

**EFFECT OF DIETARY ANTIOXIDANTS (VITAMIN C, VITAMIN E
AND SELENIUM) SUPPLEMENTATION ON PERFORMANCE AND
IMMUNE RESPONSE IN BROILERS**

T H E S I S

**Submitted
In partial fulfillment of the requirements for the Degree of**

**MASTER OF VETERINARY SCIENCE
IN
ANIMAL NUTRITION**

**BY
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I hereby declare that the experimental research work and interpretation of the thesis entitled "**EFFECT OF DIETARY ANTIOXIDANTS (VITAMIN C, VITAMIN E AND SELENIUM) SUPPLEMENTATION ON PERFORMANCE AND IMMUNE RESPONSE IN BROILERS** " or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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LIST OF ABBREVIATIONS

Abbreviation	Full form
%	- Percent
@	- At the rate of
µl	- Micro litre
µm	- Micro meter
ANOVA	- Analysis of variance
AOAC	- Association of official analytical chemist
BIS	- Bureau of Indian standard
BWG	- Body weight gain
CF	- Crude fibre
CP	- Crude protein
CRD	- Completely randomized design
d	- Day
DCP	- Dicalcium phosphate
DF	- Degree of Freedom
DLC	- Differential leucocyte count
DM	- Dry matter
EDTA	- Ethylene di-amine tetra acetate
EE	- Ether extract
<i>et al.</i>	- Co-workers
FCR	- Feed conversion ratio
Fig	- Figure
g	- Gram
g/dl	- Gram per deciliter
HA	- Haemagglutination
HI	- Haemagglutination inhibition
hr	- Hours
IBD	- Infectious bursal disease
IU	- International unit
Kcal	- Kilocalorie
Kg	- Kilogram
LSP	- Lime stone powder
ME	- Metabolizable energy
mg	- Milligram
mg/dl	- Milligram per deciliter

Abbreviation		Full form
Mm	-	Millimeter
MS	-	Mean sum of square
N	-	Nitrogen
NFE	-	Nitrogen free extract
OIE	-	Office international des epizooties
OM	-	Organic matter
PBS	-	Phosphate buffer saline
PCV	-	Packed cell volume
RBC	-	Red blood cells
RPM	-	Revolutions per minutes
Rs.	-	Rupees
SEM	-	Standard error of Mean
SS	-	Sum of square
TA	-	Total ash
TLC	-	Total leucocyte count
viz.,	-	Namely
wt.	-	Weight

CHAPTER I

INTRODUCTION

Broiler production represents the most dynamic and rapidly expanding segment of the poultry industry in India and is the one of biggest agro-based business. Poultry farming not only provides income stabilization, but also reliable and consistent cash flow source compared to the crop and other livestock industries. The broiler industry in the country has been growing steadily for the past few years, at an average of 8-10% per year. On the basis of the most recent estimates, India produces 103.32 billion tons of eggs and 2.81 billion tons of chickens (Broilers) annually (Livestock census, 2019). It is the most popular meat from all livestock species. The annual per capita per year availability is 79 eggs and 2.5 Kg of meat. The Indian egg market is the third and Broiler production is the seventh largest in the world (GOI, 2019). The market is estimated to reach INR 4,340 billion by 2024, by 16.2% of compound annual growth rate (CAGR) (Singh, 2019).

In recent years there has been tremendous increase in broilers production which has impacted on the production stress which results in pressure over producer for increasing the bodyweight of broilers in stipulated time period with good FCR. Hence, many times supplementation of anti-stress boosters i.e. antioxidants help in alleviating some of the pressure over the birds mostly during intense climatic conditions. There are certain dietary manipulations which are necessary when it comes to poultry diets, like the formulation of the diet according to the metabolism of the birds, or the addition of feed additives (antioxidants, vitamins, minerals, prebiotics, essential oils, etc.) and electrolytes supplemented in water have been successfully used for reducing stress, enhancing growth, and improving feed efficiency as well as building better immunity in broilers (Rezvani and Shojaee, 2021).

Under stress condition free radicals are generated which lead to formation of reactive oxygen species (ROS) by inhibiting the normal

metabolic processes. Free radicals are molecules with unpaired electrons and are extremely reactive species capable of destroying biologically important components such as DNA, proteins, carbohydrates, and lipids in the nucleus and cell membranes. Free radicals attack critical macromolecules, resulting in cell damage and homeostasis disruption. Antioxidant acts by decreasing free radicals and avoiding lipid peroxidation, also it serves a critical role in protecting cells from reactive oxygen species (ROS). Lower antioxidant plasma concentrations, such as Vitamin C, Vitamin E, and Selenium (Se), have been linked to increased oxidative damage in stressed chickens (Cheng *et al.*, 1990; Sahin *et al.*, 2002). Supplementing herbal anti-stress premix with natural vitamin C and bioflavonoids improved growth, feed efficiency, and hemato-biochemical profiles. The dietary inclusion of herbal anti-stress premix reduced heat stress-related production losses in broilers (Rindhe *et al.*, 2016).

Vitamin C is known as an anti-stress vitamin because it stimulates leucocyte phagocytic activity, the reticulo-endothelial system, and antibody production. Generally under normal circumstances poultry are able to synthesize vitamin 'C' but during stress condition severe depletion of vitamin 'C' takes place. Hence supplementation of vitamin 'C' as anti-stress agent is beneficial. There are organic herbal source of vitamin 'C' like '*Embllica officinalis*' commonly known as 'Amla' belongs to family 'Euphorbiaceae'. Citrus fruits are rich in Vitamin C. Vitamin C has many effects, including immunological modulation, antifungal, antibacterial, anabolic, anti-hepatotoxic, anti-inflammatory, hypolipemic, etc. Natural, herbal, and synthetic vitamin C supplementation in drinking water helps broiler chickens to cope up with summer stress and improve their immunological health (Kadam *et al.*, 2009).

Vitamin E is the most abundant lipid soluble antioxidant in cell membranes, where it functions as a chain-breaking lipid antioxidant and free radical scavenger in cell and subcellular organ membranes (Young *et al.*, 2003). Selenium (Se) is recognized as an essential trace element that plays important role in antioxidant system as a component of Se-dependent

glutathione peroxidase. This enzyme together with superoxide dismutase (SOD) and Catalase (CAT), protects cells against damage caused by free radicals and lipo-peroxides (Finkel and Holbrook, 2000). Vitamin E's ability to reduce peroxy-radicals is also enhanced by selenium. In poultry farming, herbal vitamin E-Selenium combination is more effective and helpful than synthetic vitamin E with inorganic selenium (Chatterjee, 2009).

Supplementation of Vitamin E in broiler diet increased the vitamin E contents in tissues, improved growth performance and prevented lipid oxidation in broiler meat thereby increasing the sensory quality of meat (Sahin *et al.*, 2002). Vitamin E and Selenium supplementation helped to mitigate some of the negative effects of high ambient temperature on growth performance (Sahin *et al.*, 2002). Vitamin E and Selenium acted to prevent oxidative damage to cellular membranes (Hoekstra, 1975; Combs and Scott, 1977). Selenium enhances the action of Vitamin E in reducing peroxy-radicals. Vitamin E absorption is decreased when selenium is deficient, whereas higher selenium levels result in higher absorption for chickens (Abd El-Hack *et al.*, 2017). Heat stressed birds have a larger vitamin and mineral requirement due to changes in metabolism, poorer feed intake, and decreased vitamin stability (Wasti *et al.*, 2020). Many chronic diseases are recognized to have oxidative stress as a contributing factor. Vitamin E and selenium supplementation at 150 IU/Kg and 0.1 mg/Kg, respectively, had a significant positive effect on health, disease prevention, and overall growth performance (Swain *et al.*, 2000).

Inorganic forms of selenium are selenite and selenate, and organic forms are seleno-methionine and seleno-cysteine. Compared to inorganic selenium, organic selenium was more bioavailable, safer, and less toxic. Selenium yeast was the most effective organic source of selenium (Fan and Vinceti, 2015). Selenium has a number of biological functions, including regulating glutathione peroxidase (GSH-Px), thyroid hormones, and prostaglandins; enhancing vitamin E's function; improving immunity; supporting reproductive functions; and protecting tissue membranes against injurious exposure to heavy metals, such as mercury and cadmium (Mervyn,

1985; Surai, 2002). Organic selenium derived from a yeast source with high selenium content could improve fertility compared with inorganic selenium. This was because selenium from yeast is absorbed more readily by tissues (Medina-Morales *et al.*, 2020). Nutrients like selenium, vitamins E and C, at tandem as well as in combination exhibited synergistic effects and were effective in improving broiler chicken performance under acute heat and production stress. There is evidence that selenium, vitamins E and vitamin C have close interactions to protect proteins and lipids from oxidative injuries, and a combination of them might be an effective strategy to reduce heat stress (Shakeri *et al.*, 2020).

As summarized above, Vitamin C and Vitamin E are proven antioxidants and selenium showed synergistic effect with Vitamin E, though the comparison of Vitamin C (among different forms like herbal, synthetic etc.) and Vitamin E with selenium at tandem has been assessed till now, the probable synergistic effect due to their supplementation in combination has not been reported yet. Hence, in order to understand the supplementation of certain antioxidants (Vitamin C, Vitamin E and Selenium) and their interactions on growth, immunity, hemato-biochemical profile, carcass quality trait and oxidative stability in broiler chickens was assessed with following objectives.

