Experimental Procedure
EXPERIMENTAL PROCEDURE

Oxidative stress is a condition which modifies the normal intracellular balance between oxidant substances produced during aerobic metabolism and antioxidant system processes which perform the function of neutralization, putting a series of protective mechanisms, of both an enzymatic and non-enzymatic nature in action (Tsaluchidu et al., 2008).

Diet plays a pivotal role in maintaining animal health, productive and reproductive performance of farm animals and poultry. Among many dietary factors, natural antioxidants have special importance in the maintenance of high growth levels, reproduction and immuno-competence in poultry (Surai, 2007).

The experimental procedure pertaining to the present study entitled “Effect of supplementation of vitamin E and selenium and their combinations on the growth, immune response, production performance and biochemical profile of layer chickens” was conducted in two phases.

PHASE I

3.1 GROWING PHASE OF LAYING CHICKS

The growing phase included studies on the growth performance and immune responses of the layer chickens. The growing phase was studied from 18-10-2008 to 21-2-2009 which included a total period of 18 weeks.

3.1.1 EXPERIMENTAL DESIGN

The experiment was started using two hundred and ten commercial straight run day-old layer chicks (BV-300). The chicks belonging to a single hatch were purchased from Venkateshwara Hatcheries Private Limited, Namakkal. The chicks were weighed and randomly allotted into seven treatment groups with three replicates of ten chicks each.
3.1.2 MANAGEMENT OF THE CHICKS

The layer chicks of all treatment groups were reared in cages under standard managemental conditions throughout the experimental period. The chicks were fed with weighed quantity of feed *ad libitum* and had free access to water (Plate 1).

3.1.3 PREPARATION OF EXPERIMENTAL DIET

Feed ingredients used for formulation of diets were analyzed for vitamin E and selenium content in addition to proximate composition. The investigation diet was formulated according to the standard prescribed in Bureau of Indian Standards (BIS, 1992), except the levels of vitamin E and selenium in the basal diet.

Even though most nutrients required by the immune system are present in the diet in sufficient concentrations, there is evidence that increased dietary supplementation of certain nutrients, above that needed for maximum growth and feed efficiency, is of benefit to the immune response. Inclusion of concentrations of vitamin E and selenium in the diet above the requirement is associated with variable improvements in animal performance and immune function (Rooke et al., 2004). Therefore in the present study, vitamin E and selenium were supplemented in excess of the recommended level to the poultry diet.

Vitamin E in the form of dl-α-tocopheryl acetate (Promix E) (Plate 2) and selenium in the form of Sel-plex (Alltec Inc. USA) (Plate 3) were incorporated either separately or in combination with the basal diet to form seven experimental diets (Table 2).

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. The ingredients and nutrient composition of layer chick and grower diet are presented in Table 3.
PLATE 1

4 WEEKS OLD CHICKENS ASSESSED IN PHASE I STUDY

TABLE 2

TREATMENT GROUPS OF LAYER CHICKENS BASED ON THE EXPERIMENTAL DIETS

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Experimental diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Basal diet (control)</td>
</tr>
<tr>
<td>T2</td>
<td>Basal diet + vitamin E 100mg/kg</td>
</tr>
<tr>
<td>T3</td>
<td>Basal diet + vitamin E 200mg/kg</td>
</tr>
<tr>
<td>T4</td>
<td>Basal diet + selenium 0.2mg/kg</td>
</tr>
<tr>
<td>T5</td>
<td>Basal diet + selenium 0.4mg/kg</td>
</tr>
<tr>
<td>T6</td>
<td>Basal diet + vitamin E 100mg/kg + selenium 0.2mg/kg</td>
</tr>
<tr>
<td>T7</td>
<td>Basal diet + vitamin E 200mg/kg + selenium 0.4mg/kg</td>
</tr>
</tbody>
</table>

N = 210; No. of birds in each group = 30; No. of replicates per group = 3; No. of birds per replicate per group = 10
PLATE 2
VITAMIN E (dl-α-tocopheryl acetate) ADDED TO THE FEED IN THE SUPPLEMENTATION STUDY

PLATE 3
SEL-PLEX (organic selenium) ADDED TO THE FEED IN THE SUPPLEMENTATION STUDY
TABLE 3
INGREDIENTS AND NUTRIENT COMPOSITION OF COMMERCIAL AVAILABLE LAYER CHICKEN DIET USED IN THE PRESENT STUDY

