) and quantitative agar gel precipitation test (QAGPT) antibody titres of layer chickens are shown in Table 8.

**TABLE 8**

MEAN LOG₂ HA TITRE AGAINST SRBC, HI TITRE AGAINST NDV AND QAGPT TITRE AGAINST IBDV OF LAYER CHICKENS SUPPLEMENTED WITH VITAMIN E AND SELENIUM

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>HA titre against SRBC</th>
<th>HI titre against NDV</th>
<th>QAGPT titre against IBDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁. Control</td>
<td>2.13±0.13</td>
<td>2.88±0.23</td>
<td>2.25±0.16</td>
</tr>
<tr>
<td>T₂. Vitamin E 100 mg/kg</td>
<td>3.38±0.18</td>
<td>3.63±0.18</td>
<td>2.75±0.25</td>
</tr>
<tr>
<td>T₃. Vitamin E 200 mg/kg</td>
<td>3.13±0.23</td>
<td>3.50±0.19</td>
<td>2.50±0.27</td>
</tr>
<tr>
<td>T₄. Selenium 0.2 mg/kg</td>
<td>3.75±0.25</td>
<td>4.25±0.25</td>
<td>3.00±0.19</td>
</tr>
<tr>
<td>T₅. Selenium 0.4 mg/kg</td>
<td>3.63±0.26</td>
<td>3.88±0.23</td>
<td>2.88±0.23</td>
</tr>
<tr>
<td>T₁. Vitamin E 100 mg/kg + Se-0.2 mg/kg</td>
<td>4.88±0.23</td>
<td>5.13±0.13</td>
<td>3.50±0.19</td>
</tr>
<tr>
<td>T₁. Vitamin E 200 mg/kg + Se-0.4 mg/kg</td>
<td>4.50±0.19</td>
<td>4.63±0.18</td>
<td>3.25±0.16</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>0.81</td>
<td>0.76</td>
<td>0.80</td>
</tr>
</tbody>
</table>

HA - Hemagglutination
HI - Hemagglutination Inhibition
QAGPT - Quantitative Agar Gel Precipitation Test
SRBC - Sheep Red Blood Cells
NDV - New Castle Disease Virus
IBDV - Infectious Bursal Disease Virus

Values given in each cell is the mean±SE of six birds.

AD Mean values within a column with no common superscript differ significantly (p<0.01).
The mean log₂ HA titre against SRBC was significantly (p<0.01) higher in all the treatment groups as compared to control (T₁) which recorded lowest HA titre of 2.13. The HI titre against NDV was significantly higher (p<0.01) in T₆ (5.13) and T₇ (4.63) groups that received both vitamin E and selenium in the diet. The lowest HI titre was recorded in T₁, T₃ and T₂ with mean values of 2.88, 3.50 and 3.63 respectively. The QAGPT titre against IBDV was found to be higher in T₆ (3.50) followed by T₇ (3.25) as compared to the control and other treatment groups.

Leshchinsky and Klasing (2001) found an increase in the antibody titres of broilers supplemented with 50 IU vitamin E/kg and concluded that moderate vitamin E levels (25 to 50 IU/kg) promoted better immunomodulation than high vitamin E levels (100 to 200 IU/kg), which correspond to the vitamin E levels needed for the inhibition of lipid peroxidation and for the protection of liver mitochondria against oxidative stress.

Siam et al. (2004) reported that combination of vitamin E and selenium supplementation significantly improved both humoral and cell mediated immunity in laying hens.

Singh et al. (2006) showed that broiler chicks receiving supplements of 200 mg vitamin E/kg and 0.2 mg selenium/kg diet produced significantly higher HI titres against Newcastle Disease Virus (NDV) vaccine. This was associated with an increased serum concentration of total immunoglobulins and circulatory immune complexes. The beneficial effect on immune responses by supplementing the diet with higher levels of selenium in growing Japanese quail was also confirmed by Biswas et al. (2006).

Nameghi et al. (2007) found increased antibody production to Infectious Bronchitis Virus (IBV), Newcastle Disease Virus (NDV) and Sheep Red Blood Cell (SRBC) in broilers fed different levels of dietary vitamin E and L-ascorbic acid. Increased antibody production against SRBC was observed in laying hens.
supplemented with combination of probiotics, yeast, vitamin E and vitamin C (Asli et al., 2007).

Da Silva et al. (2009) reported that supplementation of 65 mg/kg vitamin E in the diet of broiler chickens showed greater antibody titres for Newcastle disease and positively improved the humoral immune response.

Niu et al. (2009) reported that heat stress severely reduced immune response of broilers. They also found that the effect of heat stress in broilers could be improved by dietary vitamin E supplementation which increased both primary and secondary antibody responses.

On the other hand, no significant effect on antibody titres against SRBC was observed in broilers fed vitamin E by Boa-Amponsem et al. (2000) and it was concluded that vitamin E supplementation increased heterophil/lymphocyte ratio, indicating that vitamin E improved the phagocytic capacity of the immune system of the birds against the invasion of pathogenic microorganisms. The effects of vitamin E on immune function reported in the literature were highly variable, depending on the dose, the strain and age of the birds and the immune challenge.

The improvement in antibody titres might be due to antioxidant functions of vitamin E and selenium. Vitamin E prevents the free radicals (peroxides and superoxides) released during disease or vaccinal challenge, damaging the cellular and intracellular structures which also include lymphocytic cells of immune system and selenium, as a constituent of cytosolic enzyme glutathione peroxidase in the cytosol that converts free radicals to inert substances rendering them harmless. Vitamin E seems to exert a complementary effect on the immune system by inhibiting the synthesis of prostaglandins. Vitamin E and selenium would have formed a biosynergetic combination to protect the defense cells from oxidative damage.
At hatching, the immune system of birds is already partially developed and the primary organs thymus and bursa are present and populated with lymphoid cells. However, the secondary organs, such as spleen, cecal tonsils, Meckel's diverticulum and lymphoid tissues scattered in the digestive and respiratory tract are still incomplete (Dibner and Richards, 2004). The immune system benefits greatly from proper nutrition of the bird and indirectly it will also prepare the bird for periods of stress, reducing the adverse effects and enhancing recovery from stressful periods.

PHASE II

4.2 EFFECT OF VITAMIN E AND SELENIUM SUPPLEMENTATION ON THE PRODUCTION PERFORMANCE, EGG QUALITY CHARACTERISTICS AND BIOCHEMICAL PROFILE OF LAYERS

Thermo neutral (comfort) temperatures are between 18-22°C for poultry housing. Because poultry production is inevitably being carried out in warmer conditions in some regions around the world, the related problems seems to be challenged. The metabolic rate of the organism is changed by stress factors in warmer climates. Biochemical, physiological and behavioural reactions occur in order to provide homeostatic balance to the organism under heat stress (Imik et al., 2009).

When the temperature exceeds 30°C, signs of heat stress are likely to appear. It has been proposed that heat stress negatively affects egg production and egg shell quality, decreases feed consumption and live weight and disrupts the acid-base balance of the blood, thus causing some changes in metabolism and oxidative damage to cells (Ertas and Sahin, 2002).

Environmental stress has been shown to cause an increase in oxidative stress and an imbalance in antioxidant status. As a result, plasma antioxidant vitamin and mineral levels decline and oxidative damage increase in poultry. A combination of antioxidant vitamin and minerals shows greater antioxidant
stability against oxidative damage. It is known that vitamin E and selenium act synergistically as the primary members of an antioxidant defense system by quenching lipid peroxyl radicals.

4.2.1 Effect of vitamin E and selenium supplementation on the body weight of layers from 20-40 weeks of age

Vitamin E is included in animal diets to improve performance, to strengthen immunological status and to increase the vitamin E content of food of animal origin. Poultry cannot synthesize vitamin E and therefore vitamin E requirements must be met from dietary sources. Selenium is a highly effective antioxidant and essential trace mineral in animals (Sahin et al., 2003a).

The influence of vitamin E and selenium supplementation independently and in combination on the body weight of layers from 20 to 40 weeks of age is shown in Figure 7.

The mean body weight of layers from 20 to 40 weeks period of age was significantly (p<0.05) higher in T_6 and T_7 groups that received both vitamin E and selenium as compared to the control and other treatment groups. Although the body weight of layers supplemented with either vitamin E or selenium increased markedly, no significant difference was observed between vitamin E and selenium alone fed groups (T_2, T_3, T_4 and T_5) and the control group fed basal diet (T_1).

The results of the present study coincide with that of Sahin et al. (2002a) who observed that vitamin E supplementation caused significant increase in the body weight of Japanese quail layers reared under heat stress. Sahin et al. (2003a) also reported that vitamin E and selenium supplementation significantly increased the body weight of Japanese quail layers reared under cold stress.
FIGURE 7
MEAN BODY WEIGHT (g) OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 20-40 WEEKS OF AGE

Ganpule and Manjunatha (2003) reported that inclusion of organic selenium in combination with vitamin E in the basal diet improved the growth rate of broiler breeders. Kucuk et al. (2003) observed that Hy-Line laying hens fed basal diet supplemented with either vitamin E or vitamin C or a combination of vitamin E and C had significantly increased body weight which was in agreement with the result of this study. In contrary, Siam et al. (2004) and Eid et al. (2008) found no significant differences in the body weight of laying hens due to vitamin E supplementation.

The significant increase in the body weight of laying hens supplemented with both vitamin E and selenium might be due to the protection of cells and...
tissues from oxidative damage induced by free radicals. Vitamin E is an excellent biological chain breaking antioxidant in biological membranes, which prevents free radical induced oxidative damage by trapping reactive oxyradicals. Selenium plays a central role in antioxidant defense in the cell by removing hydrogen peroxide and lipid hydroperoxide formed during metabolism and superoxide radical dismutation.

4.2.2 Effect of vitamin E and selenium supplementation on livability percentage of layers from 21-40 weeks of age

The mean livability percentage of layers supplemented with vitamin E and selenium is shown in Figure 8.

FIGURE 8
MEAN LIVABILITY PERCENTAGE OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE

T1. Basal diet
T2. Basal diet+100 mg vitamin E/kg feed
T3. Basal diet+200 mg vitamin E/kg feed
T4. Basal diet+0.2 mg selenium/kg feed
T5. Basal diet+0.4 mg selenium/kg feed
T6. Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T7. Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed
During the first four weeks period of laying, no mortality was observed in any of the treatment groups of layers. However, from twenty five week onwards, mortality was observed in the control group that received only basal diet. All the supplemented groups recorded 100 percent livability throughout the laying phase of the study period.

Siam et al. (2004) observed lower mortality rate in layers which had received vitamin E supplementation, than the control which received no supplementation. Ganpule and Manjunatha (2003) reported that inclusion of organic selenium in combination with vitamin E in the basal diet improved the percentage of livability in broiler breeders.

Ciftci et al. (2005) reported that mortality rate was higher in the control group when compared to vitamin E supplemented group and concluded that the livability percentage of laying hens was improved by vitamin E supplementation which was in accordance with the results of the present study.

The results of the present study suggest that addition of vitamin E and selenium in excess of the recommended levels has beneficial effect on layers during laying period. The excess levels of added vitamin E and selenium would have worked as better antioxidants against oxidative damage and offered protection to the birds.

4.2.3 Effect of vitamin E and selenium supplementation on egg production of layers from 21- 40 weeks of age

The poultry is an important segment of the world's food industry, providing eggs and meat to a large populace. Egg is one of the most nutritious foods available to man. It provides a balanced protein which contains all the amino acids considered essential in sufficient amounts and proportion to maintain life and support growth when used as a sole source of protein food. Eggs are a good source of nutrients and play an important role as a functional food in human nutrition (Sparks, 2006).
The mean hen-day and hen-housed egg production of layers supplemented with vitamin E and selenium independently and in combination in the basal diet from 21 to 40 weeks of age are presented in Table 9 and 10 respectively.