Objectives:

- 1) To study the effect of dietary antioxidant supplementation on performance of broiler chicken
- 2) To study the effect of dietary antioxidant supplementation on carcass characteristics of meat in broiler chicken
- 3) To study the effect of dietary antioxidant supplementation on hemato-biochemical parameter, serum antioxidant enzyme status and immune response in broiler chicken.
- 4) To work out the economics of broiler production

CHAPTER II

REVIEW OF LITERATURE

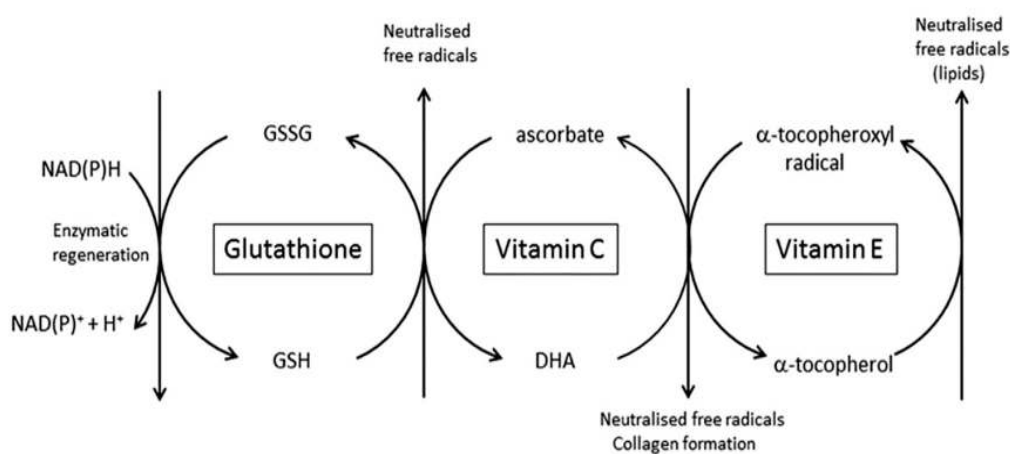
Broiler chicken's health depends upon various factors of which diet plays important role in maintaining health and prevention of various diseases. In the diet, antioxidants play a significant role in the battle for survival, the preservation of animal health, and the maintenance of reproductive and productive performance. Free radicals are detrimental to various metabolic processes mainly because of the toxic products they produce. The term 'free radical' refers to any atom, molecule, or compound whose electron is unpaired. Nitrogen and oxygen are the two most common sources of free radicals in biological system. Both these elements are important for animal life and are highly unstable, very reactive and are capable of damaging molecules such as DNA, proteins, lipids or carbohydrates.

Ascorbic acid, ascorbate (anion of ascorbic acid) or vitamin C is water-soluble antioxidants that treat oxidative stress and improves immunity (Chambial *et al.*, 2013). Dopamine beta hydroxylase converts dopamine to norepinephrine in neural tissues with vitamin C as a co-factor (May *et al.*, 2012).

Selenium is a part of several different Seleno-proteins that regulate antioxidant defense in different tissues. Feed ingredients contain variable concentration of selenium, but most of them are deficient in selenium contents. Selenium requirement is in micro amounts; however, if it is not supplied, compromises the antioxidant system and is harmful to animal health (Zoidis *et al.*, 2018).

Vitamin E is a main chain-breaking antioxidant in biological systems and it is isolated in biological membranes and lipid droplets. The increased levels of vitamin E supplementations yielded beneficial effect in broiler chicks under stress conditions (Lagana *et al.*, 2007). Whenever oxidative stress increases free radical production, major organs and

systems are not likely to sustain damage without external assistance (Maini *et al.*, 2007).



(Pullar, 2017)

The review of literature regarding effect of Vitamin C, Vitamin E and Selenium supplementation on performance, nutrient metabolism, carcass characteristics, hemato-biochemical parameters, sensory evaluation, antioxidant status, immune response and economics of broiler birds is presented in succeeding pages. The literature is reviewed with focus on the following parameters:

- 2.1 Growth Performance
- 2.2 Metabolic study
- 2.3 Carcass traits
- 2.4. Hematological Parameters
- 2.5 Serum metabolite concentration
- 2.6. Immune response
- 2.7 Economics of broiler production

2.1 Growth Performance

Kechik and Sykes (1974) assessed the effects of dietary ascorbic acid on performance of laying hens under warm environmental conditions. Three trials were conducted to investigate the effects of dietary ascorbic acid (AA) on the performance of laying hens maintained under high environmental temperatures (32.2 °C and 33.3 °C, respectively), and a third trial was carried

out under ambient conditions (12.8 °C to 17.3 °C). In first trial, the first group was fed *ad libitum* with 16.7% protein layers' ration supplemented with L-ascorbic acid (Hoffman-La Roche) at a level of 100 mg/Kg from the start of the experiment; the second group was given the same diet as the first, but commencing one week later when the warm temperature regime had been established; the third group was fed the layers' ration without added AA. In Second trial, Forty-six 10-month-old layers were divided into four equal groups. The first and second groups were fed on the control diet supplemented with dietary AA at levels of 100 mg/Kg and 500 mg/Kg respectively, and the third group was given the control diet without added AA. Third experiment was designed to determine the effects of dietary AA (100 mg/Kg and 500 mg/Kg) on egg yield and the percentage of cracked eggs of laying hens maintained under ambient conditions in the poultry house (12.0°C to 18.3°C). Forty-two 10-month-old layers were divided into three equal groups. Weekly changes in egg yield, shell deformation, percentage of cracked eggs and body weight showed an improvement in the AA-supplemented birds but the differences were non-significant. Egg yield and percentage of cracked eggs were not affected when the diet was supplemented with AA under ambient conditions.

Pardue *et al.* (1985) conducted an experiment to determine effect of dietary ascorbic acid (AA) on growth, feed efficiency, and livability in broilers following an acute heating episode. Supplementation of AA was provided in the diets at calculated levels of 0, 250, 500, and 1000 ppm, simultaneously. Females that received 1000 ppm exhibited significantly greater body weights at 2 and 4 weeks of age. No significant effects due to AA supplementation were observed in body weights of males. At 4 weeks of age, chicks were heated on two consecutive days by increasing the ambient temperature (38.3° C at bird level) in the production facility. Heating significantly reduced body weights in males, but not females, at 5 and 7 weeks of age. Feed conversions were increased after heating, but significant effects due to AA were not found. Therefore it was concluded that ascorbic acid did not improve overall

livability significantly in either sex, but heat-associated mortality was reduced in supplemented females.

Kutlu and Forbes (1993) studied three experiments which were conducted to determine whether supplemental ascorbic acid (AA) would alleviate heat-induced deterioration in performance and metabolism of broiler chicks. 96 day-old female broiler chicks were fed standard starter feed (*ad lib*) under either thermo-neutral (UH) or heated (H, thermo-neutral, heated to 36°C for 6-10 hours per day) conditions to 4 week of age. In Experiments 1 and 3 the diet was supplemented with 0, 250, 500 or 1000 mg/Kg AA. In Experiment 2, AA was given at 200 mg/Kg. In Experiment 1, which started at 7 days of age, heating significantly reduced body growth, feed intake and feed efficiency. AA supplementation, particularly at 250 mg/Kg, stimulated growth, intake and efficiency in the H birds but had no significant effect under UH conditions. AA supplementation increased growth, intake and efficiency of H birds while having no effect under UH conditions. In the third experiment, from starting at 7 days of age heating reduces growth, intake, efficiency, thyroid weight, and levels of plasma protein, potassium and calcium; while body temperature, water intake, plasma glucose, cholesterol and sodium increased. Under H conditions, AA supplementation alleviated many of these effects, particularly when included at 250 mg/Kg. No significant influence was associated with AA supplementation under UH conditions. It was demonstrated that AA, particularly at 250 mg/Kg, ameliorated the heat-induced deterioration in performance and metabolism of broiler chicks. AA supplementation of non-heat-stressed birds tended to reduce performance.

Tuleun *et al.* (2000) investigated the effects of dietary Ascorbic acid (AA) and disturbance (D) stress on the performance of broiler chickens in a tropical environment. There were four treatments consisting of two levels of disturbance (1D) and (4D) and two levels of dietary AA (0 and 250mg AA/Kg feed). Each treatment was replicated four times with 25 chicks per replicate. The starter and finisher diets were formulated to be iso-nitrogenous and iso-

caloric. Feed conversion ratios were significantly improved with AA supplementation during the starter phase only. It was concluded that supplementation of AA was beneficial in relieving not only disturbance but also performance of broilers under stressed condition.

Whitehead and Keller (2003) reviewed the effects of nutritional supply of ascorbic acid in limiting the metabolic signs of stress and alleviation of physiological consequences of stress that are manifested in performance, immunological competence and behavior of birds. They stated that optimum response in growth, feed efficiency and livability in broilers under heat stress was observed with supplementation of 250 mg/Kg of ascorbic acid.