<table>
<thead>
<tr>
<th>Ingredients (percent)</th>
<th>Chick mash (0-8 weeks)</th>
<th>Grower mash (9-18 weeks)</th>
<th>Layer mash (&gt;19 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Jowar</td>
<td>-</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>Broken Rice</td>
<td>-</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Bajra</td>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rice polish</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Deoiled rice bran</td>
<td>20</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Sunflower cake</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>15</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Fish meal</td>
<td>8</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Calcite</td>
<td>1.15</td>
<td>1.55</td>
<td>6.75</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.4</td>
<td>-</td>
<td>0.8</td>
</tr>
<tr>
<td>Coccidiostat</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Trace minerals and vitamins(^1)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Antibiotic supplement</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Nutrients (percent)**

<table>
<thead>
<tr>
<th>Nutrients (percent)</th>
<th>Metabolizable Energy(kcal/kg)</th>
<th>Crude Protein</th>
<th>Crude Fibre</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Vitamin E(mg/kg)</th>
<th>Selenium(mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2530</td>
<td>20.25</td>
<td>7.58</td>
<td>1.04</td>
<td>0.44</td>
<td>9.64</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>2440</td>
<td>16.40</td>
<td>7.8</td>
<td>1.01</td>
<td>0.3</td>
<td>10.52</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>2380</td>
<td>17.85</td>
<td>6.9</td>
<td>3.28</td>
<td>0.53</td>
<td>10.75</td>
<td>0.078</td>
</tr>
</tbody>
</table>

\(^1\) One gram of trace minerals and vitamins contained 54 mg of manganese, 52 mg of zinc, 20 mg of iron, 2 mg of iodine, 1 mg of cobalt, 82500 IU of vitamin A, 12000 IU of vitamin D₃, 10 mg of vitamin K, 8 mg of vitamin B₃, and 50 mg of vitamin B₂.
3.1.4 ASSESSMENTS MADE IN PHASE I

3.1.4.1 Body weight gain

Individual body weight of five chicks in each replicate in all the treatment groups was recorded once in every 28 days period upto 16 weeks of age. Based on these data, body weight gain was calculated.

3.1.4.2 Feed consumption and feed efficiency

Feed consumption of five chicks in each replicate in all the treatment groups was recorded once in 28 days period and the mean total feed consumption per bird was calculated. Feed intake was determined by subtracting the weight (g) of the left over feed from the weight (g) of the feed initially offered. Feed efficiency was calculated based on the data obtained from body weight gain and feed consumption (Fasuyi and Olorunfemi, 2008).

3.1.4.3 Livability

The mortality of the birds was recorded during the investigation period and the livability percentage was worked out.

\[
\text{Livability percentage} = \frac{\text{No. of birds alive}}{\text{No. of birds housed}} \times 100
\]

3.1.4.4 Immunization and measurement of antibody titres

Poultry possesses limited natural resistance against colonization or infection by pathogenic microorganisms. Increasing the resistance to infectious diseases is possible by immunostimulation, which is the enhancement of immune response by increasing the rate at which the response occurs, elevating its magnitude, thus prolonging the response or directing the response to a particular fact of the immune response (Arshad et al., 2005). Appropriate nutrition may aid in minimizing the incidence of diseases by enhancing immunity (Klasing, 2007).
Hemagglutination (HA) Test

On the 28th day of age, two chicks in each replicate totaling six birds per treatment were randomly picked up and then they were immunized with 0.5 ml of 25 per cent sheep red blood cells (SRBC) (Kundu et al., 1999) on each thigh muscle. Blood samples were collected 15 days after immunization from the wing vein of these birds and hemagglutination titre was assessed as indicated in Appendix 1 (Siegel and Gross, 1980).

Hemagglutination Inhibition (HI) Test

Blood samples were collected randomly from the six birds in each treatment group fifteen days after immunization with Newcastle Disease Vaccine (Ventri Biologicals, Pune, India). The serum samples were separated and utilized for the hemagglutination inhibition test (Allan and Gough, 1974) to find out the immunity developed against the Newcastle Disease Virus (NDV) as explained in Appendix 2.

Quantitative Agar Gel Precipitation Test (QAGPT)

Blood samples were collected randomly from the six birds in each treatment group 15 days after immunization with Infectious Bursal Disease Virus (IBDV) Vaccine (Ventri Biologicals, Pune, India). The serum samples were separated and utilized for analyzing the antibody level. Quantitative agar gel precipitation test was used to assess the antibody level against Infectious Bursal Disease Virus (Wood et al., 1979) as explained in Appendix 3.