**TABLE 9**

**MEAN HEN-DAY EGG PRODUCTION (%) OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21- 40 WEEKS OF AGE**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>21-24 weeks</th>
<th>25-28 weeks</th>
<th>29-32 weeks</th>
<th>33-36 weeks</th>
<th>37-40 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>T$_1$</td>
<td>80.06±1.07$^A$</td>
<td>83.39±0.75$^A$</td>
<td>85.12±1.07$^A$</td>
<td>82.95±0.44$^A$</td>
<td>82.31±0.45$^A$</td>
</tr>
<tr>
<td>T$_2$</td>
<td>83.03±1.18$^{ABC}$</td>
<td>85.86±1.51$^{AB}$</td>
<td>89.58±0.90$^B$</td>
<td>89.44±1.16$^{BC}$</td>
<td>88.54±1.42$^{BC}$</td>
</tr>
<tr>
<td>T$_3$</td>
<td>84.67±1.04$^{BC}$</td>
<td>88.25±1.51$^{BC}$</td>
<td>92.11±0.54$^{BC}$</td>
<td>91.67±1.57$^{BC}$</td>
<td>90.77±1.58$^{BC}$</td>
</tr>
<tr>
<td>T$_4$</td>
<td>81.10±0.98$^{AB}$</td>
<td>84.97±1.04$^{AB}$</td>
<td>88.99±0.54$^B$</td>
<td>88.69±1.30$^B$</td>
<td>87.80±1.30$^B$</td>
</tr>
<tr>
<td>T$_5$</td>
<td>81.40±1.16$^{AB}$</td>
<td>85.56±1.16$^{AB}$</td>
<td>89.88±0.39$^B$</td>
<td>89.29±1.03$^{BC}$</td>
<td>88.69±1.07$^{BC}$</td>
</tr>
<tr>
<td>T$_6$</td>
<td>85.68±0.80$^{C}$</td>
<td>89.44±0.98$^{BC}$</td>
<td>93.45±1.32$^C$</td>
<td>92.71±0.65$^{BC}$</td>
<td>92.11±1.42$^{BC}$</td>
</tr>
<tr>
<td>T$_7$</td>
<td>87.20±0.79$^{C}$</td>
<td>91.96±0.52$^{C}$</td>
<td>95.24±0.74$^C$</td>
<td>93.45±0.98$^{C}$</td>
<td>93.01±0.74$^{C}$</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>4.27</td>
<td>4.71</td>
<td>3.55</td>
<td>4.54</td>
<td>5.06</td>
</tr>
</tbody>
</table>

T$_1$. Basal diet
T$_2$. Basal diet+100 mg vitamin E/kg feed
T$_3$. Basal diet+200 mg vitamin E/kg feed
T$_4$. Basal diet+0.2 mg selenium/kg feed
T$_5$. Basal diet+0.4 mg selenium/kg feed
T$_6$. Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T$_7$. Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of five birds in three replicates.

A-C Mean values within a column with no common superscript differ significantly (p<0.01).

The results of the study showed that during 21-28 weeks, the birds in group T$_6$ and T$_7$ that were supplemented with both vitamin E and selenium recorded the highest egg production followed by T$_3$ group supplemented with 200 mg/kg vitamin E. From 29 to 40 weeks, both hen-day and hen-housed egg production were found to be significantly (p<0.01) increased in all the supplemented groups compared to the control group.
### TABLE 10

**MEAN HEN-HOUSED EGG PRODUCTION (eggs/bird) OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>21-24 weeks</th>
<th>25-28 weeks</th>
<th>29-32 weeks</th>
<th>33-36 weeks</th>
<th>37-40 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T 1</strong></td>
<td>22.42±0.30&lt;sup&gt;A&lt;/sup&gt;</td>
<td>22.83±0.68&lt;sup&gt;A&lt;/sup&gt;</td>
<td>23.11±0.43&lt;sup&gt;A&lt;/sup&gt;</td>
<td>23.23±0.13&lt;sup&gt;A&lt;/sup&gt;</td>
<td>23.05±0.13&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>T 2</strong></td>
<td>23.25±0.33&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>24.04±0.43&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>25.09±0.25&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>25.04±0.33&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>24.79±0.40&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>T 3</strong></td>
<td>23.71±0.29&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>24.71±0.42&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>25.79±0.15&lt;sup&gt;BCD&lt;/sup&gt;</td>
<td>25.67±0.44&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>25.42±0.44&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>T 4</strong></td>
<td>22.71±0.27&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>23.79±0.29&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>24.92±0.15&lt;sup&gt;B&lt;/sup&gt;</td>
<td>24.83±0.36&lt;sup&gt;B&lt;/sup&gt;</td>
<td>24.58±0.36&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>T 5</strong></td>
<td>22.79±0.33&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>23.96±0.32&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>25.17±0.11&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>25.00±0.29&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>24.83±0.30&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>T 6</strong></td>
<td>24.00±0.22&lt;sup&gt;C&lt;/sup&gt;</td>
<td>25.04±0.27&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>26.17±0.37&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>25.96±0.16&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>25.79±0.40&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>T 7</strong></td>
<td>24.42±0.22&lt;sup&gt;C&lt;/sup&gt;</td>
<td>25.75±0.14&lt;sup&gt;C&lt;/sup&gt;</td>
<td>26.67±0.21&lt;sup&gt;D&lt;/sup&gt;</td>
<td>26.17±0.27&lt;sup&gt;C&lt;/sup&gt;</td>
<td>26.04±0.21&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**CD (0.01)** | 1.19 | 1.68 | 1.11 | 1.27 | 1.41 |

T<sub>1</sub>, Basal diet
T<sub>2</sub>-Basal diet+100 mg vitamin E/kg feed
T<sub>3</sub>-Basal diet+200 mg vitamin E/kg feed
T<sub>4</sub>-Basal diet+0.2 mg selenium/kg feed
T<sub>5</sub>-Basal diet+0.4 mg selenium/kg feed
T<sub>6</sub>-Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T<sub>7</sub>-Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of five birds in three replicates.

<sup>AB</sup> Mean values within a column with no common superscript differ significantly (p<0.01).

---

Puthponsriporn et al. (2001), Kucuk et al. (2003) and Lin et al. (2004) reported that laying hens fed with diet supplemented with vitamin E caused significant increase in egg production. Broiler breeders fed with diet supplemented with vitamin E had greater percentage of hen-day egg production when compared to the control group (Siegel et al., 2001). Ganpule and Manjunatha (2003) observed that inclusion of organic selenium and vitamin E in the basal diet of broiler breeders significantly improved the egg production.

Sahin et al. (2003a) and Sahin et al. (2006) reported that Japanese quail birds fed basal diet with combination of vitamin E and selenium and lycopene...
and vitamin E respectively had significantly increased egg production. It was reported that supplementation of dietary vitamin E caused significant increase in egg production in laying hens in both poultry conditions—heat stress and normal (Bolukbasi et al., 2007).

The results of the present study were not in agreement with Andi et al. (2006) and Asli et al. (2007) who found no significant effect on egg production in broiler breeders supplemented with vitamin E and in laying hens supplemented with probiotics, yeast, vitamin E and vitamin C respectively.

Reis et al. (2009) while evaluating the effects of selenite or zinc-L-selenium-methionine in broiler breeder diets on egg production concluded that the hens fed with organic selenium for the first time produced more eggs whereas no difference in egg production was found in the later periods.

Egg production was not affected by vitamin C and vitamin E supplementation under thermo-neutral conditions as observed by Sahin et al. (2009). However, they reported that egg production was increased with vitamin C or vitamin E supplementation either singly or in combination in heat-stressed quail.

The increased egg production in the present study might be due to antioxidative properties of vitamin E and selenium, especially increased synthesis of egg yolk precursor proteins. Selenium functions throughout the cytoplasm to destroy peroxides, whereas vitamin E present in the membrane components of the cell prevents peroxide formation. In addition, selenium increases the intestinal vitamin E absorption. Dietary vitamin E supplementation would have elevated the synthesis and release of egg yolk precursor proteins vitellogenin from liver and increased ovulation. Lipovitellin, phosvitin and livetin are the main macromolecular protein components of egg yolk derived from vitellogenin (Puthpongsiriporn et al., 2001).

Heat stress was found to decrease plasma egg yolk precursor proteins vitellogenin by causing oxidative damage on the membrane of hepatic cells.
(Bollengier-Lee et al., 1998). Vitamin E and selenium spare each other in protecting the cell from the detrimental effects of peroxidation.

4.2.4 Effect of vitamin E and selenium supplementation on cumulative feed consumption and feed efficiency of layers from 21- 40 weeks of age

Feed consumption and its efficient utilization is one of the major concerns in commercial egg production. The feed consumed per dozen of eggs is the yardstick used for the measurement of feed efficiency. The feed efficiency assessed by considering the total egg production and total feed intake is perhaps the major index in egg production measurement in laying birds (Fasuyi and Olorunfemi, 2008).

The mean cumulative feed consumption and feed efficiency of layers supplemented with vitamin E and selenium from 21 to 40 weeks of age are presented in Figure 9 and Table 11 respectively.

Different levels of vitamin E and selenium supplementation in the basal diet of layers did not cause any significant difference on feed consumption from 21 to 40 weeks of age.

The layers supplemented with vitamin E and selenium either singly or in combination in the basal diet showed significant improvement in feed efficiency expressed in terms of kg of feed consumed per dozen eggs produced during laying period. From twenty ninth week onwards, significant (p<0.01) improvement in feed efficiency was observed in all the supplemented groups compared to the control group.

The present study results on feed consumption were in accordance with the report of Siam et al. (2004), Puthpongsiriporn et al. (2001) and Kucuk et al. (2003) who did not find any significant change in the feed consumption of laying hens due to vitamin E supplementation.
FIGURE 9
MEAN CUMULATIVE FEED CONSUMPTION (g/bird) OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE

T1 - Basal diet
T2 - Basal diet + 100 mg vitamin E/kg feed
T3 - Basal diet + 200 mg vitamin E/kg feed
T4 - Basal diet + 0.2 mg selenium/kg feed
T5 - Basal diet + 0.4 mg selenium/kg feed
T6 - Basal diet + 100 mg vitamin E + 0.2 mg selenium/kg feed
T7 - Basal diet + 200 mg vitamin E + 0.4 mg selenium/kg feed

Probiotics, yeast, vitamin E and vitamin C supplementation in laying hens did not cause any change in feed intake as observed by Asli et al. (2007). However, Bolukbasi et al. (2007) observed that vitamin E supplementation caused significant increase in feed intake of layers in normal poultry house but feed intake of the supplemented groups was not significantly different in birds exposed to heat stress.