Lagana *et al.* (2007) carried out research trial supplementing vitamins C and E and organic minerals Zn and Se to assess the performance of 1 to 35 day-old broilers from, which were kept under cyclic heat stress (25 to 32°C). Four levels of vitamin-mineral supplementation were used (T₁-control diet (60/30 IU of vit E for starter and growing diet, respectively, zero vit. C, 80 ppm of inorganic Zn, 0.3 ppm of inorganic Se); T₂-control diet + 100 UI vit E and 300 ppm vit C/Kg; T₃control diet + 40 ppm Zn and 0.3 ppm Se/Kg; T₄-control diet + T₂ and T₃ levels) and two environments – thermo-neutral and cyclic heat stress (TN and HS) from 14 to 35 days of age. In the period when part of the birds was submitted to HS, from 14 to 35 days, it was observed lower feed intake (FI) and better feed conversion (FC) for HS birds receiving supplementation compared to the group without supplementation. Evaluating the total period, all the types of supplementation provided lower FI and better FC than the control treatment, but not affected weight gain (WG). Hence, the supplementation of vitamins C and E and/or organic minerals Zn and Se improved the performance of birds due to a lower FI resulting in better FC, independently of the environment.

Özkan *et al.* (2007) studied the effect of dietary supplementation with organic and inorganic selenium (Se) sources plus controlled and large amounts of vitamin E (α -tocopherol acetate) in broilers raised at control (20 to 24°C) or low (14.5 to 16.8°C) temperatures after 2 weeks of age. 780 day old

chicks of Ross 308 feather-sexing line were supplemented with following diets. Diet 1, the control diet, comprised a commercial diet containing 0.15mg/Kg inorganic Se and 50mg vitamin E/Kg feed. Diet 2 was the same as diet 1, supplemented with 0.15mg/Kg inorganic Se. Diet 3 was the same as diet 2 but was supplemented with 200mg/Kg vitamin E. Diet 4 was the same as diet 1, but inorganic Se was replaced with 0.30mg/Kg organic Se. Diet 5 was the same as diet 4, supplemented with 200mg/Kg vitamin E. Low temperature reduced the growth rate of broilers; however, at 6 weeks, there were no differences in the body weights of birds fed on organic Se supplemented diets housed at low or controlled temperature. The results demonstrated that feed conversion ratio was significantly affected by low temperature but not by diet.

Shisodiya *et al.* (2008) compared the study of Ashwagandha and commercial synthetic compound on performance of broilers during hot weather. The trial was conducted for a period of six weeks on 225 day old broiler chicks, uniformly distributed into four groups of 75 chicks in each T₁, T₂ and T₃ group. The chicks were fed with standard starter mash which contained crude protein 22.84% and metabolizable energy 2852.5 Kcal / Kg (calculated value) up to three weeks of age. For next 3 weeks i.e. from 4 to 6 weeks of age with finisher mash which contained crude protein 20% and metabolizable energy 2966 Kcal / Kg (calculated value). Group T₁ received standard broiler diet. Group T₂ and T₃ received standard broiler diet supplemented with Ashwagandha and commercial synthetic compound @ 0.05 % of feed respectively. The supplementation of Ashwagandha and commercial synthetic compound recorded significant improvement in all studied growth parameter i.e. live body weights, weekly gain in body weights and feed conversion ratio was observed in all the supplemented groups over the control group. However, feed consumption in control group was significantly higher (P<0.05) than supplemented group. The economic returns of supplemented groups were more than the unsupplemented group. The net profit per bird was maximum in the commercial synthetic compound

supplemented group followed by Ashwagandha supplemented group and lowest was recorded in control group (T₁).

Niu *et al.* (2009a) assessed the effect of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. Birds raised in either a thermoneutral (23.9°C constant) or HS (23.9 to 38°C cycling) environment were fed a corn-soybean meal basal diet supplemented with vitamin E at 0, 100, or 200 mg/Kg, respectively. Two hundred forty 1-d-old male broiler chicks were randomly assigned to 6 groups; each group had 4 replicates of 10 birds. Humoral immunity was assessed by i.v. injection of 7% SRBC followed by evaluation of serum for antibody titers in primary and secondary responses. Cell-mediated immunity was assessed by using a Sephadex stimulation method to recruit abdominal exudate cells (AEC) to evaluate macrophage phagocytic ability. Body weight and feed intake were not significantly influenced by dietary vitamin E ($P > 0.05$), whereas feed conversion was significantly affected by vitamin E at 100 mg/Kg ($P < 0.05$) supplementation. Heat stress significantly reduced BW, feed intake, and feed conversion ($P < 0.05$). Numbers of AEC, percentage of macrophages in AEC, phagocytic macrophages, and internalized opsonized and unopsonized SRBC were increased by dietary vitamin E ($P < 0.05$). Both primary and secondary antibody responses were significantly increased by dietary vitamin E when birds were exposed to HS ($P < 0.05$). Lymphoid organ weights, antibody responses, incidence of macrophages in AEC, and phagocytic ability of macrophages were all significantly reduced under HS. These results indicated that HS severely reduced growth performance and immune response of broilers, whereas the immune response of broilers could be improved by dietary vitamin E supplementation under HS.

Khajali *et al.* (2010) evaluated the effect of an organic selenium supplementation at various levels of dietary protein. Two levels of supplemental selenium (0 and 0.3 mg/Kg) from zinc-L-seleno-methionine were tested at two levels of dietary protein (normal and reduced) in a completely randomized design with 2x2 factorial layout. The experiment

lasted up to 49 d of age during which all birds were exposed to $31\pm 1^{\circ}\text{C}$. The effects of selenium or its interaction with CP on growth performance and carcass characteristics were not significant. However, feeding the reduced-CP diet decreased weight gain in the starting period and increased liver and abdominal fat weights relative to body weight.

Gaikwad *et al.* (2014) investigated the effect of feeding Shatavari Root Powder (SRP) and Amla Fruit Powder (AM) on broiler performance. Two hundred and fifty day old straight run commercial broilers chicks were randomly distributed into five treatment groups with four replicates and 12-13 chicks in each replicate. The dietary treatments consisted of control without SRP or AM (T_1), 0.50% SRP (T_2), 1.00% SRP (T_3), 0.50% AM (T_4) and 1.00% AM (T_5). At six weeks of age, the average weekly gain was 467.53, 528.79, 513.88, 466.3 and 502.15g in T_1 to T_5 treatment groups, respectively. The cumulative feed consumption at sixth week of age was 3965.30, 3877.91, 3794.77, 3891.22 and 3832.35g, respectively, in T_1 - T_5 treatments groups. The cumulative feed conversion efficiency of various groups T_1 to T_5 was 1.85, 1.75, 1.70, 1.76 and 1.71, respectively. The results showed that the use of Shatavari root powder and Amla fruit powder were found to be beneficial. The overall performance was better at 1.00% level of inclusion of these herbs compared to 0.50% level.

Rajashree *et al.* (2014) compared the effects of organic selenium on hen performance and productivity of broiler breeders. A 10-week experiment was conducted with Ross 308 broiler breeder chickens in cages to evaluate the influence of organic and inorganic sources of selenium (Se) supplementation. A total of 600 birds at 29 weeks of age were divided at random into 4 groups and fed on a maize-soya basal diet supplemented with different forms of Se. The first (control) group was given the basal diet without Se supplementation, whereas the second, third and fourth groups were given the basal diet with 0.3 mg/Kg of inorganic Se in the form of sodium selenite or 0.3 and 0.5 mg/Kg of organic Se in the form of Se enriched yeast (Se-yeast). The experiment was carried out for 10 weeks to compare and evaluate the influence of Se supplementation on breeder performance, egg production, hatchability and the

quality of eggs. Samples were collected for analysis at weeks 0, 5 and 10 of the experimental period. At the end of the experiment (39 weeks), there was a reduction in mortality in breeders given diets supplemented with 0.5 mg/Kg of Se-yeast. Supplementation of feed with 0.5 mg/Kg organic Se increased egg production, percentage of settable eggs and hatchability. Selenium supplementation increased egg weight and specific gravity compared to the control diet. However, no significant variation was found in albumen or yolk protein content at the end of week 10. Selenium accumulation and glutathione peroxidase (GSH-Px) activity were lower in the egg albumen and yolk of control compared with Se-supplemented treatments. Se accumulation and GSH-Px activity were higher in the group given 0.3 mg/Kg organic compared to 0.3 mg/Kg of inorganic Se. The results favoured the use of Se-yeast at the dose of 0.5 mg/Kg in broiler breeder diets for better productivity of eggs, settable eggs, hatchability and higher Se accumulation and antioxidant status in eggs.

