Summary and Conclusion
Poultry birds encounter numerous stressors during their lives. These stressors cause hormonal changes, declines in feed intake, altered nutrient metabolism and suppressed immune function. The adverse effects of the stressors are additive and every attempt should be made in all poultry operations to lessen the number and intensity of these stressors. High ambient temperature is considered as a potent climatic stressor causing impaired antioxidant status in poultry. Proper nutrition lessens the immune suppression associated with the stress response in the bird. Stressed birds require higher vitamin and mineral supply due to changes in metabolism and decrease in feed intake.

Animal and human health starts at the cellular level. Preventing cell damage therefore promotes and maintains healthy cellular and immune function. Scientists are now manipulating feed antioxidant content to improve bird health and efficiency and to add value to poultry products by offering an added benefit to consumer's health.

Antioxidants are involved in the prevention of cellular damage, which is commonly responsible for aging and a variety of diseases. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient antioxidants are vitamin E and selenium. Vitamin E is present in the membrane components of the cell and prevents peroxide formation whereas selenium functions throughout the cytoplasm to destroy peroxides.

The objective of the study was to evaluate the effect of vitamin E and selenium supplementation in the feed on the growth performance, immune response, production performance, egg quality characteristics and biochemical profile of layer chickens. The study was conducted in two phases.
PHASE I

Effect of vitamin E and selenium supplementation on the growth performance and immunological response of layer chickens

The experiment was started using two hundred and ten commercial straight run day-old layer chicks (BV-300). The chicks were weighed and randomly allotted into seven treatment groups with three replicates of ten chicks each.

Vitamin E in the form of dl-α-tocopheryl acetate (Promix E) and selenium in the form of Sel-plex (Alltec Inc. USA) were incorporated either separately or in combination with the basal diet to form seven experimental diets. The seven treatment groups were chicks fed basal diet (T_1), basal diet with 100 mg/kg vitamin E (T_2), basal diet with 200 mg/kg vitamin E (T_3), basal diet with 0.2 mg/kg selenium (T_4), basal diet with 0.4 mg/kg selenium (T_5), basal diet with 100 mg/kg vitamin E plus 0.2 mg/kg selenium (T_6), and basal diet with 200 mg/kg vitamin E plus 0.4 mg/kg selenium (T_7). The chicks in all the treatment groups were fed chick mash diet from 0-8 weeks followed by grower diet upto 18 weeks of age.

Individual body weight of the chicks in each replicate in all the treatment groups was recorded once in every 28 days period up to 16 weeks of age. Based on these data, body weight gain was calculated. Feed consumption of all the treatment groups was recorded once in 28 days period and the mean total feed consumption per bird was calculated. Feed efficiency was calculated based on the data obtained from body weight gain and feed consumption. The mortality of the birds was recorded on its occurrence during the investigation period and the livability percentage was worked out.

Immunological parameters were analyzed in their serum to assess the antibody titre against selected antigens. This included assessment of hemagglutination titre against sheep red blood cells, hemagglutination...
inhibition test against the Newcastle Disease Virus and quantitative agar gel precipitation test against Infectious Bursal Disease Virus.

PHASE II

Effect of vitamin E and selenium supplementation on the production performance, egg quality characteristics and biochemical profile of layers

At the end of eighteenth week, one hundred and sixty eight ready to lay layer birds were wing banded and distributed into seven treatment groups with three replicates having eight birds each. The birds were fed with weighed quantity of layer feed supplemented with vitamin E and selenium as indicated in phase I till forty weeks of age.

Individual body weight of the pullets in each replicate in all the treatment groups was recorded at the 20th week of age and subsequently once in every 28 days period up to 40 weeks of age. Feed consumption of all the treatment groups was recorded once in 28 days period and the mean total feed consumption per bird was calculated. The feed efficiency was also calculated and expressed as kilograms of feed consumed to produce a dozen eggs. Livability percentages were calculated based on the mortality of the layer birds.

The egg production was recorded daily during the experimental period and based on the data, hen-day (percent) and hen-housed (number) egg production were calculated. Egg quality traits namely egg weight, shape index, albumen index, yolk index, yolk colour, Haugh unit score and shell thickness were measured at the end of every 28 days period. α-tocopherol content in egg yolk and selenium content in egg albumen and egg yolk were estimated at the end of the 30th and 40th week of age.

Selected biochemical parameters were analyzed in the blood and liver of the experimental birds in order to assess the effect of vitamin E and selenium supplementation in the feed on the metabolism of birds during hot summer. The biochemical parameters assessed were lipid peroxidation (thiobarbituric
acid reactive substances in plasma and liver), antioxidant status (superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione in liver), lipid profile (triglyceride, total, HDL, LDL and VLDL cholesterol in plasma), hematological profile (hemoglobin content, red blood corpuscles and white blood corpuscles count, packed cell volume and red cell indices), serum marker enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and creatine kinase), mineral status (calcium, phosphorus, iron, copper and zinc in serum), plasma glucose, protein profile (total protein, albumin and globulin), blood urea and serum uric acid and creatinine.