Kucuk et al. (2003) and Ganpule and Manjunatha (2003) observed significant improvement in feed efficiency by supplementing vitamin E and selenium in Japanese quail layers and broiler breeders respectively which supports the results of the present study.
TABLE 11
MEAN FEED EFFICIENCY (kg of feed/dozen eggs) OF LAYERS
SUPPLEMENTED WITH VITAMIN E AND SELENIUM
FROM 21-40 WEEKS OF AGE

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>21-24 weeks</th>
<th>25-28 weeks</th>
<th>29-32 weeks</th>
<th>33-36 weeks</th>
<th>37-40 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_1</td>
<td>1.43±0.02^C</td>
<td>1.56±0.05^C</td>
<td>1.63±0.03^C</td>
<td>1.64±0.01^B</td>
<td>1.66±0.01^B</td>
</tr>
<tr>
<td>T_2</td>
<td>1.36±0.02^ABC</td>
<td>1.48±0.02^ABC</td>
<td>1.50±0.02^B</td>
<td>1.52±0.02^A</td>
<td>1.55±0.03^A</td>
</tr>
<tr>
<td>T_3</td>
<td>1.34±0.02^AB</td>
<td>1.44±0.03^AB</td>
<td>1.46±0.01^AB</td>
<td>1.48±0.03^A</td>
<td>1.51±0.03^A</td>
</tr>
<tr>
<td>T_4</td>
<td>1.39±0.01^BC</td>
<td>1.49±0.02^BC</td>
<td>1.51±0.01^B</td>
<td>1.53±0.03^A</td>
<td>1.56±0.03^A</td>
</tr>
<tr>
<td>T_5</td>
<td>1.39±0.02^BC</td>
<td>1.48±0.02^ABC</td>
<td>1.50±0.01^B</td>
<td>1.52±0.02^A</td>
<td>1.54±0.02^A</td>
</tr>
<tr>
<td>T_6</td>
<td>1.32±0.01^AB</td>
<td>1.41±0.02^AB</td>
<td>1.44±0.02^AB</td>
<td>1.46±0.01^A</td>
<td>1.48±0.03^A</td>
</tr>
<tr>
<td>T_7</td>
<td>1.30±0.02^A</td>
<td>1.37±0.01^A</td>
<td>1.41±0.01^A</td>
<td>1.45±0.02^A</td>
<td>1.47±0.01^A</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>0.07</td>
<td>0.11</td>
<td>0.07</td>
<td>0.08</td>
<td>0.10</td>
</tr>
</tbody>
</table>

T_1. Basal diet
T_2. Basal diet+100 mg vitamin E/kg feed
T_3. Basal diet+200 mg vitamin E/kg feed
T_4. Basal diet+0.2 mg selenium/kg feed
T_5. Basal diet+0.4 mg selenium/kg feed
T_6. Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T_7. Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of five birds in three replicates.

A-C Mean values within a column with no common superscript differ significantly (p<0.01).

Decreased values denotes increased feed efficiency.

Better feed efficiency was also observed by Sahin et al. (2002a) and Sahin et al. (2003a) due to vitamin E and vitamin E plus selenium supplementation in Japanese quail layers reared under heat stress and cold stress respectively. Feed efficiency was significantly improved in pullets fed with supplemental vitamin E as observed by Lin et al. (2004). A similar improvement in feed efficiency was also reported in laying hens supplemented with vitamin E and C by Ciftci et al. (2005).

The variation in the feed consumption of laying hens might be due to season, housing designs, dosage of vitamin E and selenium supplementation.
and weather patterns that prevailed during the study period. The significant improvement in feed efficiency of layers might be due to the protection of cell membranes against oxidative damage by vitamin E and selenium supplementation and might have promoted proper utilization of the nutrients.

4.2.5 Effect of vitamin E and selenium supplementation on egg quality characteristics of layers from 21-40 weeks of age

All food has a limited shelf life, which will vary depending on the type of food and storage conditions. The egg is a perishable food product, which could lose its quality rapidly during the period between storage and consumption. Egg quality can be affected by the environmental conditions such as temperature and humidity of storage, as well as the gaseous environment and storage time (Akyurek and Okur, 2009). Dietary antioxidants are essential for maintaining egg quality characteristics by preventing the damaging effects of free radicals.

The egg quality characteristics of layers supplemented with vitamin E and selenium are depicted in Figures 10 to 16.

Supplementation of the combination of vitamin E and selenium in the diet of layers significantly (p<0.05) increased the egg weight compared to vitamin E and selenium alone fed groups and the control group that received no supplementation. However, the two different levels of vitamin E and selenium supplementation in layers did not cause any significant difference in egg quality characteristics such as shape index, albumen index, yolk index, yolk colour and shell thickness. Higher levels of vitamin E and selenium supplementation in the layers significantly (p<0.01) improved the egg Haugh units.

Puthponsiriporn et al. (2001) reported that the eggs of White Leghorn hens fed with basal diet supplemented with vitamin E (65 IU/kg) had significantly greater Haugh units when compared to those eggs from hens supplemented with 25 and 45 IU of vitamin E per kg diet.
FIGURE 10
MEAN EGG WEIGHT (g) OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE

FIGURE 11
MEAN EGG SHAPE INDEX OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE
**FIGURE 12**

**MEAN EGG ALBUMEN INDEX OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE**

**FIGURE 13**

**MEAN EGG YOLK INDEX OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE**
FIGURE 14
MEAN EGG YOLK COLOUR VALUES OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE

FIGURE 15
MEAN EGG HAUGH UNITS OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE
FIGURE 16
MEAN EGG SHELL THICKNESS (mm) OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM FROM 21-40 WEEKS OF AGE

Sahin et al. (2002a) found that supplementation of vitamin E significantly increased the egg weight, egg specific gravity, egg shell thickness and Haugh units of Japanese quail eggs. A similar effect of vitamin E and selenium on egg quality characteristics was observed by Sahin et al. (2003a) in Japanese quail layers. An increase in the egg weight of laying hens due to organic selenium supplementation was found by Payne et al. (2005).

Skrivan et al. (2006) reported that organic selenium in the diet of the laying hens significantly increased selenium content in the edible part of the egg and had a better effect on egg freshness (Haugh unit) when compared to the control group.
Broiler breeder supplemented with increasing dietary levels of vitamin E significantly increased the egg Haugh unit than the control with no supplementation (Andi et al., 2006). Haugh units were greater in Japanese quail layer supplemented with vitamin E or lycopene or as a combination of vitamin E and lycopene groups compared with the control group (Sahin et al., 2006).

Arpasova et al. (2009a) and Arpasova et al. (2009b) reported significant increase in egg weight of birds supplemented with selenium yeast compared to sodium selenite and the control group while the former also revealed higher Haugh units (HU) score in the groups of birds fed with the selenized yeast supplemented diet.

Fresh eggs of the group supplemented with 0.4 ppm organic selenium had higher Haugh unit values than those of the 0.2 ppm supplemented group, which might be due to reduced metabolic processes in eggs, possibly due to higher content of selenium in eggs of that group and its antioxidative effect (Gajcevic et al., 2009).

In contrary to the present study results, Galobart et al. (2001) observed that supplementation of hen diet containing five percent linseed oil with α-tocopheryl acetate (50,100 and 200 mg/kg) had no effect on egg weight, Haugh units, shell thickness and yolk colour values.

The significant increase in egg weight and egg Haugh units in the present study might be due to the fact that supplementation of vitamin E and selenium in the diet of laying hens would have helped to protect egg materials inside the egg and to maintain egg freshness. The antioxidant features of vitamin E and selenium are able to reduce the production of harmful free radicals, which are initiators of uncontrolled oxidation processes. Free radicals primarily affect lipids causing lipid peroxidation of unsaturated fatty acids. The damage of lipids can induce further damage of proteins and DNA.
The quality of the edible part of the egg is considered to be affected by many factors namely period and temperature of storage, age of laying hens, layer diets, health condition of a flock and dietary supplements.

4.2.6 Effect of vitamin E and selenium supplementation on α-tocopherol content in the egg yolk of layers

Today, consumers are much more concerned about the influence of diets on health which has encouraged the nutritional quality improvement of food of animal origin and the development of products such as vitamin E enriched eggs. Vitamin E is an essential multifunctional nutrient, well known for its antioxidative properties, inhibiting lipids of cell membrane peroxidation by free radicals. Vitamin E has been recognized as a modulator of the immune system that confers improved disease resistance in animals and humans (Siegel et al., 2001). The incorporation of vitamin E into the egg may both increase oxidative stability and provide a source of tocopherols for human nutrition and health.

The mean α-tocopherol content in the egg yolk of layers supplemented with vitamin E and selenium in the basal diet are presented in Table 12 and Figure 17.

The effect of vitamin E and selenium supplementation in layers showed significant (p<0.01) increase in egg yolk α-tocopherol content in all the treatment groups compared to the control. The α-tocopherol content in egg yolk of layers supplemented with vitamin E and selenium showed significant variation between treatment groups. The results of the present study showed that the amount of vitamin E in the egg yolk was related to the amount of α-tocopherol in the diet and increased linearly as dietary dl-α-tocopheryl acetate increased.

However, no significant difference was observed in egg yolk α-tocopherol content in all the treatment groups between 30th and 40th week of age in layers supplemented with vitamin E and selenium.
TABLE 12
MEAN EGG YOLK α-TOCOPHEROL CONTENT (μg/g of egg yolk) IN LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>30&lt;sup&gt;th&lt;/sup&gt; week</th>
<th>40&lt;sup&gt;th&lt;/sup&gt; week</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>87±2.0</td>
<td>84±2.0</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>185±3.0</td>
<td>181±3.0</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>318±3.0</td>
<td>315±2.0</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>113±2.0</td>
<td>110±2.0</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>135±2.0</td>
<td>132±2.0</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>220±2.0</td>
<td>217±2.0</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>357±3.0</td>
<td>353±3.0</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td></td>
<td>8.7</td>
</tr>
</tbody>
</table>

T<sub>1</sub>. Basal diet
T<sub>2</sub>-Basal diet+100 mg vitamin E/kg feed
T<sub>3</sub>-Basal diet+200 mg vitamin E/kg feed
T<sub>4</sub>. Basal diet+0.2 mg selenium/kg feed
T<sub>5</sub>. Basal diet+0.4 mg selenium/kg feed
T<sub>6</sub>-Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T<sub>7</sub>. Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of six birds.

Meluzzi <i>et al.</i> (2000), Surai (2000), Galobart <i>et al.</i> (2001) and Puthpongsiriporn <i>et al.</i> (2001) reported that inclusion of vitamin E in the commercial diet of laying hens significantly increased α-tocopherol content in the egg yolk compared to the unsupplemented group.

Siegel <i>et al.</i> (2001) observed that broiler breeders fed basal diet with higher vitamin E supplementation had increased the content of α-tocopherol in egg yolk when compared to lower dietary vitamin E supplemented group.
FIGURE 17
MEAN EGG YOLK α-TOCOPHEROL CONTENT (μg/g of egg yolk)
IN LAYERS SUPPLEMENTED WITH VITAMIN E
AND SELENIUM

Flachowsky et al. (2002), Grobas et al. (2002), Pal et al. (2002) and Mori et al. (2003) found a significant increase in α-tocopherol concentration in egg yolk of laying hens due to vitamin E supplementation.

Lin et al. (2005) reported that dietary supplementation of vitamin E affected the α-tocopherol content in egg yolk of laying hens. Egg yolk vitamin E was increased as supplemental vitamin E increased from 0 to 120 mg/kg. However, a further increase in supplemental vitamin E (160 mg/kg) did not affect its concentration in the egg yolk.

An increase in egg yolk α-tocopherol content of laying hens was observed by Bolukbasi et al. (2007). It was also reported that supplementation

T₁. Basal diet
T₂. Basal diet+100 mg vitamin E/kg feed
T₃. Basal diet+200 mg vitamin E/kg feed
T₄. Basal diet+0.2 mg selenium/kg feed
T₅. Basal diet+0.4 mg selenium/kg feed
T₆. Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T₇. Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed
of laying hens diet with vitamin E was a novel way of increasing the intrinsic amount of vitamin E in egg yolk.

Shahriar et al. (2007) reported that the amount of vitamin E in the yolk was strictly related to the amount of α-tocopherol in the diet and was increased linearly as dietary dl-α-tocopheryl acetate increased in broiler breeders.

Skrivan et al. (2008) found that dietary selenium supplementation increased the α-tocopherol content of egg yolk and concluded that the inclusion of organic dietary selenium sources in the diets of laying hens would enhance the nutritional value (vitamin E content) of eggs for human consumption.