Tawfeek *et al.* (2014) examined the effects of dietary supplementation with some antioxidants (vitamins & minerals) against the deleterious impacts of heat stress on broilers. One hundred and twenty eight broiler chicks were randomly assigned into 4 dietary groups with 4 replicates (8 chicks each). The chicks were raised for 6 weeks under natural summer months (36 °C and 75% RH) during which the birds received either a basal diet only (control group), or the basal diet supplemented with either 250 mg vitamin C+ 250 mg vitamin E/ Kg diet, or organic micro minerals: 40 mg Zn + 0.30 mg Se/ Kg diet, or with 0.50 mg Cr/Kg diet. Another group of birds which consisted of 32 broiler chicks (4 replicates, 8 birds each) was reared under natural autumn conditions (25 C° and 67% RH) and fed on the same control diet. It was found that high ambient temperature severely reduced body weight, feed intake, and feed efficiency as well as increased abdominal fat and mortality rate. The result indicated that the supplementation of diets with antioxidants, especially vitamins and chromium, was necessary to overcome the deleterious effects of heat stress on broilers' performance.

Dhore *et al.* (2015) conducted a study to assess the effect of herbal and synthetic vitamin C supplementation on performance of broilers under intense summer condition. The body weight gain was significantly ($P<0.01$) higher and better FCR, DM digestibility and nitrogen retention in broilers fed with 200 mg/Kg herbal vitamin C. It was concluded that the feeding of synthetic and herbal vitamin C had beneficial effects on body weight of broilers.

Adeyemi *et al.* (2015) studied effect of ascorbic acid (vitamin C) supplementation on performance of laying birds reared under harsh environmental conditions. A total of 96 unsexed 22 days-old Marshall Broiler chickens of a commercial strain were distributed to six treatments. Birds in treatments 1 and 2 were fed *ad libitum*, and those in treatments 3 and 4 were placed on 85% *ad libitum* while those on treatments 5 and 6 were offered 70% *ad libitum*. Diets fed to birds on treatments 1, 3 and 5 were formulated without ascorbic acid while those on treatments 2, 4 and 6 had their diets supplemented with 200 mg/Kg ascorbic acid. Performance indices (feed intake, weight gain and intake: gain ratio) were significantly ($P<0.05$) influenced by quantitative feed restriction. Feed intake (3256.93, 2799.74 and 2360.05 g/bird) and weight gain (1134.70, 1023.88 and 968.86 g/bird) had highest values recorded for the birds fed *ad libitum* while those on 70% *ad libitum* feeding recorded the best intake: gain ratio (2.87, 2.74 and 2.44). Ascorbic acid supplementation significantly ($P<0.05$) affected the growth performance of broilers. It was concluded that quantitative feed restriction and ascorbic acid supplementation influenced the growth performance of broilers.

Vasanthakumar *et al.* (2015) assessed the effect of Ashwagandha on alleviating heat stress using 90 numbers of day old broiler chicks divided in 3 treatment groups containing 3 replicates in each group during summer season. The dietary treatments included T₁ (Control) without any herbal supplementation, T₂ - 1.0 % Ashwagandha root powder and T₃ - 0.15 % Ashwagandha root extract. At the end of 42 days of age, the body weight, feed intake, antioxidant level and immune status were significantly ($p<0.05$) better in Ashwagandha root powder and extract supplemented groups when

compared to the control group. Based on the above said results, Ashwagandha root powder and extract were found beneficial to alleviate heat stress in broilers during summer season.

Rindhe *et al.* (2016) investigated the effect of supplementation of herbal antistress product and synthetic Vitamin C on performance of broiler birds under physiological heat stress. An experiment was carried out on 135 day old Vencob straight run commercial broiler chicks for a period of 42 days (6 weeks). The chicks were randomly divided into three groups (n=45) of three replicates each. Untreated control group (T₀) was fed standard basal diet without any supplement, treatment group T₁ was supplemented with Ayucee premix at the rate of 100gm/tonne of feed from 0-42 days and treatment group T₂ supplemented with synthetic ascorbic acid @100g/tonne of feed. Record of temperature were maintained on daily basis with mean maximum daily temperature of 39±2°C, relative humidity (RH) 82.57 ± 1.40 % (Recorded twice daily at a fixed time by hygrometer). Ayucee premix was a polyherbal formulation containing natural vitamin C and bioflavonoids, scientifically well known for their antioxidant and free radical scavenging activities. The mean live body weight (g) at 6th week was significantly (P<0.05) improved in Ayucee supplemented group T₁ birds (2503.66) as compared to control group (2385) and varied non significantly from synthetic ascorbic acid supplemented group T₂ birds (2527.16). FCR on 42nd day was significantly (P<0.05) better in Ayucee supplemented group T₁ birds (2.08) than synthetic ascorbic acid supplemented group T₂ birds (2.12) and control group (2.25). Hematological and biochemical parameters, which were altered under stressful conditions, also improved in Ayucee supplemented group T₁ birds.

Abd El-hack *et al.* (2017) investigated the experiment to alleviate the negative biohazards of high ambient temperature on the productive performance and physiological status of laying hens. A total of 135 Bovans laying hens were distributed into nine groups in a 3 × 3 factorial design experiment. Basal diet was supplemented with vitamin E at levels of 0, 250, and 500 mg /Kg diet. Within each dietary vitamin E level, each diet was supplemented with sodium selenite as a source of selenium (Se) to supply 0,

0.25, and 0.50mg Se/Kg diet. Results showed that supplementing layer's diet with 500mg vitamin E/Kg was accompanied with the lowest feed consumption (FC) and feed conversion ratio (FCR). The interaction among vitamin E and Se levels exerted significant effects only on FC and FCR. In conclusion, the combination between vitamin E and Se showed a good ability to alleviate the harmful impacts of heat stress and produced the highest productive performance when compared with the other groups, which exhibit the synergistic effect between the two antioxidants.

Marković *et al.* (2018) studied the effects of selenium (Se)-yeast supplementation on glutathione peroxidase activity, Se levels in tissues, growth performance, carcass, and meat composition in broilers. A total of 275 one-d-old Cobb 500 broilers of both sexes were randomly allotted to 1 of 5 treatments during a 42-d period. The 5 treatments differed only in Se content: group 1 had no additional Se (background only); groups 2, 3, and 4 received 0.3 mg/Kg of added Se from the beginning of the trial until d 21, whereas in the second half of the study (from d 22 to 42), these groups received 0.3, 0.6, and 0.9 mg/Kg of added Se, respectively; and group 5 received 0.9 mg/Kg of Se for the entire experimental period. At the end of the study, the control group showed significantly lower ($P<0.01$) glutathione peroxidase activity in blood plasma compared to Se-supplemented groups. Regarding Se concentration in various tissues, the groups receiving Se yeast showed higher plasma, feces, and meat Se contents than the control group ($P<0.01$). Supplementation of Se improved broilers' body weight, weight gain and feed conversion ratio ($P<0.01$). Dressing percentage was lower in the control group and the group with 0.3 mg/Kg of added Se compared to other experimental groups (0.6 and 0.9 mg/Kg of dietary Se). The proportion of less valuable carcass parts (wings and legs) was higher ($P<0.01$) in the group fed the basal diet compared to groups supplemented with 0.9 mg/Kg of Se. Initial and ultimate pH values differed among experimental groups ($P<0.05$). Supplementation of Se improved the broiler's antioxidative resistance, growth performance, carcass quality, and chemical composition of meat.

Aikpitanyi *et al.* (2019) investigated the effect of oral administration of lime fruit on growth performance in broiler chicken. A total of 150, one day-old unsexed broiler chickens of Anak 2000 strain, were used in a completely randomized design (CRD). The birds were randomly allotted to five treatments with each group having 30 birds, replicated three times to give 10 birds per replicate. Treatment 1 was the control and had no additive; Treatment 2, had 5 ml of lime juice per litre of water every day; Treatment 3, had 7.5g of diced whole lime fruit per litre of water every day; treatment 4, had 5ml of lime juice per litre of water in alternate day while Treatment 5, had 7.5g of diced whole lime fruit per litre of water in alternate day. The experimental trial was for six (6) weeks after a brooding period of two weeks. Significant differences were observed in the final weight, feed intake, weight gain, and feed conversion ratio. Treatment 4 with 5ml of lime juice per litre of water in alternate day recorded better growth performance in the study, with weight gain and feed conversion ratio of 2963.35g/bird and 1.91, respectively. The study showed the use of lime fruit (juice or whole fruit) to promote growth characteristics of broiler chickens which ultimately contribute to the improvement of the bird's overall productivity.

Saiz del Barrio *et al.* (2020) tested the effect of a mineral and vitamin mix (AHS) added to drinking water on the performance of broiler chickens. In total, 1,824 one-day-old birds were randomly allocated to 48 pens. Maximum animal density was 26.5 Kg/m². The control group received no additive; AHS-1 and -2 groups received the AHS mix at a concentration of 1 and 2 Kg/1000 L in drinking water, respectively; and the Vit-C group received vitamin C in drinking water at 200 g/1000 L. All birds were fed the same diets based on a 3-phase feeding program; feed and water were given on ad libitum basis. To mimic heat stress conditions, temperature in the barn was raised to 35⁰C from 08:00 to 14:00 hrs each day. For the overall growing period (0 to 35 D), adding AHS to drinking water increased final BW, ADG, and ADFI linearly ($P_{Linear} < 0.05$); FCR was decreased linearly with AHS supplementation ($P_{Linear} < 0.05$). Final BW, ADG, and FCR for the Vit-C group were intermediate between AHS-2 and the control groups ($P > 0.10$). It was

concluded that the combined use of supplementary levels of minerals and vitamins could alleviate the effects of heat stress on broilers chickens.