PHASE II

3.2 LAYING PHASE OF THE BIRDS

The laying phase of the birds included studies on the production performance, egg quality characteristics and biochemical profile in blood and tissues. The laying phase of the birds was studied from 22-2-2009 to 1-8-2009 which included a total period of 22 weeks in hot climate (Plate 4).
PLATE 4

20 WEEKS OLD LAYERS ASSESSED IN PHASE II STUDY

PLATE 5

USE OF VERNIER CALIPER IN THE DETERMINATION OF WIDTH OF EGG YOLK
3.2.1 EXPERIMENTAL DESIGN

At the end of the eighteenth week, one hundred and sixty eight ready to lay layer birds were wing banded and distributed to seven treatment groups with three replicates having eight birds each.

3.2.2 MANAGEMENT OF THE BIRDS

The birds were fed with weighed quantity of layer feed *ad libitum* and had free access to water. Sixteen hours light was provided daily during the laying period. The feed ingredients used for formulation of layer diets were analysed for vitamin E and selenium content in addition to proximate composition.

The layer diet was formulated as per the standard prescribed in Bureau of Indian Standards (BIS, 1992), except the levels of vitamin E and selenium in the basal diet. Vitamin E in the form of dl-α-tocopheryl acetate (Promix E) and selenium in the form of Sel-plex (Altec Inc. USA) were incorporated either separately or in combination with the basal diet to form seven experimental diets as in phase I (as indicated in Table 2 on page 40).

3.2.3 PRODUCTION TRAITS

3.2.3.1 Body weight

Individual body weight of five 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.

3.2.3.2 Feed consumption and feed efficiency

Feed consumption of five layer birds in each replicate in 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.
3.2.3.3 Livability

The mortality of the layer birds was recorded during the experimental period and livability percentage was calculated.

3.2.3.4 Egg production

The egg production was recorded daily during the experimental period. Based on the data, hen-day (percent) and hen-housed (number) egg production were calculated.

The percent hen-day egg production was computed as the percentage of the total number of eggs over the total number of days by number of hens (Fasuyi and Olorunfemi, 2008).

\[
\text{% hen-day egg production} = \frac{\text{Total number of eggs}}{\text{Total number of days} \times \text{No. of hens}} \times 100
\]

Hen-housed egg production was worked out using the following formula (Shah et al., 2004):

\[
\text{Hen-housed egg production} = \frac{\text{Total number of eggs produced}}{\text{Total number of hens housed}}
\]

3.2.4 EGG QUALITY TRAITS

Egg quality is defined as the characteristics of an egg that affect its acceptability to the consumers. Evaluation of the external and internal quality of chicken eggs is important because of consumer preferences for better quality eggs. It is generally agreed that all characteristics of egg quality have a genetic basis. Egg quality is the most important price contributing factor in table eggs and hatching eggs. Therefore, the economic success of a laying flock solely depends on the total number of quality eggs produced. Quality of eggs may vary due to several factors like rearing, temperature, relative humidity and season (Parmar et al., 2006).
The eggs laid from each replicate in all the treatment groups were collected during the last three days of 28 days laying period. From these eggs, two eggs per treatment were randomly picked up from each day's collection and were used to measure egg quality parameters such as egg weight, shape index, albumen index, yolk index, yolk colour, Haugh unit score and shell thickness.

3.2.4.1 Egg weight

Individual egg weight (g) was recorded with an accuracy of 0.01g using electronic monopan balance. The mean egg weight was calculated (Singh and Panda, 1987).

3.2.4.2 Shape index

The length and width of the eggs were measured by dial vernier caliper with 0.05 mm accuracy. The shape index was calculated as follows (Singh and Panda, 1987):

\[
\text{Shape index} = \left( \frac{\text{Greatest width of the egg (mm)}}{\text{Greatest length of the egg (mm)}} \right) \times 100
\]

3.2.4.3 Albumen index

The eggs were broken on a glass plate, laid evenly on the table and the width of the thick albumen was measured at two places using a dial vernier caliper with 0.05 mm accuracy and their mean width was calculated. Height of the thick albumen was measured to 0.1 mm accuracy using Ames tripod micrometer. Albumen index was calculated by using the following formula (Heiman and carver, 1936):

\[
\text{Albumen index} = \frac{\text{Maximum height of the thick albumen (mm)}}{\text{Mean width of the thick albumen (mm)}}
\]
3.2.4.4 Yolk index

The width of the yolk was measured at two places using a dial vernier caliper with 0.05 mm accuracy and their mean width was calculated (Plate 5). Height of the yolk was measured to 0.01 mm accuracy using Ames tripod micrometer. Yolk index was calculated as per Sauter et al. (1951).

\[
\text{Yolk index} = \frac{\text{Maximum height of the yolk (mm)}}{\text{Mean width of the yolk (mm)}}
\]

3.2.4.5 Yolk colour

The intensity of yolk colour was determined by visual comparison with the colour numbers in the Roche yolk colour fan (Plate 6).