The oxidative damage of lipids can be prevented or limited by the natural antioxidant, tocopherol. Supplementation of vitamin E in the hen’s diet seems to be the most suitable choice to increase the content of this vitamin and to decrease the amount of primary (lipid hydroperoxides) and secondary oxidation products (thiobarbituric acid reactive substances) in eggs and egg products (Galobart et al., 2001).

In the present study, the increased α-tocopherol content in the egg yolk of the supplemented group might be due to an effective absorption of α-tocopherol through the membranes of intestinal tract, transportation in blood and active deposition in the egg yolk. The inclusion of organic selenium in the diet through its sparing effect for vitamin E might help in the absorption of α-tocopherol, retention of α-tocopherol in plasma and deposition of α-tocopherol in the egg yolk. Selenium is a component of glutathione peroxidase enzyme, which actively participates in lipid peroxide removal (Surai, 2000).

4.2.7 Effect of vitamin E and selenium supplementation on selenium content in the egg albumen and egg yolk of layers

As a microelement, selenium has manifold importance in animal feed. Its antioxidant properties help to protect animals from free radicals caused by
oxygen metabolism. Selenium reduces negative effects of stress in chickens kept in intensive production conditions and enhances the quality of poultry products. One of the possibilities of enriching the feed with selenium is to design animal products that differ in their nutritive values from other conventional products available in the market (Kralik et al., 2009).

The mean selenium content in egg albumen and egg yolk of layers supplemented with vitamin E and selenium in the basal diet is presented in Table 13 and Figure 18.

**TABLE 13**

**MEAN EGG ALBUMEN AND YOLK SELENIUM CONTENT (ng/g of albumen and yolk) IN LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Egg albumen</th>
<th>Egg yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30th week</td>
<td>40th week</td>
</tr>
<tr>
<td><strong>T1</strong> Basal diet</td>
<td>40±1.83</td>
<td>39±2.07</td>
</tr>
<tr>
<td><strong>T2</strong> Basal diet+100 mg vitamin E/kg feed</td>
<td>52±2.07</td>
<td>50±2.25</td>
</tr>
<tr>
<td><strong>T3</strong> Basal diet+200 mg vitamin E/kg feed</td>
<td>59±1.79</td>
<td>57±1.90</td>
</tr>
<tr>
<td><strong>T4</strong> Basal diet+0.2 mg selenium/kg feed</td>
<td>155±2.40</td>
<td>154±1.96</td>
</tr>
<tr>
<td><strong>T5</strong> Basal diet+0.4 mg selenium/kg feed</td>
<td>211±2.63</td>
<td>211±1.83</td>
</tr>
<tr>
<td><strong>T6</strong> Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed</td>
<td>163±2.34</td>
<td>162±2.55</td>
</tr>
<tr>
<td><strong>T7</strong> Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed</td>
<td>221±2.45</td>
<td>220±2.48</td>
</tr>
<tr>
<td><strong>CD (0.01)</strong></td>
<td>8.2</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Values given in each cell is the mean±SE of six birds.
Layers supplemented with different levels of vitamin E and selenium had recorded significant (p<0.01) increase in selenium content in the egg albumen and egg yolk in all the treatment groups compared to the control. Significant variation in selenium content in the egg albumen and egg yolk was observed between treatment groups supplemented with vitamin E and selenium. Higher concentration of selenium was accumulated in egg yolk compared to egg albumen. The results of this study showed that the amount of selenium in the egg albumen and yolk were related to the amount of selenium in the diet and increased linearly as dietary selenium increased.
However, no significant difference was observed in selenium content in egg albumen and egg yolk in all the treatment groups between 30th and 40th week of age in layers supplemented with vitamin E and selenium.

Surai and Dvorska (2001) and Paton et al. (2002) reported that supplementation of selenium in the basal diet of layers significantly increased the content of selenium in the eggs. Kenyon and spring (2003) and Karadas et al. (2004) observed that sel-plex supplemented group had significantly higher content of selenium in both egg yolk and albumen, when compared to the sodium selenite supplemented group.

Pappas et al. (2004) found that supplementation of sel-plex singly or in combination with fish oil significantly increased the content of selenium in the egg yolk and egg albumen compared to the control group. An increase in egg selenium content by supplementing both organic and inorganic selenium in the diet of laying hens was observed by Payne et al. (2005) and Utterback et al. (2005).

Organic selenium from selenium-enriched yeast had higher availability in laying hens than inorganic selenium from sodium selenite, resulting in higher egg selenium content (Skrivan et al., 2006; Pan et al., 2007).

Leeson et al. (2007) compared three different sources of selenium (Sodium selenite, Selenium yeast and B-TRAXIM ® Se) in broiler breeder hens and concluded that selenium content in egg yolk was highest in hens fed B-TRAXIM ® Se, whereas selenium content in albumen was highest in hens fed with selenium yeast.

Reis et al. (2009) evaluated the effects of selenite or zinc- L- selenium -methionine in broiler breeder diets and found that selenium content in eggs increased regardless of selenium source. Kralik et al. (2009) reported that selenium concentration in diet of Hy line brown laying hens affected the content of selenium in albumen and yolk significantly.
The laying hens supplemented with higher levels of selenium in the diet had significantly higher portion of selenium in egg yolk and albumen than the hens that received lesser dose of selenium as observed by Gajcevic et al. (2009).

The high content of selenium in the egg yolk and albumen might be due to the active absorption of organic selenium, bioavailability and accumulation in the body tissues. Organic selenium from yeast is a highly available form of selenium for chickens and other livestock and provides antioxidant protection at levels greater than inorganic selenium. Since the main form of selenium in the egg is selenomethionine and chickens cannot synthesize this aminoacid, selenomethionine from sel-plex might effectively get transfer to egg albumen and yolk.

A significant (p<0.05) positive correlation (r=0.957) was observed between the selenium content of egg albumen and egg yolk (Figure 19).

FIGURE 19
CORRELATION BETWEEN SELENIUM CONTENT IN EGG ALBUMEN AND EGG YOLK

* is significant at 5% level
Positive correlation ($r=0.0536$) was observed between vitamin E and selenium contents of egg yolk which was not found to be significant (Figure 20).

**FIGURE 20**
CORRELATION BETWEEN VITAMIN E AND SELENIUM CONTENTS IN EGG YOLK

ns – Not significant

4.2.8 **Effect of vitamin E and selenium supplementation on lipid peroxidation in the plasma and liver of layers**

Biological specimens contain a mixture of thiobarbituric acid reactive substances (TBARS), including lipid hydroperoxides and aldehydes, which increase as a result of oxidative stress. Plasma concentrations of thiobarbituric acid reactive substances (TBARS) are an index of lipid peroxidation and oxidative stress. In practice, TBARS are expressed in terms of malondialdehyde (MDA) equivalents.

The mean levels of plasma and liver TBARS in layers supplemented with vitamin E and selenium are shown in Table 14.
### TABLE 14

**MEAN LEVELS OF PLASMA AND LIVER THIOBARBITURIC ACID REACTIVE SUBSTANCES IN LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Plasma TBARS (nmoles of MDA/ml plasma)</th>
<th>Liver TBARS (nmoles of MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_1$</td>
<td>9.55±0.19$^E$</td>
<td>1.77±0.03$^E$</td>
</tr>
<tr>
<td>$T_2$</td>
<td>8.25±0.32$^D$</td>
<td>1.54±0.04$^D$</td>
</tr>
<tr>
<td>$T_3$</td>
<td>7.20±0.24$^C$</td>
<td>1.20±0.04$^{BC}$</td>
</tr>
<tr>
<td>$T_4$</td>
<td>8.60±0.20$^D$</td>
<td>1.60±0.03$^D$</td>
</tr>
<tr>
<td>$T_5$</td>
<td>7.88±0.14$^{CD}$</td>
<td>1.32±0.03$^C$</td>
</tr>
<tr>
<td>$T_6$</td>
<td>6.14±0.27$^B$</td>
<td>1.07±0.04$^B$</td>
</tr>
<tr>
<td>$T_7$</td>
<td>4.88±0.28$^A$</td>
<td>0.93±0.02$^A$</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>0.93</td>
<td>0.13</td>
</tr>
</tbody>
</table>

$T_1$: Basal diet  
$T_2$: Basal diet+100 mg vitamin E/kg feed  
$T_3$: Basal diet+200 mg vitamin E/kg feed  
$T_4$: Basal diet+0.2 mg selenium/kg feed  
$T_5$: Basal diet+0.4 mg selenium/kg feed  
$T_6$: Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed  
$T_7$: Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed  

Values given in each cell is the mean±SE of six birds.  
A-E Mean values within a column with no common superscript differ significantly (p<0.01).

The antioxidants vitamin E and selenium supplementation in laying hens had significantly (p<0.01) decreased plasma and liver TBARS compared to the control group which received no antioxidant supplementation. Among the supplemented groups, the birds that received higher levels of both vitamin E and selenium in the feed had significantly lower levels of plasma and liver TBARS.
El-Sebai (2000) found a pronounced decrease in malondialdehyde content in blood and liver homogenates with increasing selenium and vitamin E levels in broiler chickens. Maternal dietary vitamin E and selenium supplementation was found to significantly decrease the chick liver susceptibility to peroxidation (Surai, 2000).

Laying hens supplemented with vitamin E were found to maintain lower levels of TBA in yolk and plasma, which might be due to the antioxidant property of vitamin E. It has been shown that supplementation of vitamin E leads to increased deposition of α-tocopherol in laying hens and thus inhibits the chain reaction of peroxidation in yolk and plasma of hens exposed to heat stress (Puthpongsiriporn et al., 2001).

The results of the present study coincide with that of Sahin et al. (2001c) who reported that increased supplemental vitamin E linearly decreased serum and liver MDA in broilers reared under heat stress. Greater vitamin E and selenium inclusions in the diet of Japanese quails resulted in lower serum and liver MDA concentrations (Sahin et al., 2002c). A similar decrease in MDA concentration was also observed by supplementing vitamin E and vitamin C in laying hens reared at high ambient temperature (Sahin et al., 2002b).

Pullets given 160 mg/kg supplemental vitamin E had lower plasma MDA concentrations than those given 0-80 mg/kg. Moreover, it was also reported that MDA concentrations in chick brain were decreased as the maternal supplementation of vitamin E increased (Lin et al., 2005). Separately or as a combination, supplemental lycopene and vitamin E were found to decrease serum and liver MDA levels in Japanese quails (Sahin et al., 2006).

Maini et al. (2007) observed significantly low concentration of MDA in vitamin E fed groups in broilers during summer. Reddy et al. (2007) showed that there was a significant decrease in lipid peroxidation as indicated by MDA levels in Ocimum sanctum and selenium supplemented broilers. Ocimum
sanctum and selenium were suggested to work together to minimize oxidative stress through their action in blocking the formation of lipid peroxides from phospholipids.

Eid et al. (2008) reported significant reduction in yolk and hepatic MDA in vitamin E supplemented laying hens compared to the control group which received no supplementation. It was also stressed that dexamethasone induced oxidative stress in laying hens was relieved by vitamin E supplementation as indicated by significantly lower yolk, plasma and liver malondialdehyde. These researchers opined that free radicals are neutralized by α-tocopherol before lipid oxidation propagates among highly unsaturated fatty acids in cellular and sub-cellular membranes.

Yardibi and Turkay (2008) reported that higher vitamin E supplementation in laying hens under heat stress resulted in a significantly reduced MDA level. Selenium supplementation at 0.3 and 0.6 mg/kg feed in Lang-shan breeding hens led to significantly decreased malondialdehyde content compared with that of the control group which received no selenium in the feed (Wang et al., 2009).