Romero-Sanchez *et al.* (2020) studied the effects of antioxidants (selenium, vitamin C and vitamin E) and feed allocation on broiler breeder egg production, fertility and hatchability. In Experiment 1, males were divided assigned to a large comb (LC) or small comb (SC) height group at 20 week and fed a layer diet with either organic high selenium yeast or inorganic selenium [sodium selenite (NaSe)]. In Experiment 2, all birds were randomly assigned to receive a High (Vitamins C, E and HiSe) or Normal antioxidant layer diet. Results showed that experiment 1: LC males receiving NaSe exhibited reduced fertility that was related to a significantly heavier BW relative to the feed allocation. Similarly, the SC males that received more ME relative to BW responded with improved fertility but significantly increased BW at 40 week of age. An interaction between comb size and selenium source suggested that when higher BW males (LC) were underfed, the HiSe Yeast maintained high fertility irrespective of gain in BW. Experiment 2: Overall, High antioxidant levels increased fertility. Further, after 38 week of age, there was a decrease in fertility due to an inadequate feed allocation for males that had a reduced effect on the High antioxidant group. However, when male feed allocation was increased at 54 week of age the differences due to antioxidant level were diminished. It was concluded that a consistent feed increment that maintained a consistent male BW gain and elevated antioxidant levels is important for maintaining fertility.

2.2 Metabolic study

Goni *et al.* (2007) studied the effect of dietary grape pomace and vitamin E on nutrient digestibility in chickens. An experiment was conducted with inclusion of GP at levels of 5, 15, and 30 g/Kg and α -tocopheryl acetate (200 mg/Kg) in a corn-soybean basal diet on growth performance, protein and amino acid digestibilities. Growth performance and protein and amino acid digestibilities were not affected among the different treatments. Total intake and digestibility of extractable polyphenols in the birds fed the GP diet were

increased compared with birds fed supplemented and unsupplemented vitamin E diets. In conclusion, the results showed that a dietary inclusion rate up to 30 g/Kg of GP did not impair chickens growth performance and protein and amino acids digestibilities and increased antioxidant activity in diet and excreta.

Seven *et al.* (2012) examined the effects of Propolis and vitamin C supplemented feed on nutrient utilization in broilers exposed to lead. 360 three-days-old broiler chicks (Ross 308) were randomly divided into four treatment groups each containing 90 chicks, including six replicate groups for each treatment. The experimental groups were designed for a 3–42 days period as follows: no supplement to basal ration (Control; group I); supplement of 500 ppm vitamin C and 200 ppm lead (as lead acetate) to basal ration (Vitamin C/Pb; group II); supplement of 1 g/Kg propolis and 200 ppm lead (as lead acetate) to basal ration (Propolis/Pb; group III); and supplement of 200 ppm lead (as lead acetate) to basal ration (Pb; group IV). Feed intake was reduced with lead inclusion in the diet ($P < 0.05$). Utilization of crude protein improved with both vitamin C and Propolis supplementation in comparison to the lead administrated group ($P < 0.05$). The results suggested that lead inclusion in diet of birds had negative effects on the performance while Propolis supplements improved the growth, nutrient utilization and carcass yield.

Ashour *et al.* (2019) evaluated the effect of dietary inclusion of dried brewers' grains (DBG) on nutrient digestibility of broiler chickens. A total of 300 unsexed one-week-old Hubbard chicks were randomly allotted to five treatment groups. The dietary treatments consisted of a basal diet as the control and DBG groups (3%, 6%, 9% and 12%, respectively). Broilers fed a diet containing 3% DBG had significantly higher of digestion coefficients for crude protein than those fed on the other experimental diets. Based on growth performance and health status, 3% or 9% DBG may be the optimum percentages for inclusion in the diets of poultry until six weeks old. In addition, DBG exerted several health benefits in meat, which would be reflected positively on human health.

Rezvani and Shojaee (2021) assessed the effect of adding Watermelon juice, vitamin E-Selenium, and vitamin C into the drinking water prececal nutrient digestibility in broilers under heat stress. Two hundred and fifty Hubbard F15 day-old broilers were allocated to five treatments with five replicates of 10 broilers per each replicate, based on a completely randomized design. The birds in the control treatment did not receive any supplement in drinking water and reared in thermo-neutral temperature. Birds in the other four treatments received watermelon juice at 60% or 30%, vitamin C at 50 mg/L, or vitamin E-Selenium at 1 mL/L of drinking water during the heat stress period. Heat stress applied for 9 hours from day 12 onward. Results indicated birds that received 60% watermelon juice had similar body weight and feed intake compared to control birds. The relative weight of proventriculus, intestine length, and prececal dry matter nutrient digestibility was significantly higher in 60% watermelon juice treatment than other treatments. It was concluded that watermelon juice supplementation at 60% concentration into the drinking water could improve the performance of birds reared under heat stress as a result of restoring water and feed intake and also better prececal dry matter digestibility.

2.3 Carcass traits

Sahin *et al.* (2003) studied the effect of chromium (chromium picolinate, CrPic) and vitamin C (L-ascorbic acid) supplementation on carcass characteristics in broiler chickens (Ross) reared under heat stress (32°C). One day-old 120 male broilers were randomly assigned to 4 treatment groups, 3 replicates of 10 birds each. The birds were fed either a basal diet or the basal diet supplemented with either 400 mg of Cr/Kg of diet, 250 mg of L-ascorbic acid/Kg of diet, or 400 mg of Cr plus 250 mg of L-ascorbic acid/Kg of diet. The supplements also increased hot and chilled carcass weights and yields ($P>0.05$) as well as liver, heart, spleen, and gizzard weights ($P>0.05$). However, abdominal fat decreased ($P>0.05$) upon dietary chromium and vitamin C supplementation. The results showed that, separately or as a

combination, vitamin C and chromium supplementation resulted in an improved live weight gain and carcass traits.

Choct *et al.* (2004) examined the effect of dietary source and concentration of selenium on broiler performance and meat quality. Increased dietary selenium content markedly reduced feed conversion ratio (FCR) as a result of significantly lower feed intakes of birds while maintaining the same weight gains. Selenium supplementation increased feathering, with organic selenium (selenised yeast) being superior to inorganic selenium (sodium selenite). Birds receiving organic selenium in their diets had improved eviscerated weight, breast yield and reduced drip loss. There were significant concentration \times source interactions on yields of breasts and Marylands (thigh plus drumstick), with elevated levels of organic selenium increasing the yields, whereas the opposite was true for the inorganic selenium.

Deniz *et al.* (2005) supplemented broiler diets with two different supplemental dietary selenium (Se) sources in broilers and evaluated whole carcass drip-loss. A total of 273 one-day-old male broiler chicks (Avian Farms) were randomly divided into 3 groups of 91 birds each according to the dietary Se supplementation. The first group received no supplemental Se into starter (from 1st to 21st day), grower (from 22nd to 35th day) and withdrawal (from 36th to 42nd days) diets. The second and third groups were fed with the different dietary regimes supplemented with 0.3 ppm mineral Se (Sodium selenite) or organic Se (Se enriched yeast) respectively. Carcass traits (chilled carcass weight and chilled carcass yield, whole carcass drip-loss) were measured after slaughtering on the 42nd day. Chilled carcass weights and their yield were also unaffected at the end of experiment. In contrast, whole carcass drip-loss was significantly reduced in birds receiving 0.3 ppm organic Se in comparison to the other groups ($P < 0.01$). These results indicated that supplementation of broiler diets with organic Se decreased drip-loss, leading to increased meat quality and economical gain. However, carcass weight and carcass yield were unaffected by selenium sources. It was concluded, that the

use of organic Se instead of inorganic Se in broiler diets may increase both meat quality and economic gain by reducing drip-loss.

Dhaliwal *et al.* (2005) studied the effect of supplementation of synthetic vitamins C, E and combination of herbal C & E on the performance of Japanese quail during dry hot conditions. Vitamin E (SE) and herbal CE supplementations increased ($P<0.05$) the proportion of leg and breast meat. It was concluded that the supplementation of herbal CE as well as synthetic vitamin C and E alleviated some of the negative effects of heat stress in Japanese quail.

Khajali *et al.* (2010) evaluated the effect of an organic selenium supplement at various levels of dietary protein among three hundred chicks were randomly assigned among four treatments groups. Two levels of supplemental selenium (0 and 0.3 mg/Kg) from zinc-L-seleno-methionine were tested at two levels of dietary protein (normal and reduced) in a completely randomized design with 2x2 factorial layout. The experiment lasted up to 49 d of age during which all birds were exposed to $31\pm 1^{\circ}\text{C}$. The effects of selenium or its interaction with CP on growth performance and carcass characteristics were not significant. However, feeding the reduced-CP diet decreased weight gain in the starting period and increased liver and abdominal fat weights relative to body weight.