Salient findings of the study

PHASE I

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 higher in T6 and T7 groups that received both vitamin E and selenium in the diet as compared to the control and other treatment groups. No significant differences could be observed in the feed consumption of chickens throughout the growing period. Significantly better feed efficiency was observed in T6 and T7 groups that were supplemented with both vitamin E and selenium and also in T3 and T5 that received 200mg/kg vitamin E and 0.4 mg/kg selenium respectively in the diet. Livability percentages were found to be significantly higher in all the supplemented groups compared to the control group.

The mean log_{2} HA titre against SRBC was significantly higher in all the treatment groups as compared to the control (T1). The HI titre against NDV was significantly higher in T6 and T7 groups that received both vitamin E and selenium in the diet followed by selenium supplemented groups (T4 and T5). The QAGPT titre against IBDV was significantly higher in T6 and T7 groups as compared to the control and other treatment groups.
PHASE II

The mean body weight of layers from 20 weeks to 40 weeks period of age was significantly higher in T₆ and T₇ groups that received both vitamin E and selenium as compared to the control and other treatment groups. All the supplemented groups recorded 100 percent livability throughout the laying phase.

Birds in T₆ and T₇ that were supplemented with both vitamin E and selenium recorded the highest egg production followed by T₃ group supplemented with 200mg/kg vitamin E during 21-28 weeks and thereafter, both hen-housed and hen-day egg production were found to be significantly increased in all the supplemented groups compared to the control group. No significant differences could be observed in the feed consumption of the layers. Better feed efficiency (kg of feed consumed per dozen eggs produced) were observed in birds that received the supplementation of both vitamin E and selenium in the diet.

A significant increase in egg weight and Haugh units were recorded in both vitamin E and selenium supplemented groups. No significant differences were observed in the other egg quality characteristics such as shape index, albumen index, yolk index, yolk colour and shell thickness. α-tocopherol content in egg yolk and selenium content in egg albumen and egg yolk were found to be increased linearly as vitamin E and selenium level in the diet were increased. A significant positive correlation was observed between the selenium content of egg albumen and egg yolk. Positive correlation was observed between vitamin E and selenium contents of egg yolk which was not found to be significant.

The antioxidants vitamin E and selenium supplementation in laying hens had significantly decreased plasma and liver TBARS status compared to the control group which received no antioxidant supplementation. The activities of enzymic antioxidants namely superoxide dismutase, catalase and glutathione...
peroxidase and non-enzymic antioxidant, reduced glutathione were significantly increased in all the groups supplemented with vitamin E and selenium.

The mean plasma total cholesterol levels in layers was found to be significantly 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. A significant reduction in plasma triglyceride was observed in T6 and T7 groups that received both vitamin E and selenium in the diet and also in T3 group supplemented with 200 mg vitamin E/kg feed suggesting hypolipidemic effect of vitamin E and selenium. Vitamin E and selenium supplementation caused significant increase in plasma HDL cholesterol with significant decrease in VLDL and LDL cholesterol.

A significant increase in blood hemoglobin level, red blood corpuscle and white blood corpuscle count and packed cell volume were observed in both vitamin E and selenium supplemented groups (T6 and T7). No significant differences could be observed in red cell indices. The layer birds supplemented with vitamin E and selenium showed significant decrease in serum enzyme activities of aspartate transaminase, alanine transaminase and creatine kinase. However, no significant changes were observed in alkaline phosphatase and lactate dehydrogenase activities.

Calcium, phosphorus, iron and zinc were significantly increased in serum in all the supplemented groups except copper which was found to be decreased in the supplemented groups compared to the control. Vitamin E and selenium supplementation in layers caused a significant reduction in plasma glucose in all the treatment groups compared to the control. Plasma total protein, albumin and globulin were found to be significantly increased in all the treatment groups supplemented either with vitamin E, selenium or both vitamin E and selenium. Blood urea and serum uric acid and creatinine levels were found to be significantly reduced in layers supplemented with dietary vitamin E and selenium suggesting decreased protein catabolism.
The following conclusions may be drawn from the present investigation:

**PHASE I**

Increased body weight gain, better feed efficiency, reduced mortality and increased antibody titres were observed in layer chickens due to vitamin E and selenium supplementation.

**PHASE II**

Increased growth rate, reduced mortality, increased egg production, better feed efficiency, increased egg weight and Haugh units, increased retention of α-tocopherol and selenium content in the egg, decreased lipid peroxidation, increased antioxidant status and improved biochemical profiles were observed in layers supplemented with vitamin E and selenium during hot summer.

To conclude, supplementation of both vitamin E and selenium in poultry feed in both the dosages tried in the present study proved to be efficient in improving the growth, immune response, production performance and biochemical profile among the layer chickens.

**Scope for future work**

- A long term study can be attempted in laying hens to alleviate the negative effects of heat stress with suitable dietary supplementations.
- A study can be made to find the effect of vitamin E and selenium supplementation on gene expression in developing embryo.
- The role of vitamin E and selenium in fertility and hatchability can be studied.
- A study can be conducted to find the mechanism of action of vitamin E and selenium on metabolic profiles of chickens.