Vitamin E and selenium supplementation in the birds might have reduced the synthesis of MDA in the liver by protecting the liver from lipid peroxidation and cell membranes from damage. It is known that vitamin E diminishes the peroxidation of polyunsaturated lipids via scavenging free radicals. It is also an important structural component of biological membranes, contributing to their stability. The methyl groups of tocopherol interact with the cis double bonds of the fatty acids to form a stable complex in membrane phospholipids. Selenium is a component of the enzyme glutathione peroxidase and is involved in the catabolism of peroxides formed from tissue lipid oxidation. Thus, selenium also is proved to play a very important role in maintaining integrity of cellular membranes (Sahin et al., 2002c).
4.2.9 Effect of vitamin E and selenium supplementation on the enzymic and non-enzymic antioxidant status in the liver of layers

Environmental stress causes oxidative stress in vivo resulting in an imbalance in the antioxidant status due to excessive formation of free radicals. The effect of reactive oxygen and nitrogen species is balanced by the action of non-enzymic antioxidants, as well as antioxidant enzymes. The most efficient non-enzymic antioxidants include vitamin C, vitamin E, carotenoids, thiols and natural flavonoids while enzymic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Barciela et al., 2008).

The effect of vitamin E and selenium supplementation on enzymic and non-enzymic antioxidant activity in the liver of layers is shown in Table 15.

The activities of enzymic antioxidants namely superoxide dismutase, catalase and glutathione peroxidase and the non-enzymic antioxidant - reduced glutathione were significantly (p<0.01) increased in all the vitamin E and selenium supplemented groups either singly or in combination compared to the control group that received only the basal diet. The groups T_6 and T_7 that received both vitamin E and selenium in the diet showed higher SOD, CAT, and GSH activities compared to the treatment groups that were supplemented with either vitamin E or selenium. GPx activity was found to be higher in group T_7 that received maximum level of vitamin E and selenium in the diet followed by T_6 (vitamin E 100 mg/kg and selenium 0.2 mg/kg), T_5 (selenium 0.4 mg/kg) and T_4 groups (selenium 0.2 mg/kg).

A significant increase in the activities of GPx, Cu, Zn-SOD and Mn-SOD in the fourth week and an increase in CAT activity in the sixth week of age in the heart muscle were observed in the chickens fed with supplementary organic selenium (Milinkovic-Tur et al., 2009).

SOD is the main enzyme participating in the organism's complex defence against oxidative stress. SOD catalyses the reaction, which
eliminates superoxide anion radical and leads to the formation of hydrogen peroxide and molecular oxygen (Mates et al., 1999). Hydrogen peroxide is then removed by CAT and GPx. At low $H_2O_2$ concentrations, it is eliminated mostly by GPx and when intracellular concentration of $H_2O_2$ is high, it is removed by CAT, which is not saturated even at very high $H_2O_2$ concentration (Lledias et al., 1998). Since GPx reacts also with other hydroperoxides, it is believed that this enzyme plays a key role in antioxidant protection, particularly during oxidative stress of low intensity.

**TABLE 15**

**MEAN ACTIVITIES OF SUPEROXIDE DISMUTASE (SOD), CATALASE (CAT), GLUTATHIONE PEROXIDASE (GPx) AND REDUCED GLUTATHIONE IN THE LIVER OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>SOD @ Units/min/mg protein</th>
<th>CAT #</th>
<th>GPX $^$</th>
<th>GSH (mmoles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_1$</td>
<td>14.86±0.90$^A$</td>
<td>51.17±1.84$^A$</td>
<td>24.85±1.23$^A$</td>
<td>0.52±0.02$^A$</td>
</tr>
<tr>
<td>$T_2$</td>
<td>20.96±1.23$^B$</td>
<td>57.83±1.92$^B$</td>
<td>32.12±1.45$^B$</td>
<td>0.64±0.02$^B$</td>
</tr>
<tr>
<td>$T_3$</td>
<td>27.58±1.32$^{CD}$</td>
<td>62.22±1.02$^B$</td>
<td>34.76±1.72$^{BC}$</td>
<td>0.70±0.02$^B$</td>
</tr>
<tr>
<td>$T_4$</td>
<td>22.15±1.30$^{BC}$</td>
<td>58.36±1.53$^B$</td>
<td>37.69±2.24$^{BCD}$</td>
<td>0.66±0.02$^B$</td>
</tr>
<tr>
<td>$T_5$</td>
<td>25.86±2.47$^{BC}$</td>
<td>60.61±2.37$^B$</td>
<td>39.65±2.16$^{CD}$</td>
<td>0.69±0.03$^B$</td>
</tr>
<tr>
<td>$T_6$</td>
<td>30.72±1.58$^{GD}$</td>
<td>68.81±0.84$^C$</td>
<td>43.03±1.41$^{DE}$</td>
<td>0.81±0.02$^C$</td>
</tr>
<tr>
<td>$T_7$</td>
<td>33.04±1.19$^{D}$</td>
<td>72.10±1.73$^C$</td>
<td>48.43±1.68$^E$</td>
<td>0.87±0.04$^C$</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>5.78</td>
<td>6.47</td>
<td>6.68</td>
<td>0.10</td>
</tr>
</tbody>
</table>

$T_1$, Basal diet  
$T_2$ - Basal diet+100 mg vitamin E/kg feed  
$T_3$ - Basal diet+200 mg vitamin E/kg feed  
$T_4$ - Basal diet+0.2 mg selenium/kg feed  
$T_5$ - Basal diet+0.4 mg selenium/kg feed  
$T_6$ - Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed  
$T_7$ - Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of six birds  
$^A-E$ Mean values within a column with no common superscript differ significantly (p<0.01)

$^@$ 1 enzyme unit is enzyme amount that gives 50% inhibition of nitroblue tetrazolium reduction  
$^#$ 1 enzyme unit is pmole of hydrogen peroxide decomposed  
$^\$ 1 enzyme unit is µg of glutathione utilized
El-Sebai (2000) reported a significant increase in GPx activity in blood, kidney and liver homogenates in broiler chickens supplemented with increasing levels of vitamin E and selenium. Elevation of blood GSH levels by supplementing organic selenium in the feed of chickens was observed by Mahmoud and Edens (2003).

Lin et al. (2005) observed that the maternal supplementation with high levels of vitamin E (120-160mg/kg diet) enhanced the antioxidant capability and depressed the oxidative stress in chicks. The results of the present study coincide with that of Yoon et al. (2007) and Reddy et al. (2007) who reported that broilers supplemented with organic selenium showed significantly increased GPx activity and blood GSH levels.

Leeson et al. (2007) observed in broiler breeders that GPx in liver and plasma were affected by the sources of selenium such as sodium selenite, selenium yeast or B-TRAXIM ® Se and not by the dosage. They found out that GPx in liver was higher in hens fed selenite or selenium yeast while in plasma, GPx activity was higher in hens fed selenite compared to B-TRAXIM ® Se or selenium yeast.

Ozkan et al. (2007) reported that at low temperature, supplementation with organic selenium alone or with inorganic selenium and vitamin E increased the GPx activity and GSH concentration in the liver of broilers, which might indicate increased activity of the bird’s antioxidant defense against suboptimal environments.

Salman et al. (2007) reported that GPx activity in plasma, kidney, leg muscle, heart and liver of broilers was elevated in all the treatment groups that were supplemented with vitamin E or vitamin E and organic selenium / inorganic selenium. Treatment group supplemented with vitamin E and organic selenium had higher liver GPx activity than the rest of the groups.
Chantiratikul et al. (2008) observed that GPx activity in RBC of laying hens fed control diet was significantly lower than that of hens fed with selenium (sodium selenite and zinc-L-selenomethionine) supplemented diets indicating that sources of selenium did not markedly alter GPx activity.

Pirsljin et al. (2008) reported that dietary organic selenium supplementation manifested higher activity of GPx during fattening and maintaining its activity in stress conditions provoked by fasting.

As elevated GPx activity is indicative of oxidative stress, Upton et al. (2009) concluded that dietary sel-plex supplementation resulted in better selenium and redox status in broilers than did sodium selenite. Han et al. (2009) showed that selenium yeast had better effects compared with sodium selenite on increasing serum SOD activities in rats. However, the activity of serum GPx was significantly increased by the addition of selenium yeast or sodium selenite.

Gajcevic et al. (2009) observed higher GPx activity in the blood of hens supplemented with 0.4 ppm of organic selenium in the diet than the hens that received 0.2 ppm of selenium. They suggested that GPx enzyme activity depended on the concentration of selenium in an organism as aminoacid selenocysteine maintains the active centre of an enzyme.

The enzyme GPx, together with SOD and CAT protects cells from (hydrogen or lipid) peroxidation (Milinkovic-Tur et al., 2009). Dietary antioxidant supplementation of vitamin E and selenium with levels exceeding nutritional requirement in the present study would have improved the bird's antioxidant defense system and decreased oxidative stress.

4.2.10 Effect of vitamin E and selenium supplementation on plasma lipid profile in the layers

The mean plasma lipid profile of layers supplemented with vitamin E and selenium are shown in Table 16.
Table 16
MEAN LIPID PROFILE OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Total cholesterol mg/dl</th>
<th>Triglyceride mg/dl</th>
<th>HDL-cholesterol mg/dl</th>
<th>VLDL-cholesterol mg/dl</th>
<th>LDL-cholesterol mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>155±1.16E</td>
<td>113±1.13D</td>
<td>30.1±0.70A</td>
<td>22.7±0.22D</td>
<td>102.6±1.38F</td>
</tr>
<tr>
<td>T2</td>
<td>148±1.17CD</td>
<td>109±1.19BCD</td>
<td>35.0±0.89BCD</td>
<td>21.8±0.23BCD</td>
<td>91.5±1.73CD</td>
</tr>
<tr>
<td>T3</td>
<td>146±1.03BC</td>
<td>107±1.28ABC</td>
<td>35.7±0.68CD</td>
<td>21.5±0.25ABC</td>
<td>89.1±1.03BC</td>
</tr>
<tr>
<td>T4</td>
<td>151±1.05DE</td>
<td>111±1.15CD</td>
<td>31.9±0.90AB</td>
<td>22.2±0.23CD</td>
<td>97.1±1.01E</td>
</tr>
<tr>
<td>T5</td>
<td>150±1.12CD</td>
<td>109±1.00BCD</td>
<td>32.5±0.54ABC</td>
<td>21.9±0.21BCD</td>
<td>95.4±1.59DE</td>
</tr>
<tr>
<td>T6</td>
<td>143±1.10AB</td>
<td>106±1.25AB</td>
<td>37.9±1.04DE</td>
<td>21.1±0.25AB</td>
<td>84.5±1.69AB</td>
</tr>
<tr>
<td>T7</td>
<td>141±1.17A</td>
<td>104±1.27A</td>
<td>39.2±1.03E</td>
<td>20.8±0.26A</td>
<td>81.0±1.28A</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>4.30</td>
<td>4.55</td>
<td>3.25</td>
<td>0.91</td>
<td>5.44</td>
</tr>
</tbody>
</table>

T1 - Basal diet
T2 - Basal diet+100 mg vitamin E/kg feed
T3 - Basal diet+200 mg vitamin E/kg feed
T4 - Basal diet+0.2 mg selenium/kg feed
T5 - Basal diet+0.4 mg selenium/kg feed
T6 - Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T7 - Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of six birds
A-F Mean values within a column with no common superscript differ significantly (p<0.01).