Skřivan *et al.* (2012) investigated the combined effect of dietary vitamin C and Se on the composition and oxidative stability of meat of broilers. Male broiler chickens were fed a maize–wheat–soya diet supplemented with vitamin C at 280 and 560 mg/Kg of diet, and Se (sodium selenite or selenised yeast; Se) at 0.3 mg/Kg for 5 weeks. After slaughter, samples of thigh meat were analysed. The supplementation of diets with vitamin C or Se increased the protein concentration of the meat at the expense of fat. Vitamin C supplementation increased the vitamin C content of the meat in a dose dependent manner and decreased the vitamin A concentration in the meat of broilers fed diets with sodium selenite or without a Se supplement. In the meat of the broilers that were fed these diets, the vitamin C decreased the

lipid oxidation in meat that was stored for 5 days. No sparing effect of vitamin C was apparent on the amount of vitamin E in the meat. Both Se sources increased the activity of glutathione peroxidase and the oxidative stability of the meat.

Kumari *et al.* (2012) evaluated the effects of Amla (*Emblica officinalis*) pomace feeding on growth performance of commercial broilers. 120 day-old commercial broiler chicks, were fed with Amla pomace @ 250g/quintal (AM1), 500g/quintal (AM2) and 750g/quintal (AM3) and it was compared with a control group (C). Each treatment had 30 broilers with three replicates of 10 chicks per replicate. The broilers were offered starter diets up to 4 weeks of age and were reared in battery brooders, thereafter they were offered finisher diets in 5th and 6th week of age and were reared in Californian cages. At the end of 6th week, 6 birds from each treatment were sacrificed to record dressing percentage, weights of liver, spleen, gall bladder, bursa, thymus, and abdominal fat. There was a significant improvement in FCR but no effect of feeding on weights of liver, spleen, bursa, thymus and abdominal fat percentage was observed. It may be concluded that the feeding of Amla pomace significantly improved the performance of broilers, thereby improving the profitability of broiler industry.

Kale *et al.* (2014) conducted an experiment on the performance of broilers supplemented with *Emblica officinalis* (amla) and vitamin E under nutritional stress during 0–6 weeks of age. Feed intake, and feed conversion ratio was similar in all the treatment groups upto 6th week of age, however, average body weights of the birds were higher ($P < 0.05$) in group T₀ and T₃ compared to other two groups showing positive effect of vitamin E supplementation.

Tawfeek *et al.* (2014) evaluated the effects of dietary supplementation with some antioxidants (vitamins & minerals) against the deleterious impacts of heat stress on broilers. One hundred and twenty eight broiler chicks were randomly assigned into 4 dietary groups with 4 replicates (8 chicks each). The chicks were raised for 6 weeks under natural summer

months (36 °C and 75% RH) during which the birds received either a basal diet only (control group), or the basal diet supplemented with either 250 mg vitamin C+ 250 mg vitamin E/ Kg diet, or organic micro minerals: 40 mg Zn + 0.30 mg Se/ Kg diet, or with 0.50 mg Cr/Kg diet. Another group of birds which consists of 32 broiler chicks (4 replicates, 8 birds each) was reared under natural autumn conditions (25 C° and 67% RH) and fed on the same control diet. This group was used to stand out the effects of heat stress per se through comparing its results with those of the previous control one. It was found that at high ambient temperature increased abdominal fat. The present results indicated that the supplementation of diets with antioxidants, especially vitamins and chromium, is necessary to overcome the deleterious effects of heat stress on broilers' performance.

Mahmoud *et al.* (2016) examined the effects of nano-selenium on growth performance, antioxidative status, and immune function in broiler chickens reared under thermoneutral (22±1°C) or high ambient temperature (35±1°C) conditions. Thirty-six broiler chicks at 15d old were randomly divided into 6 treatments in a 3 ×2 factorial design. The main factors included the dietary supplementation (basal diet without Se supplementation [control], basal diet with 0.3mg of nano-elemental Se per kilogram of diet [nano-Se], and basal diet with 0.3mg of sodium selenite per kilogram of diet [SSe] and the ambient temperature challenge (22±1°C or 35±1°C). The birds were given the experimental diets from 15 to 30 d of age. High ambient temperature significantly depressed body weight gain, feed intake, feed conversion ratio, breast muscle weight, and abdominal fat weight, while feeding nano-Se clearly alleviated these negative effects of high ambient temperature. Under thermo-neutral and high-temperature conditions, broiler chickens fed nano-Se had higher Se and vitamin E concentrations in breast muscle than broiler chickens fed the control diet. In contrast, feeding Se at the same dose as nano-Se did not alleviate the negative effects of high ambient temperature on broiler chickens. In conclusion, dietary supplementation with nano-Se at 0.3mg/Kg diet might enhance growth performance by improving antioxidative or immune properties in broilers reared under high ambient temperature.

Singh *et al.* (2019) studied the effect of density, protein and Amla fruit powder (*Emblica officinalis*) on broiler performance and litter parameters. Four hundred eighty day old mediocre broiler chicks were reared in D₁ (500 cm²/bird), D₂ (700 cm²/bird) and D₃ (900 cm²/bird) densities. Two protein levels i.e. P₁ (21% cp), P₂ (23% cp) with isocaloric energy (2800 - 3000 Kcal ME/Kg) was utilized to prepare rations. Each ration was supplemented with Amla fruit powder (AFP) at A₁ level (1 Kg 100 Kg ration) and A₀ level (without AFP). Experiment was conducted in factorial design. Broiler in D₃, P₂, A₁, treatment attained higher body weight with significantly superior feed efficiency than all other treatments. Similarly main effect showed that D₁ density broilers has inferior body wt., Feed efficiency, carcass yield and high percentage of moisture and nitrogen in litter with increase incidences of foot pad lesions. P₂ protein has significantly higher body wt. and FE whereas A₁ AFP revealed significantly improved FE. Effect of protein and AFP on % litter moisture, nitrogen, foot pad lesions and carcass yield was non-significant.

2.4. Hematological Parameters

Ahmed *et al.* (2007) evaluated the effect of enzyme and vitamin supplementation on physio-biochemical parameters in broiler chickens. A total of 20 broiler birds of 20 day old were divided into four equal groups as A, B, C and D. Group A was maintained as control and was fed only with commercial broiler ration throughout the experimental period. Group B was treated with enzymes @ 1 gm per litre of drinking water, group C was treated with vitamins @ 0.5 gm per litre of drinking water and group D was treated with enzymes and vitamins combinely at the rate of above mentioned dose. Body weight was recorded at 7 days interval up to the end of the 21 days of experimental period and the birds were sacrificed on the final day of experiment to collect blood sample for haematological (TEC, Hb, PCV and ESR) and biochemical study (SGOT and SGPT). TEC, PCV and Hb content increased significantly (P<0.01) in the treated groups as compared to that of control group but ESR, SGOT and SGPT values decreased significantly

($P < 0.01$) in all the treated groups as compared to that of control group. The result revealed that use of enzymes and vitamins is an effective way of getting best result in terms of haemato-biochemical profiles in broiler birds.

Kadam *et al.* (2009) compared the efficacy of supplementation of natural (Citrus lemon Juice), herbal and synthetic vitamin C on the immune response of broiler chicken during summer stress. The hematological and immunological study was undertaken on 488 (Ven- Cobb) straight run commercial broiler chicks for period of one week divided into four treatments viz., T_0 , T_1 , T_2 and T_3 on the onset of fifth week of age. The treatment T_0 was the control without supplementation of vitamin C. The birds in treatment T_1 , T_2 and T_3 were supplemented with 5 ml Citrus lemon juice, 2 ml herbal vitamin C and 1 g synthetic vitamin C per 100 birds, respectively through drinking water. The results of the study indicated that Heterophil percentage was significantly ($P < 0.05$) decreased in treatment T_1 , T_2 and T_3 as compared to control T_0 . Similarly, the Lymphocyte percentage was significantly ($P < 0.05$) increased in treatment T_1 , T_2 and T_3 than the control T_0 . The Heterophil: Lymphocyte ratio was significantly ($p < 0.05$) lower in treatment T_1 , followed by T_3 , T_2 , and T_0 . The weight of bursa of fabricious and spleen was significantly ($p < 0.05$) higher in T_1 , followed by T_3 , T_2 and T_0 . The rectal temperature of birds was decreased significantly ($p < 0.05$) T_1 , T_2 and T_3 as compared to control group. Carcass traits did not differ significantly among the different groups except the percent giblet weight was significantly higher in T_1 . The overall study concluded that, the Citrus lemon juice, herbal vitamin C and synthetic vitamin C had significant beneficial effect in alleviating the summer stress in broiler chicken. However, the better results were loam with Citrus lemon juice as compared to herbal and synthetic vitamin C.

Shlig (2009) investigated the sufficiency of vitamin E and Selenium supplementation to diets against containing aflatoxins on the relative blood parameters. One hundred and twenty unsexed Ross birds were used from 3 to 7 weeks of age. Birds were randomly distributed and subjected to five nutrition treatments as follows: (T_1 - Control Group: 0.0 AF + 0.0 Se +0.0 Vit.