3.2.4.6 Haugh unit

Haugh unit was measured directly by adjusting the weight of the egg with albumen height by using Ames Haugh unit micrometer (Plate 7).

3.2.4.7 Shell thickness

The shells were washed to remove the sticking albumen and dried in a hot air oven at 102°C±5°C overnight. Later the shell thickness was measured at three places viz equatorial region, narrow and broad ends by using screw gauge with 0.01 mm accuracy and mean thickness was calculated (Singh and Panda, 1987) (Plate 8).

3.2.5 DETERMINATION OF ALPHA-TOCOPHEROL AND SELENIUM CONTENT IN EGGS

Eggs were collected from all treatment groups in the last three days of 30th and 40th week. Six eggs per treatment were randomly selected to analyse α-tocopherol by using High Performance Liquid Chromatography system as described by AACC (2000) and selenium by Atomic Absorption Spectrophotometry as described by AOAC (2000). The methodologies followed for α-tocopherol and selenium estimation are detailed in Appendix 4 and Appendix 5 respectively.
3.2.6 BIOCHEMICAL PROFILE OF BLOOD AND LIVER OF LAYERS

Blood analyses have been performed much less often in avian medicine in comparison to its routine use in large animal practices in veterinary medicine (Talebi, 2006). Serum biochemical parameters may provide valuable information for differential diagnosis of nutritional disorders, anti-toxic effects of probiotics (Aqawane and Lonkar, 2004) and evaluation of health status of the birds (Kral and Suchy, 2000).

At the end of the study period, blood samples were collected from the wing vein of six birds in each treatment group. 2.0 ml of blood was collected in two sets of heparinised vials separately. One set was used for the separation of plasma by centrifugation and used for the analysis of thiobarbituric acid reactive substances, lipid profile, glucose and protein profile. Hematological parameters were assessed using the other set of heparinised blood. Whole blood obtained in screw cap tubes were allowed to clot for one hour and serum was separated and used for the remaining biochemical analyses.

Six birds were randomly selected from each treatment group and subjected to humane method of slaughter. The liver was collected from all the slaughtered birds, washed in ice-cold saline and used for the assessment of lipid peroxidation and antioxidant status.

3.2.6.1 Assessment of plasma and tissue lipid peroxidation of layers

Thiobarbituric acid assay is the most prominent and currently preferred test used as an index for lipid peroxidation products. The plasma thiobarbituric acid reactive substances were estimated by the method of Yagi (1987) as detailed in Appendix 6. The level of lipid peroxidation in tissue was estimated by the method of Nichans and Samuelson (1968) as explained in Appendix 7.

3.2.6.2 Assessment of antioxidant status of layers

Living organisms have evolved specific antioxidant protective mechanisms to deal with free radicals which are constantly produced in the
cells. Biological antioxidants react with free radicals or precursor metabolites
converting them into less reactive molecules and preventing or delaying
oxidation of biological molecules. Therefore the presence of natural
antioxidants in living organisms is the major factor that enables their survival in
oxygen rich environment (Sural, 2007).

The methods followed for the estimation of selected enzymic and non-
enzymic antioxidants in liver tissue are presented in Table 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Reference</th>
<th>Appendix no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymic antioxidants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Spectrophotometry</td>
<td>Kakkar et al., (1984)</td>
<td>8</td>
</tr>
<tr>
<td>Catalase</td>
<td>Spectrophotometry</td>
<td>Sinha (1972)</td>
<td>9</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>Spectrophotometry</td>
<td>Rotruck et al., (1973)</td>
<td>10</td>
</tr>
<tr>
<td>Non-enzymic antioxidant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced glutathione</td>
<td>Spectrophotometry</td>
<td>Ellman (1959)</td>
<td>11</td>
</tr>
</tbody>
</table>

3.2.6.3 Assessment of plasma lipid profile of layers

Lipid accumulation leads to oxidative stress which may contribute to
deradicalization of LDL. These peroxidative fatty acids and reactive oxygen
species induce hepatic damage. It has been reported that dietary antioxidant
supplementation causes significant improvement in blood lipid parameters of
humans (Miller et al., 1997).

Plasma triglycerides, total cholesterol and HDL cholesterol were
measured according to the instruction manuals accompanying the diagnostic
kits obtained from Qualigens Diagnostics, Mumbai, India as indicated in
Appendix 12, 13 and 14 respectively.
VLDL and LDL cholesterol were calculated using Friedwald formula (1972).

\[
VLDL = \frac{\text{TG}}{5}
\]

\[
LDL = \text{Total cholesterol} - (\text{HDL} + VLDL).
\]

The values are expressed as mg/dl plasma.