The mean plasma total cholesterol levels in layers was found to be significantly (p<0.01) lower in all the supplemented groups except T4 group that received 0.2 mg/kg selenium compared to the control group which received no vitamin E and selenium supplementation in the feed. Among the treatment groups, T6 and T7 groups that received both vitamin E and selenium in the diet had lower total cholesterol levels in plasma. Plasma triglyceride and VLDL cholesterol levels were found to be lower in T6 and T7 groups that received both
vitamin E and selenium in the diet followed by T₃ group supplemented with
200 mg vitamin E / kg feed.

The mean plasma HDL-cholesterol was found to be higher in layer birds
receiving both vitamin E and selenium (T₆ and T₇) and vitamin E alone (T₂ and
T₃) in the diet. The plasma LDL-cholesterol level was found to be decreased in
all the treatment groups as compared to the control.

The results of the present study were in agreement with the
hypcholesterolemic effect of α-tocopherol observed in rats (Liu and Boylan,
1994 and Al-Juary et al., 2006) and other animal models (Khoja and Marzouki,
1994; Chien et al., 1972; Jack and Desai, 1977; Raederstorff et al., 2002;
Hidiroglou et al., 2004).

Ozturk et al. (2000) reported that the chickens fed on standardized diet
with antioxidant vitamin supplementation (vitamin C-200 mg/kg/day and vitamin
E-100 mg/kg/day) resulted in significant reduction in plasma triglyceride but the
total cholesterol remained unchanged. They also observed significant elevation
in serum HDL-cholesterol and HDL/LDL cholesterol ratio in the broiler chickens
compared with the control group.

Sahin et al. (2001b) observed a significant decrease in serum
cholesterol and triglyceride concentrations when both dietary vitamin E and
vitamin A were supplemented in broilers reared under heat stress. Sahin et al.
(2002b) demonstrated that application of vitamins E and C decreased the
cholesterol levels in laying hens under heat stress. Significant decrease
in both cholesterol and triglyceride levels were observed when both dietary
vitamin E and selenium were increased in Japanese quails under heat stress
(Gursu et al., 2003). It was also reported that combination of dietary lycopene
and vitamin E supplementation caused a reduction in serum cholesterol
concentrations in Japanese quails (Sahin et al., 2006).
Selenium is an essential trace element that is an integral part of many selenoproteins (Alissa et al., 2003). It has been observed that selenium has an antiatherogenic action and suppresses peroxidation of lipids (Kurtsikidze, 2006).

Supplementation of selenium, zinc and vitamin E (0.42, 68, and 60 mg per day, respectively) in the diet of lamb significantly decreased the cholesterol content of blood plasma with no changes in the blood plasma content of triglycerides (Gabryszuk et al., 2007).

Vitamin E was found to bring a significantly decreased plasma triglyceride concentration in both dexamethasone treated or untreated laying hens as compared to the control group which suggested that the antioxidants like vitamin E play an important role in lipid metabolism (Eid et al., 2008).

Imik et al. (2009) reported significantly lower triacylglycerol percentage in vitamin E and vitamin C supplemented quails under heat stress. However, significantly higher cholesterol level was observed in vitamin E and vitamin C supplemented quails compared to the control group that received only the basal diet.

Significant decrease in the total cholesterol, triglyceride, VLDL and LDL cholesterol levels and an increase in the HDL cholesterol level in the present study might be due to the effect of vitamin E and selenium supplementation on lipoprotein metabolism either through their antioxidant activity or by some other related mechanism.

Lower plasma cholesterol concentrations could be accounted for an inhibition of the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in endogenous cholesterol synthesis by α-tocopherol (Oriani et al., 1997; Xu et al., 2000; Pal et al., 2003).

Changes in the plasma cholesterol concentration might also result from the effect of vitamin E on liver cholesterol metabolism. This effect might be
ascribed to the higher levels of cholesterol-7-α-hydroxylase, which is involved in the conversion of cholesterol into bile acids in the liver (Wojcicki et al., 1991). Bile acids synthesis and subsequent excretion in the feces might represent a significant mechanism for the elimination of excess cholesterol (Al-Juary et al., 2006).

Plasma VLDL and LDL- cholesterol represent mobilization of fats from the liver to adipose tissue. HDL removes the excess or unused cholesterol from cells. Cholesterol, after being taken up by HDL, is converted to cholesteryl esters by the enzyme lecithin cholesterol acyltransferase.

Selenium deficiency seemed to result in increased total cholesterol and LDL levels and a significant decrease in HDL levels in rats and it has been proposed that this might be related to an increased HMG CoA reductase activity (Qu et al., 2000).

The reason for the contradiction among the results might be due to difference in the species used, age of animals, duration and the dosage of vitamin E and selenium supplemented in the diet. Unfortunately, there is a scarcity in information with respect to the lipid parameters of layer chickens.

4.2.11 Effect of vitamin E and selenium supplementation on hematological parameters in the layers

Hematological evaluation in poultry represents a valuable aid in the diagnosis of many diseases and determination of the extent to which blood cells have been damaged. Hematological studies become important when dealing with life as the various constituents of blood would change in direct relation to the physiological conditions of health, well being and age of the individual (Togun et al., 2006).

Although widely used in large in animal medicine, they have not yet found the place they deserve in avian medicine.
The hematological parameters and red cell indices of layers supplemented vitamin E and selenium in the feed are presented in Table 17 and 18.

TABLE 17
MEAN HEMATOLOGICAL PARAMETERS OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Hb (g/dl)</th>
<th>RBC (10⁶/mm³)</th>
<th>WBC (10³/mm³)</th>
<th>PCV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>9.75±0.21ᵃ</td>
<td>3.10±0.03ᵃ</td>
<td>26.35±1.02ᴬ</td>
<td>29.1±0.87ᴬ</td>
</tr>
<tr>
<td>T₂</td>
<td>10.23±0.24ᵇᶜ</td>
<td>3.31±0.08ᵇᶜ</td>
<td>28.65±0.79ᴬᴮᶜ</td>
<td>31.17±0.50ᴬᴮᶜ</td>
</tr>
<tr>
<td>T₃</td>
<td>10.47±0.24ᵇᶜ</td>
<td>3.37±0.10ᵇᶜ</td>
<td>29.07±0.67ᴬᴮᶜ</td>
<td>31.70±0.71ᴬᴮᶜ</td>
</tr>
<tr>
<td>T₄</td>
<td>10.15±0.21ᵇᶜ</td>
<td>3.25±0.10ᵇᶜ</td>
<td>27.22±0.57ᴬ'Bᶜ</td>
<td>30.02±0.65ᴬ'Bᶜ</td>
</tr>
<tr>
<td>T₅</td>
<td>10.20±0.29ᵇᶜ</td>
<td>3.32±0.12ᵇᶜ</td>
<td>27.93±0.69ᴬ'Bᶜ</td>
<td>30.68±0.77ᴬ'Bᶜ</td>
</tr>
<tr>
<td>T₆</td>
<td>10.67±0.27ᵇᶜ</td>
<td>3.55±0.08ᵇᶜ</td>
<td>30.13±1.07ᴮᶜ</td>
<td>32.17±0.75ᴮᶜ</td>
</tr>
<tr>
<td>T₇</td>
<td>10.90±0.24ᵇᶜ</td>
<td>3.56±0.13ᵇᶜ</td>
<td>31.17±0.93ᶜ</td>
<td>33.03±0.54ᶜ</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td>0.70</td>
<td>0.28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>-</td>
<td>-</td>
<td>3.23</td>
<td>2.68</td>
</tr>
</tbody>
</table>

T₁, Basal diet
T₂ - Basal diet+100 mg vitamin E/kg feed
T₃ - Basal diet+200 mg vitamin E/kg feed
T₄ - Basal diet+0.2 mg selenium/kg feed
T₅ - Basal diet+0.4 mg selenium/kg feed
T₆ - Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T₇ - Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of six birds.
ᵃ⁻ᶜ Mean values within a column with no common superscript differ significantly (p<0.05).
ᴬ⁻ᶜ Mean values within a column with no common superscript differ significantly (p<0.01).

Increase in blood hemoglobin level was observed in both vitamin E and selenium supplemented groups (T₆ and T₇) followed by T₃ group that received 200 mg vitamin E per kg diet. No significant difference was noticed between the control and other treatment groups.
Total red blood cell count was found to be increased in T₆ and T₇ groups that received both vitamin E and selenium in the diet as compared to the control. No significant difference was observed between the control and the treatment groups (T₂, T₃, and T₄ and T₅) that were supplemented with either vitamin E or selenium. White blood cell count (WBC) and packed cell volume (PCV) were found to be increased in layer birds supplemented with both vitamin E and selenium (T₆ and T₇) when compared to the control.

**TABLE 18**

**MEAN RED CELL INDICES OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>MCV(fl)</th>
<th>MCH(pg)</th>
<th>MCHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>94.02±3.51</td>
<td>31.49±0.93</td>
<td>33.61±0.97</td>
</tr>
<tr>
<td>T₂</td>
<td>94.40±2.46</td>
<td>30.97±0.78</td>
<td>32.86±0.80</td>
</tr>
<tr>
<td>T₃</td>
<td>94.59±3.34</td>
<td>31.29±1.39</td>
<td>33.16±1.40</td>
</tr>
<tr>
<td>T₄</td>
<td>92.80±3.26</td>
<td>31.35±0.86</td>
<td>33.94±1.28</td>
</tr>
<tr>
<td>T₅</td>
<td>92.79±3.24</td>
<td>30.83±1.02</td>
<td>33.30±0.98</td>
</tr>
<tr>
<td>T₆</td>
<td>90.80±2.84</td>
<td>30.10±0.92</td>
<td>33.33±1.48</td>
</tr>
<tr>
<td>T₇</td>
<td>93.55±4.68</td>
<td>30.85±1.46</td>
<td>33.07±1.07</td>
</tr>
</tbody>
</table>

T₁. Basal diet  
T₂. Basal diet+100 mg vitamin E/kg feed  
T₃. Basal diet+200 mg vitamin E/kg feed  
T₄. Basal diet+0.2 mg selenium/kg feed  
T₅. Basal diet+0.4 mg selenium/kg feed  
T₆. Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed  
T₇. Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of six birds.

Red blood cell indices namely Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) did not differ significantly among the treatment groups.
A positive effect of selenium on haematological indicators was observed by several authors (Horton et al., 1978; Sehgal et al., 1980; Doni et al., 1984; Li et al., 1990; Chen and Lin, 2000).

Hematological parameters namely erythrocyte count, hemoglobin and haematocrit values were significantly increased in broiler chickens supplemented with vitamin E and selenium compared to the control. Similarly, selenium and/or vitamin E seemed to affect leukocyte count in broilers (El-Sebai, 2000).

Significantly higher hemoglobin, WBC and PCV were observed in parenterally vitamin E and selenium supplemented calves than in the control (Mohri et al., 2005). However, Tras et al. (2000) found that none of the haematological indicators (red blood cell count, haemoglobin or packed cell volume) were affected by a diet supplemented with vitamin E and selenium in male broiler chicken.

In another study, WBC counts were found to be significantly higher in vitamin E and selenium injected groups of rats than the control, but RBC count, hemoglobin, PCV, MCV, MCH and MCHC values were apparently not influenced by the injection of vitamin E and selenium (Cay and Naziroglu, 1999).

Faixova et al. (2007) reported that total erythrocyte count and osmotic resistance of red blood cells of lambs were increased by selenium supplementation but found no significant changes in white blood cell count.

In the present study, the higher levels of RBC, hemoglobin, PCV and WBC in the vitamin E and selenium supplemented group could be related to the protection of cell membrane and intracellular organelles by the antioxidant effects of vitamin E and selenium accounting for an increase in the life span of RBC and leukocytes. The increase in hemoglobin
concentration might reflect improved integrity of erythrocyte cellular membranes and thus potentially better tissue oxygenation.