E, T₂- 2.5 mg AF/Kg diet, T₃- 2.5 mg AF /Kg diet + 0.18mg/Kg Se +10 I.U Vit. E, T₄- 2.5 mg AF/Kg diet + 0.32mg/Kg Se +30 I.U Vit.E, T₅- 2.5mg AF /Kg diet + 0.50mg/Kg Se +50 I.U Vit. E). Moreover existence of aflatoxins caused a significant decrease in values of Packed cell volume (PCV), Red blood cells counts (RBC), Hemoglobin (Hb), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) significantly moreover, it has been noticed a significant increase occurrence in values of total white blood cell (WBC) and ration of Heterophils to lymphocytes in second treatment birds influenced by Aflatoxins. Addition of both the Vitamin E and Selenium (T₃, T₄, T₅) to the provender containing by aflatoxins, had no effects on blood parameters to their natural averages at control group.

Mansoub *et al.* (2010) determined the effects of vitamin E (α TA; dL- α -Tocopheryl acetate) and zinc (Zn; ZnCl₂) on some blood measures of broiler chickens under normal and hot temperatures. One hundred sixty 10-day-old male chicks were assigned to four groups in four replicates. The birds received two diets, control and enriched with two levels of vitamin E and Zn (100 and 50 mg/Kg of diet, respectively) under two ambient temperatures (22 °C and 35 °C). The hematocrit and heterophil : lymphocyte ratio levels were significantly lower in birds reared under 22 °C compared with the group under 35 °C but the hematocrit was not significantly lower in groups fed enriched diets and exposed to normal conditions. The hematocrit and heterophil : lymphocyte ratio levels were significantly lower in birds reared under 22 °C compared with the group under 35 °C but the hematocrit was not significantly lower in groups fed enriched diets and exposed to normal conditions (P>0.05). It was concluded that a combination of 100 mg of vitamin E and 50 mg of Zn provided the better blood picture and the least blood C and TG levels accompanying the better oxidative stability in male broilers under normal temperature (22 °C) and this combination can be considered as a protective strategy in broiler diets for reducing the negative effects of heat stress.

Tayeb and Qader (2012) studied the effect of supplementation Selenium and Vitamin E in the diets on hematological parameters of broiler

chick. This study was conducted at the poultry production farm in Akre region for a period 49 days using 420 Ross day old broiler chicks. The chicks were distributed randomly in to seven treatments. Selenium and Vitamin E added to the diet according to as follows: T₁- (control group), no supplemented Se and Vitamin E; T₂- 0.15 mg Se + 100 mg Vit.E/Kg feed; T₃- 0.3 mg Se + 100 mg Vit.E/Kg feed; T₄- 0.15 mg Se+ 150 mg Vit.E/Kg feed; T₅- 0.3 mg Se + 150 mg Vit.E/Kg feed; T₆- 0.45 mg Se+ 100 mg Vit.E/Kg feed and T₇- 0.45 mg Se +150 mg Vit.E/Kg feed. Results showed that significant differences (P<0.05) appeared in packed cell volume, hemoglobin for T₇ compared to other treatments and T₁ at 42, 49 days of age. While, there were no significant differences in lymphocytes between all treatments compared to control group except T₆ at 42 days of age, this treatment was significantly superior to control regarding heterophil %, H/L. Significant differences (P<0.05) in monocyte, eosinophil, basophil were resulted among all treatments at 42 days of age.

Shirzadegan and Falahpour (2014) evaluated the antioxidative potential, indices such as quality of the thigh meat and liver of broiler chickens fed with a dietary medicinal herb extract mixture (HEM, consisting: Iranian green tea, cinnamon, garlic and chicory at a ratio of 25:15:45:15). A total of 320, one-d-old Ross (male) broiler chickens were used to investigate the effects of 0.0, 2.5, 5.0 and 7.5 g/Kg HEM in the diet, on aforementioned factors. The HEM supplementation did not influence the composition of raw thigh meat except for the total phenols and crude ash (P<0.05). Furthermore, pH, water-holding capacity (WHC) and acceptability of thigh meat were affecting by administration of HEM in diets (P<0.05). Meat flavor increased in the supplemented groups (P<0.05). According to our data, HEM supplementation decreased the amount of thiobarbituric acid reactive substance (TBARS) in various times of storage and improved the liver lipid peroxides and superoxide dismutase (SOD) activities at week 6 (P<0.05), but did not influence the catalase activity. Results revealed that the addition of 7.5 g/Kg or higher HEM in diet could be sufficient to increase the antioxidative activity and 2.5 g/Kg for meat taste of broilers in maximum levels.

Dhore *et al.* (2015) assessed the effect of herbal and synthetic vitamin C supplementation on performance of broilers under intense summer condition. The haematological study revealed significant ($P<0.01$) rise in Hb, PCV, TEC, TLC, lymphocytes and significantly decreased neutrophils in vitamin C supplemented groups as compared to control. It was concluded that the feeding of synthetic and herbal vitamin C has beneficial effects on haemobiochemical of broilers.

2.5 Serum metabolite concentration

Kutlu and Forbes (1993) studied three experiments which were conducted to determine whether supplemental ascorbic acid (AA) would alleviate heat-induced deterioration in performance and metabolism of broiler chicks. Day-old female broiler chicks were fed standard starter feed (*ad lib*) under either thermo-neutral (UH) or heated (H, thermo-neutral, heated to 36°C for 6-10 hours per day) conditions to 4 weeks of age. In Experiments 1 and 3 the diet was supplemented with 0, 250, 500 or 1000 mg/Kg AA. In Experiment 2, AA was given at 200 mg/Kg. They reported that high environmental temperature increased level of plasma glucose and cholesterol and reduced protein level in broiler chicks. In the third experiment, from starting at 7 days of age heating reduces growth, intake, efficiency, thyroid weight, and levels of plasma protein, potassium and calcium; while body temperature, water intake, plasma glucose, cholesterol and sodium increased. They also observed that total cholesterol, VLDL cholesterol, and triglycerides in blood plasma were not significantly influenced by heat stress.

Maini *et al.* (2007) conducted trial on anti-oxidative effects of vitamin E, amla-electrolyte (Electrolyte mix of *Emblica officinalis*) and mint (*Mentha longifolia*) supplementation in broilers. Day old broiler chicks (n=75) were randomly divided into five groups. Control group was given basal diet and treatment groups were supplemented with either Vitamin E (200mg/Kg feed), dried mint leaves (10g/Kg and 30g/Kg feed) or amla-electrolyte mix (1g/Kg feed) from day 1 to seven weeks of age. Control group at 3rd and 5th week showed high concentration of lipid peroxidation and low activity of

glutathione (GSH) and superoxide dismutase (SOD) in erythrocytes, heart, liver and brain cortex. Antioxidants supplemented groups showed significantly reduced level of lipid peroxidation in erythrocytes both at 3rd and 5th week and in tissues. The GSH, catalase, SOD and glutathione reductase activities were elevated in erythrocytes whereas GSH and SOD activities were depressed in treated birds. It was concluded that oxidative stress in broilers during summer could be ameliorated using herbal antioxidants.

Monsoub *et al.* (2010) determined the effects of vitamin E (α -TA; dL- α -Tocopheryl acetate) and zinc (Zn; ZnCl₂) on some blood measures of broiler chickens under normal and hot temperatures. One hundred sixty 10-day-old male chicks were assigned to four groups in four replicates. The birds received two diets, control and enriched with two levels of vitamin E and Zn (100 and 50 mg/Kg of diet, respectively) under two ambient temperatures (22 °C and 35 °C). The serum malondialdehyde (MDA), cholesterol (C), triglycerides (TG), glucose (G) and total protein (TP) levels were significantly lower in birds reared under 22 °C compared with the group under 35 °C. It was concluded that a combination of 100 mg of vitamin E and 50 mg of Zn provided better oxidative stability in male broilers under normal temperature (22 °C) and also this combination could be considered as a protective strategy in broiler diets for reducing the negative effects of heat stress.

Ghazi Harsini *et al.* (2012) evaluated the effects of dietary vitamin E, selenium (Se), and a combination of the two, on the performance, serum metabolites and oxidative stability of skeletal muscle of broilers during heat stress. The broilers raised in either a thermoneutral (23.9°C constant) or heat stress (23.9°C to 37°C cycling) environment were assigned to 6 dietary treatments (0, 0.5, or 1 mg/ Kg Se; 125 and 250 mg/Kg vitamin E; or 0.5 mg/Kg Se plus 125 mg/Kg vitamin E) from 1 to 49 days of age. At the end of the experiment, blood samples were collected from chicks, the chicks sacrificed, and pectoralis superficialis muscle was used for measurement of malondialdehyde (MDA) concentration and enzyme activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD). The serum concentrations

of copper (Cu), glucose, and uric acid were significantly increased under heat stress ($P < 0.05$). The GPx activity remained relatively constant ($P > 0.05$), though SOD activity and MDA levels in skeletal muscle were enhanced on exposure to heat stress ($P < 0.05$). The heat-stressed chicks that received the combined supplementary level of vitamin E and Se had the lowest concentration of MDA and the highest activity of SOD in the skeletal muscle ($P < 0.05$). Dietary Se also caused a significant increase in enzyme activity of GPx in the skeletal muscle ($P < 0.05$). The results indicated that the derangement of blood parameters and oxidative stability in broilers under heat stress were improved by supplemental vitamin E and Se.