### 3.2.6.4 Assessment of hematological parameters of layers

Analysis of normal hematological parameters of chickens is very much essential in diagnosing the various pathological and metabolic disorders. It can be used as a diagnostic tool in order to assess the health status of an individual and/or a flock. Hematological changes are routinely used to determine the clinical status of the body and to determine stresses due to environmental, nutritional and/or pathological factors (Islam et al., 2004b).

Hemoglobin levels of the layer birds were analysed by Cyanmethemoglobin method (Samuel, 1989) as shown in Appendix 15. Total RBC and WBC counts were determined using the improved Neubauer hemocytometer by the method of Raghuramulu et al. (2003) as detailed in Appendix 16 and 17 respectively. Packed cell volume was determined using the microhematocrit method as indicated in Appendix 18.

Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are the red cell indices which were determined using the appropriate formulae (Raghuramulu et al., 2003) as indicated in Appendix 19.

### 3.2.6.5 Assessment of serum marker enzymes of layers

The enzymes are indicators of different aspects of metabolism and they have been used to evaluate physiological, biochemical and metabolic defects.
in the cells. Assessment of liver damage can be made by estimating the activities of serum aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate dehydrogenase. Creatine kinase is considered to be a muscle specific enzyme and used to test for muscle damage (Samudram et al., 2009).

The methods followed for the estimation of serum marker enzymes are presented in Table 5.

**TABLE 5**

ASSESSMENT OF SERUM MARKER ENZYMES OF LAYERS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Reference</th>
<th>Appendix no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate transaminase</td>
<td>Spectrophotometry</td>
<td>Reitman and Frankel (1957)</td>
<td>20</td>
</tr>
<tr>
<td>Alanine transaminase</td>
<td>Spectrophotometry</td>
<td>Reitman and Frankel (1957)</td>
<td>21</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Spectrophotometry</td>
<td>King and Armstrong (1934)</td>
<td>22</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Spectrophotometry</td>
<td>King (1965)</td>
<td>23</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>Spectrophotometry</td>
<td>Okinaka et al. (1961)</td>
<td>24</td>
</tr>
</tbody>
</table>

3.2.6.6 Assessment of serum mineral levels and selected biochemical parameters of layers

Serum mineral levels and selected biochemical parameters were estimated by using a commercial kit and the methods followed are presented in Table 6.
### TABLE 6

**ASSESSMENT OF SERUM MINERAL LEVELS AND SELECTED BIOCHEMICAL PARAMETERS OF LAYERS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Reference</th>
<th>Appendix no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Spectrophotometry</td>
<td>Gitelman (1967) and Baginski <em>et al.</em> (1973)</td>
<td>25</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Spectrophotometry</td>
<td>Teitz (1976)</td>
<td>26</td>
</tr>
<tr>
<td>Iron</td>
<td>Spectrophotometry</td>
<td>Siedel <em>et al.</em> (1984)</td>
<td>27</td>
</tr>
<tr>
<td>Copper and Zinc</td>
<td>Atomic Absorption Spectrophotometry</td>
<td>Piper (1969)</td>
<td>28</td>
</tr>
<tr>
<td>Glucose</td>
<td>Spectrophotometry</td>
<td>Trinder (1969)</td>
<td>29</td>
</tr>
<tr>
<td>Total protein, albumin and globulin</td>
<td>Spectrophotometry</td>
<td>Doumas <em>et al.</em> (1971) and Webster (1977)</td>
<td>30</td>
</tr>
<tr>
<td>Blood urea</td>
<td>Spectrophotometry</td>
<td>Chaney and Marbach (1962) and Searcy <em>et al.</em> (1967)</td>
<td>31</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Spectrophotometry</td>
<td>Fossati <em>et al.</em> (1980)</td>
<td>32</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Spectrophotometry</td>
<td>Henry <em>et al.</em> (1974)</td>
<td>33</td>
</tr>
</tbody>
</table>

### 3.3 STATISTICAL ANALYSIS

The data obtained were statistically analyzed by one way analysis of variance to compare the individual parameters of the seven different treatment groups. Values are expressed as Mean ± Standard Error (SE).
Two way analysis of variance was performed to compare (i) the vitamin E content of egg yolk in two different periods (30th week and 40th week) and also between the seven different treatments (ii) the selenium content of egg albumen and egg yolk.

Correlation analysis was done between selenium contents of egg albumen and egg yolk and also between vitamin E and selenium contents of egg yolk.