Interpretation and sensible utilization of the results of hematological examinations are often limited by the lack of physiological data relevant to the individual avian species, breeding lines, production types, sexual maturation and nutrition (Kral and Suchy, 2000). Hematological values of chickens are influenced by age, sex, breed, climate, geographical location, season, day length, time of the day, nutritional status, life habit of species, present status of individual birds and such other physiological factors.

4.2.12 Effect of vitamin E and selenium supplementation on selected serum marker enzymes in the layers

Each cell within an organ has a specific function and contains enzymes designed to perform those functions. In some situations, enzymes are unique to specific cells within an organ and in other cases enzymes are found in numerous cells from various organs. These enzymes enter the blood only when the cells to which they are confined are damaged or destroyed. The presence of significant quantities of these specific enzymes in the blood indicates the probable site of tissue damage (Celebi et al., 2009). Organic lesions and metabolic disorders of many organs, especially the liver, are followed by changes in some enzyme activities in blood plasma of domestic animals and poultry (Kraljevic et al., 2008).

The serum enzyme activities of layers supplemented with vitamin E and selenium are shown in Table19.

The layer birds supplemented with vitamin E and selenium in the feed caused a significant (p<0.01) decrease in serum enzyme activities of AST, ALT and CK. The birds that were fed with a combination of vitamin E and selenium at different levels and higher levels of vitamin E showed a significant reduction in the activities of AST and ALT in serum. The activity of CK was found to be significantly reduced in all the supplemented groups compared to the control.
### TABLE 19

**MEAN ACTIVITIES OF SERUM MARKER ENZYMES (IU/L) OF LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>LDH</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>182±2.91&lt;sup&gt;C&lt;/sup&gt;</td>
<td>16.85±0.43&lt;sup&gt;D&lt;/sup&gt;</td>
<td>27.25±1.33</td>
<td>274.02±3.92</td>
<td>161±1.20&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₂</td>
<td>171±3.38&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>15.75±0.37&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>28.93±2.04</td>
<td>268.50±4.76</td>
<td>152±2.70&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₃</td>
<td>164±3.20&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>14.57±0.67&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>29.35±2.24</td>
<td>264.73±5.40</td>
<td>147±1.86&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₄</td>
<td>172±2.88&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>16.60±0.48&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>29.28±2.41</td>
<td>266.75±6.25</td>
<td>149±3.12&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₅</td>
<td>168±3.64&lt;sup&gt;B&lt;/sup&gt;</td>
<td>15.18±0.87&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>30.77±1.86</td>
<td>262.58±6.05</td>
<td>148±2.32&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₆</td>
<td>153±4.02&lt;sup&gt;A&lt;/sup&gt;</td>
<td>12.72±0.48&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>32.32±1.40</td>
<td>259.53±4.55</td>
<td>141±2.14&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₇</td>
<td>151±3.46&lt;sup&gt;A&lt;/sup&gt;</td>
<td>12.33±0.52&lt;sup&gt;A&lt;/sup&gt;</td>
<td>33.58±2.25</td>
<td>254.63±5.68</td>
<td>137±2.17&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>13.00</td>
<td>2.19</td>
<td>-</td>
<td>-</td>
<td>8.81</td>
</tr>
</tbody>
</table>

AST - Aspartate transaminase
ALT - Alanine transaminase
ALP - Alkaline phosphatase
LDH - Lactate dehydrogenase
CK - Creatine kinase

One International Unit (IU) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

T₁ - Basal diet
T₂ - Basal diet+100 mg vitamin E/kg feed
T₃ - Basal diet+200 mg vitamin E/kg feed
T₄ - Basal diet+0.2 mg selenium/kg feed
T₅ - Basal diet+0.4 mg selenium/kg feed
T₆ - Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T₇ - Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of six birds.

<sup>A-D</sup> Mean values within a column with no common superscript differ significantly (p<0.01).

On the other hand, no significant difference was noticed in the serum enzyme activities of ALP and LDH in layers supplemented with vitamin E and selenium.

Cay and Naziroglu (1999) reported a significant increase in serum ALP activity in rats supplemented with vitamin E and selenium and stated that ALP...
activity would have been induced by increased cellular activity rather than cell damage. Franchini et al. (1988) opined that the increase in ALP activity of broilers fed with excess dietary vitamin E could be related to osteoblastic activity.

Swain and Johri (2000) found a significant decrease in the serum glutamate oxaloacetate transaminase activity in broilers supplemented with combinations of different levels of vitamin E and selenium. It has also been indicated that for maintenance of normal muscle structure and tonicity, higher than the recommended levels of vitamin E and selenium are required.

Activity of alkaline phosphatase in ruminal fluid was found to be significantly higher in organic selenium supplemented lambs compared with the lambs given basal diet. It was also reported that lambs fed organic selenium had significantly lower activity of creatine kinase in serum with no change in lactate dehydrogenase activity (Faixova et al., 2007).

Elaroussi et al. (2007) found significant reduction in AST activity in Japanese quail as the levels of selenium and/or vitamin E was increased in the diet. The activities of liver enzymes (AST, ALT and ALP) seemed to decrease in Japanese quails fed vitamin E, C and α-lipoic acid compared with the control group without having any statistical significance. It is known biochemically that increased liver enzymes are indicators of disorders and increased activity of the liver (Imik et al., 2009).

Peric et al. (2009) observed significant reductions in both ALT and AST enzyme activities in broiler chickens fed organic selenium, which suggested less oxidative damage within sensitive tissues such as liver in birds receiving organic selenium in the feed.

On the other hand, Arslan et al. (2001) observed no significant difference in plasma AST, ALT and ALP activities in broilers supplemented with vitamin E. Siam et al. (2004) also found no significant changes in serum AST and ALT
activities in laying hens supplemented with vitamin E and selenium under hot conditions.

Sahin et al. (2001b) and Gursu et al. (2003) reported that activities of AST and ALT were not influenced by dietary vitamin E and a combination of vitamin E and selenium supplementation in broilers and Japanese quails under heat stress respectively. However, there was an increase in ALP activity.

Lower AST and ALT activities of layers supplemented with vitamin E and selenium in the present study might indicate a lower degree of liver damage in vitamin E and selenium supplemented groups as compared to the control group fed with basal diet. CK and LDH, associated with muscle cell damage, might indicate diminished muscle damage in layers. Significantly lower CK enzyme activity might also suggest that there were no clinical signs of nutritional muscular dystrophy. The reduced liver and muscle enzyme levels in the supplemented groups might suggest that vitamin E and selenium supplementation could be effective in reducing the effect of stressors that directly affect physiological functions in layers.

4.2.13 Effect of vitamin E and selenium supplementation on serum mineral status in the layers

The biological availability of minerals from the diet is marked by the efficiency with which the body utilizes the dietary minerals. Minerals are accumulated in the tissues and organs of birds and also as the content of egg and egg shell in quite different concentrations, dependent on the dose and form of the elements as well as many other factors, including physiological conditions (Dobrzanski et al., 2008).

The mean levels of serum calcium, phosphorus, iron, copper and zinc in layers supplemented with vitamin E and selenium are presented in Table 20.
TABLE 20
MEAN SERUM MINERAL LEVELS IN LAYERS SUPPLEMENTED
WITH VITAMIN E AND SELENIUM

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Calcium (mg/dl)</th>
<th>Phosphorus (mg/dl)</th>
<th>Iron (µg/dl)</th>
<th>Copper (µg/dl)</th>
<th>Zinc (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>9.78±0.20^A</td>
<td>5.22±0.08^A</td>
<td>770±1.67^A</td>
<td>31.80±0.24^F</td>
<td>378±1.15^A</td>
</tr>
<tr>
<td>T2</td>
<td>12.88±0.28^C</td>
<td>6.87±0.16^C</td>
<td>785±2.68^B</td>
<td>28.13±0.21^C</td>
<td>392±1.56^C</td>
</tr>
<tr>
<td>T3</td>
<td>13.93±0.33^D</td>
<td>7.02±0.14^C</td>
<td>805±2.63^C</td>
<td>25.42±0.19^B</td>
<td>404±1.40^D</td>
</tr>
<tr>
<td>T4</td>
<td>11.35±0.27^B</td>
<td>5.93±0.17^B</td>
<td>781±3.94^B</td>
<td>30.88±0.35^E</td>
<td>383±1.06^B</td>
</tr>
<tr>
<td>T5</td>
<td>11.52±0.21^B</td>
<td>6.22±0.10^B</td>
<td>783±1.80^B</td>
<td>30.00±0.18^D</td>
<td>387±0.83^B</td>
</tr>
<tr>
<td>T6</td>
<td>15.22±0.23^E</td>
<td>7.63±0.23^D</td>
<td>817±2.08^D</td>
<td>24.22±0.12^A</td>
<td>414±1.17^E</td>
</tr>
<tr>
<td>T7</td>
<td>16.83±0.30^F</td>
<td>8.28±0.12^E</td>
<td>828±2.50^E</td>
<td>23.95±0.22^A</td>
<td>422±1.33^F</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>1.01</td>
<td>0.57</td>
<td>9.89</td>
<td>0.86</td>
<td>4.76</td>
</tr>
</tbody>
</table>

T1: Basal diet
T2: Basal diet+100 mg vitamin E/kg feed
T3: Basal diet+200 mg vitamin E/kg feed
T4: Basal diet+0.2 mg selenium/kg feed
T5: Basal diet+0.4 mg selenium/kg feed
T6: Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T7: Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of six birds.
A-F: Mean values within a column with no common superscript differ significantly (p<0.01).

Vitamin E and selenium supplementation in layers caused a significant increase (p<0.01) in serum calcium, phosphorus, iron and zinc in all the treatment groups except serum copper which was found to be significantly (p<0.01) reduced in all the supplemented groups when compared to the birds which received only basal diet without any supplementation. Among the treatment groups, the birds that received higher levels of both vitamin E and selenium in the feed showed significant elevation in the mineral levels of calcium, phosphorus, iron and zinc, which indicated the synergistic action of vitamin E and selenium.
Supplemental dietary vitamin E and vitamin A resulted in an increase in serum concentrations of both calcium and phosphorus in broilers reared under heat stress (Sahin et al., 2001b). A similar increase in plasma calcium and phosphorus concentrations was reported by Sahin et al. (2001a) in heat stressed Japanese quails supplemented with vitamin E.

The increase in calcium and phosphorus in the present study also coincides with that of Kucuk et al. (2003) who stated that supplementation of vitamin C or vitamin E, particularly a combination of the two vitamins significantly increased the serum concentrations of calcium and phosphorus in laying hens maintained at low ambient temperature. Gursu et al. (2003) also found significant increase in serum calcium and phosphorus by increasing both dietary vitamin E and selenium in Japanese quails under heat stress.

Quails supplemented with a combination of vitamin E, C and α-lipoic acid caused a significant increase in serum phosphorus level (Imik et al. 2009). However, Arslan et al. (2001) found an insignificant increase in serum calcium and phosphorus in broilers supplemented with vitamin E and related this increase to the age of the hens.

Increasing dietary vitamin E caused a linear increase in serum concentrations of iron and zinc, but a decrease in serum concentration of copper in broilers under heat stress (Sahin et al., 2001c). A similar observation was also made by Sahin et al. (2002c) who demonstrated that increase in both dietary vitamin E and selenium caused an increase in serum concentrations of iron and zinc, but a decrease in serum concentration of copper in Japanese quails under heat stress.

The increase in serum calcium and phosphorus levels in the present study could be related to osteoblastic activity. Iron is an essential constituent of the heme portion of hemoglobin. Vitamin E is thought to have similar effects to that of ascorbic acid causing iron release into the serum, thus increasing the serum iron concentration.
Zinc stabilizes the red cell membrane against cellular changes caused by peroxidations and it plays a role similar to that of vitamin E in reducing peroxidative damage on cellular membrane. The reason for a decrease in serum copper level in the present study is unclear. It might be due to the fact that stress increases mineral excretion in birds. The available information about the nutritional interrelationships between vitamin E, selenium and mineral levels in birds is very limited.

The reason for the contradiction between the results of this study and the results of other studies might be due to difference in species used, different age of animals, amount and duration of vitamin E and selenium supplemented in the diet. The breed and genetic line might also influence these parameters as suggested by Gyenis et al. (2006).

4.2.14 Effect of vitamin E and selenium supplementation on plasma glucose levels in the layers

Glucose is continuously required as an energy source by all the body cells and must be maintained at adequate levels in plasma. Glucose levels are maintained principally through the conversion of liver glycogen, with some being derived from non-carbohydrate sources (hepatic gluconeogenesis). Chickens have a blood glucose level that is twice as high as that of most mammals (Akiba et al., 2004).

The mean plasma glucose levels in layers due to dietary vitamin E and selenium supplementation are shown in Figure 19.

The results of the present study indicate that vitamin E and selenium supplementation in layers caused a significant (p<0.01) reduction in plasma glucose in all the treatment groups compared to the control. However, no significant difference was observed between the control and the treatment group (T4) that was supplemented with 0.2 mg/kg selenium. Among the treatment groups, T6 and T7 that received both vitamin E and selenium supplementation showed significant reduction in plasma glucose concentration.
According to Sahin et al. (2001a), Sahin et al. (2001b), Sahin et al. (2002b) and Kucuk et al. (2003), dietary vitamin E supplementation caused a significant reduction in plasma glucose concentration in chickens compared to the control. A similar decrease in plasma glucose level was also observed when both dietary vitamin E and selenium supplementation was increased in Japanese quails reared under heat stress (Gursu et al., 2003).

The mean levels of plasma glucose were found to be significantly reduced in Japanese quails under heat stress supplemented with vitamin E, vitamin C and α-lipoic acid. The stress factors usually cause an increase in free radicals and the release of ACTH and cortisol hormones inhibiting insulin release. Antioxidant vitamins counteract the free radicals and decrease the ACTH and cortisol levels protecting the metabolism from the effect of stress (Imik et al., 2009).

Inclusion of increased vitamin E in the feed would have reduced oxidative stress and improved the action of insulin. Antioxidant effects of vitamin E would have the protective effect on β-cell destruction which occurred due to lipid peroxidation.

Selenium is reported to mimic the action of insulin. Selenium mediates a number of insulin-like actions such as stimulating glucose uptake and regulating metabolic processes including glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway. Although the exact mechanism of the insulin mimicking action of selenium has yet to be
elucidated, it is reported that these actions are mediated through the activation of key proteins involved in the insulin-signal cascade (Stapleton, 2000). The plasma glucose concentration of the birds varies with species, nutritional status and environmental condition.

4.2.15 Effect of vitamin E and selenium supplementation on plasma total protein, albumin and globulin levels in the layers

The evaluation of the levels of total protein and its fractions supply the information required to interpret the occurrence of dehydration, infections, immune diseases and inflammatory responses (Silva et al., 2007).

Total protein content in plasma is often used as an indicator for the health status of a bird. Determination of plasma protein concentrations may be of value in diagnosing gastrointestinal, hepatic or renal diseases. Furthermore, plasma proteins will be abnormal in infectious diseases that cause a stimulation of the immune system. Although determination of plasma proteins seldom leads to a specific diagnosis, it will help the clinician to evaluate the severity and progression of a disease.

The mean values of plasma total protein, albumin and globulin in layers supplemented with vitamin E and selenium are shown in Table 21.

Total protein was significantly (p<0.01) increased in all the treatment groups supplemented either with vitamin E, selenium or both vitamin E and selenium compared to the control which received only the basal diet. A similar increase was also observed in albumin and globulin levels in layers that received supplementation of vitamin E and selenium in the basal diet.

Sahin et al. (2001a), Sahin et al. (2001b) and Sahin et al. (2002b) reported that chickens fed dietary vitamin E supplementation caused a significant increase in plasma total protein and albumin compared to the control. A similar increase in protein level was also observed by Gursu et al.
(2003) when both dietary vitamin E and selenium supplementation were increased in Japanese quails reared under heat stress.

TABLE 21
MEAN PLASMA TOTAL PROTEIN, ALBUMIN AND GLOBULIN LEVELS IN LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>5.1±0.08&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.3±0.06&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.8±0.03&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>5.9±0.13&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>2.6±0.05&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.3±0.08&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>T3</td>
<td>6.1±0.18&lt;sup&gt;C&lt;/sup&gt;</td>
<td>2.7±0.08&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.4±0.12&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td>5.6±0.12&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.5±0.04&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.1±0.08&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>T5</td>
<td>5.8±0.11&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>2.6±0.05&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.2±0.06&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>T6</td>
<td>6.7±0.10&lt;sup&gt;D&lt;/sup&gt;</td>
<td>3.0±0.06&lt;sup&gt;C&lt;/sup&gt;</td>
<td>3.7±0.05&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>T7</td>
<td>6.9±0.15&lt;sup&gt;D&lt;/sup&gt;</td>
<td>3.1±0.09&lt;sup&gt;C&lt;/sup&gt;</td>
<td>3.8±0.07&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>0.49</td>
<td>0.24</td>
<td>0.26</td>
</tr>
</tbody>
</table>

T<sub>1</sub> - Basal diet
T<sub>2</sub> - Basal diet+100 mg vitamin E/kg feed
T<sub>3</sub> - Basal diet+200 mg vitamin E/kg feed
T<sub>4</sub> - Basal diet+0.2 mg selenium/kg feed
T<sub>5</sub> - Basal diet+0.4 mg selenium/kg feed
T<sub>6</sub> - Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed
T<sub>7</sub> - Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed

Values given in each cell is the mean±SE of six birds.
A-D Mean values within a column with no common superscript differ significantly (p<0.01).

Islam et al. (2004a) observed that total serum protein, albumin and globulin were significantly increased in broilers supplemented with 2% and 4% vitamin-mineral premix and they suggested that vitamin-mineral premix supplementation in commercial poultry feed is essential for proper growth and body resistance of poultry.
Seyrek et al. (2004) found that vitamin C supplements in the Japanese quails improved the albumin level and did not change the globulin level. It was observed that, during heat stress (35°C), the total protein and albumin concentrations in laying hens were increased compared to those in laying hens under optimal environmental temperature (21°C) (Yardibi et al., 2009).

Total protein and globulin levels were significantly increased in Japanese quails that were supplemented with a combination of vitamin E and vitamin C. Moreover, it was also stated that albumin level was significantly higher in vitamin E supplemented quails compared to the control (Imik et al., 2009). However, Arslan et al. (2001) found insignificant increase in total protein of broilers supplemented with different levels of vitamin E.

The synthesis of proteins in the liver of layers would have been supported by the addition of antioxidants (vitamin E and selenium) increasing the plasma total protein, albumin and globulin.

Plasma proteins are a significant indicator of the health condition and production features of the organisms because of their numerous roles in physiology. They play roles in the maintenance of colloid osmotic pressure. The relation between individual fractions of proteins reflects the functional, metabolic and health status of birds (Filipovic et al., 2007).

4.2.16 Effect of vitamin E and selenium supplementation on blood urea, serum uric acid and creatinine levels in the layers

The avian kidney has a mixture of cortical (reptilian) and medullary (mammalian) nephrons. Nitrogenous wastes excreted by the avian kidney include variable amounts of uric acid, urea, creatinine and ammonia, depending upon the animal's natural environment. Uric acid is the primary catabolic end product of protein metabolism and represents the bulk of the waste nitrogen eliminated by the birds. Relatively small amounts of urea may be found in the blood, the level being influenced by age, sex and production. Plasma urea
appears to be the single most useful variable for early detection of pre-renal causes of renal failure (Kaneko et al., 1997).

The mean blood urea, serum uric acid and creatinine levels in layers supplemented with vitamin E and selenium are presented in Table 22.

**TABLE 22**

**MEAN BLOOD UREA, SERUM URIC ACID AND CREATININE LEVELS IN LAYERS SUPPLEMENTED WITH VITAMIN E AND SELENIUM**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_1</td>
<td>3.82±0.06^C</td>
<td>5.15±0.08^E</td>
<td>0.84±0.03^E</td>
</tr>
<tr>
<td>T_2</td>
<td>3.28±0.15^B</td>
<td>4.58±0.09^CD</td>
<td>0.63±0.03^BC</td>
</tr>
<tr>
<td>T_3</td>
<td>3.13±0.09^B</td>
<td>4.00±0.06^B</td>
<td>0.59±0.02^B</td>
</tr>
<tr>
<td>T_4</td>
<td>3.35±0.09^B</td>
<td>4.77±0.06^D</td>
<td>0.75±0.02^D</td>
</tr>
<tr>
<td>T_5</td>
<td>3.40±0.06^B</td>
<td>4.45±0.04^C</td>
<td>0.71±0.01^CD</td>
</tr>
<tr>
<td>T_6</td>
<td>2.63±0.13^A</td>
<td>3.70±0.07^A</td>
<td>0.49±0.03^A</td>
</tr>
<tr>
<td>T_7</td>
<td>2.50±0.10^A</td>
<td>3.55±0.04^A</td>
<td>0.44±0.02^A</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>0.40</td>
<td>0.25</td>
<td>0.08</td>
</tr>
</tbody>
</table>

T_1 Basal diet  
T_2 Basal diet+100 mg vitamin E/kg feed  
T_3 Basal diet+200 mg vitamin E/kg feed  
T_4 Basal diet+0.2 mg selenium/kg feed  
T_5 Basal diet+0.4 mg selenium/kg feed  
T_6 Basal diet+100 mg vitamin E+0.2 mg selenium/kg feed  
T_7 Basal diet+200 mg vitamin E+0.4 mg selenium/kg feed  

Values given in each cell is the mean±SE of six birds.  
^A^-^E_ Mean values within a column with no common superscript differ significantly (p<0.01).

Blood urea and serum uric acid, the end products of protein metabolism were found to be significantly (p<0.01) reduced in layers supplemented with dietary vitamin E and selenium compared to the control. A similar trend was also observed in serum creatinine levels of layer birds that received vitamin E.
and selenium supplementation. The groups T6 and T7 that received both vitamin E and selenium in the feed showed significant reduction in uric acid, urea and creatinine levels among all the supplemented groups.

Gursu et al. (2003) reported a significant decrease in blood urea level when both vitamin E and selenium were increased in the diet of Japanese quails reared under heat stress. Serum uric acid concentration was found to be decreased when both dietary vitamin E and vitamin A were supplemented in broilers under heat stress (Sahin et al., 2001b).

Ammonia, urea and other soluble urinary nitrogenous wastes require large amounts of water for excretion. Therefore, to conserve water, birds produce more insoluble nitrogenous wastes in the form of uric acid and urate salts, which are eliminated in a semisolid state. Birds excrete moderate quantities of filtered urea when water availability is not a problem (Skadhauge and Schmidt-Nielsen, 1967).

Creatinine is a normal constituent of the urine of mammals, but the amount formed in most birds is negligible (<1 mg/dl). Blood creatinine is derived mainly from the catabolism of phosphocreatine found in muscle tissue. Creatinine level in serum is directly related to the muscle volume and activity and therefore its lower level in the present study might speculate that muscles of laying hens are functioning in good condition due to the antioxidative effect of vitamin E and selenium.

Increased synthesis of protein in the liver and decreased protein catabolism by the antioxidants might be the reason for the decreased blood urea and serum uric acid levels in layers.