Habibian *et al.* (2013) assessed the effects of dietary vitamin E (0, 125 and 250 mg/Kg), selenium (Se, 0, 0.5 and 1 mg/Kg), or their different combinations on immune response and blood biological parameters of 360 broiler chickens raised under either thermo-neutral (23.9 °C) or heat stress (23.9 to 37 °C) conditions. Heat stress also resulted in a significant increase in H/L ratio ($P < 0.05$). The serum concentrations of glucose, triglycerides, total cholesterol, and LDL-cholesterol were increased but serum HDL-cholesterol decreased in HS broilers ($P < 0.05$).

Habibi *et al.* (2014) performed an experiment to evaluate the impact of ginger (*Zingiber officinale*) feed supplementation on growth performance, antioxidant status, carcass characteristics and blood parameters in broiler chicks under conditions of heat stress ($32 \pm 2^\circ\text{C}$ for 8 h per d). A total of 336 d-old male broiler chicks (Cobb-500) were randomly assigned to one of 6 dietary groups representing: basal diet with no supplement as control, basal diet containing 100 mg/Kg vitamin E as positive control, basal diets containing either 7.5 or 15 g/Kg of ginger root powder, and diets containing 75 or 150 mg/Kg of ginger essential oil. The results indicated that at 22 d of age, the group receiving 7.5 g/Kg of ginger root powder experienced significantly increased body weight (BW) and body weight gain (BWG) compared to the control group. There were no significant difference among the diet groups regarding BW, BWG, feed intake (FI) or feed conversion ratio

(FCR) at 42 and 49 d of age. The inclusion of powder and essential oil of ginger in broiler diets did not affect carcass characteristics and blood parameters of the chickens. However, in the group receiving 150 mg/Kg ginger essential oil, the total superoxide dismutase (TSOD) activity in liver increased compared to the control group. Malondialdehyde (MDA) concentrations in liver also decreased in the groups receiving ginger powder and essential oil compared to that in the control group. There were no significant difference between experimental groups regarding glutathione peroxidase (Gpx), TSOD and catalase (CAT) enzymes in red blood cells. All dietary groups increased total antioxidant capacity (TAC) and decreased MDA concentration in serum compared to the control group. The results suggested that ginger powder and essential oils might be a suitable replacement for synthetic antioxidants in broiler diets. Results also suggested that ginger powder might be better than extracted essential oil for improving antioxidant status in broilers.

Perai *et al.* (2014) evaluated the effects of supplemental vitamin C and chromium on metabolic and hormonal responses and antioxidant status. Three hundred female broilers were assigned to five groups with six replicates and were fed with either a basal diet (two control groups) or the basal diet supplemented with 800-mg vitamin C/Kg (Vit C group), 1,200- μ g Cr +3 from chromium (Cr) chloride/Kg (Cr group) or 800-mg Vit C and 1,200- μ gCr+3 from Cr chloride/Kg (VitC+Cr group) from 42 to 49 days of age. Transport decreased insulin level in the control and Cr groups and increased glucose/insulin (G/I) ratio in the groups. The level of insulin was higher in the VitC+Cr group than those in the control and Cr groups after the transport. The G/I ratio was lowest in the VitC+Cr group after the transport. Alkaline phosphatase activity was decreased in the Vit C+Cr group due to transport. Transport increased malondialdehyde concentration in the control and Vit C groups and also increased glutathione peroxidase (GPx) activity in the Cr-fed groups. The GPx activity was higher in the Vit. C+Cr group than those in the control and Cr supplemented groups after the transport. Ferric reducing/antioxidant power (FRAP) value was decreased in the Vit C and Cr

groups by transport. Either alone or in combination, Cr increased the FRAP value before the transport.

Tawfeek *et al.* (2014) examined the effects of dietary supplementation with some antioxidants (vitamins & minerals) against the deleterious impacts of heat stress on broilers. One hundred and twenty eight broiler chicks were randomly assigned into 4 dietary groups with 4 replicates (8 chicks each). The chicks were raised for 6 weeks under natural summer months (36 °C and 75% RH) during which the birds received either a basal diet only (control group), or the basal diet supplemented with either 250 mg vitamin C+ 250 mg vitamin E/ Kg diet, or organic microminerals: 40 mg Zn + 0.30 mg Se/ Kg diet, or with 0.50 mg Cr/Kg diet. Another group of birds which consists of 32 broiler chicks (4 replicates, 8 birds each) was reared under natural autumn conditions (25 C° and 67% RH) and fed on the same control diet. The heat stress condition significantly increased serum cholesterol, glucose, and malondialdehyde (MDA) and decreased protein and glutathione peroxidase. Even though, the used supplements improved the blood profile parameters and the oxidative status of birds.

Habibian *et al.* (2015) conducted the experiment on 360 broiler chickens to evaluate effects of dietary vitamin E (0, 125, and 250 mg/Kg), selenium (0, 0.5, and 1 mg/Kg), or their different combinations on performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers raised under either a thermo-neutral (TN, 24 °C constant) or heat stress (HS, 24 to 37 °C cycling) condition. The malondialdehyde (MDA) content of the breast meat was increased ($P < 0.05$), but its selenium content was decreased ($P < 0.05$) by exposure to HS. The breast meat MDA content was not affected ($P > 0.05$) by dietary treatments under TN condition. However, the breast meat MDA content was decreased ($P < 0.05$) by both vitamin E and selenium supplementation under HS condition, and the lowest MDA content was observed in the breast meat of broilers receiving combination of 125 mg/Kg vitamin E and 1 mg/Kg selenium. The results

showed that supplementation of selenium and vitamin E improves the lipid oxidation of the breast meat when broilers reared under HS condition.

Leskovec *et al.* (2018) performed an experiment investigating the effects of supplementation with α -tocopherol, ascorbic acid, selenium, or their combination in linseed oil-enriched diets on the oxidative status in broilers. A trial was conducted to investigate whether, and if so to what extent, the combined supplementation of vitamin E, vitamin C, and selenium was superior to their sole supplementation concerning the oxidative stress induced by a high n-3 dietary polyunsaturated fatty acids (PUFA) intake in broilers. Four hundred 21-day-old Ross 308 male broilers were allocated to 5 experimental groups fed the following linseed oil (5%)-enriched finisher diets: Cont (no supplement), +E (200 IU vitamin E/Kg feed), +C (250 mg vitamin C/Kg feed), +Se (0.2 mg selenium/Kg feed), or +ECSe (concentrations as in the sole supplementation, combined). Analyses of malondialdehyde (MDA), vitamin C, and α - and γ -tocopherols in plasma, antioxidant capacity of water- (ACW) and lipid- (ACL) soluble compounds in serum and glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities in whole blood were performed. In breast muscle, analyses of MDA, α - and γ -tocopherols, ACW, selenium, and fatty acid (FA) composition were carried out. Birds fed the combination of antioxidants showed reduced average daily gain (ADG) and average daily feed intake (ADFI) and, as +E, lower MDA and γ -tocopherol, together with raised α -tocopherol levels in plasma and lower MDA and raised α -tocopherol levels in breast muscle compared to the control. The combination of antioxidants in the +ECSe group raised GPx activity in whole blood compared to the control. In conclusion, results indicated that vitamin E was the most effective antioxidant to alleviate oxidative stress caused by high dietary PUFA and that the supplementation with additional vitamin C and selenium did not have clear synergistic effect.

Begum *et al.* (2019a) conducted the experiment to investigate the effect of dietary supplementation of amla powder on biochemical profile characteristics of commercial broiler chickens. A total of 180 broiler chicks were randomly divided into four groups consisting of 45 numbers, sub divided

into 3 replicates consisting of 15 chicks. The four groups T₀, T₁, T₂ and T₃ were offered basal diet with supplementation of amla powder at the rate of 0.00, 0.25, 0.50 and 0.75% in the feed (on dry matter basis), respectively. The result showed that total serum cholesterol and triglycerides, except total serum protein, AST and ALT differed significantly (P<0.05; 0.01) among the experimental groups. The antioxidant biomarkers viz. Superoxide Dismutase (SOD) and Glutathione peroxidase (GSH-Px) differed significantly (P<0.05; 0.01) among the experimental groups. It was concluded that supplementation of feeding the birds with Amla powder improved the antioxidant status and decreased the lipids profile such as total serum cholesterol and triglycerides.

2.6 Immune response

Bashir *et al.* (1998) carried out experiment to study the immune-regulatory effects of water soluble Vitamins B₂, B₆, B₁₂ and Vitamin C and Cyclophosphamide (known immunosuppressant). The birds which received ration with all vitamins had higher mean body and lymphoid organ weights. The bird receiving cyclophosphamide and ration without vitamins showed poor results. Moreover, cyclophosphamide treatment resulted in bursal atrophy. The highest-hemagglutination-inhibition (HI) titre was recorded in the vitamin C treated vaccinated birds. The vitamin C treated birds (vaccinated/unvaccinated) also had significantly higher mean body and lymphoid organ weights than the birds received vitamins B₂, B₆ and B₁₂ separately or ration without vitamins and those given cyclophosphamide.

Swain *et al.* (2000) examined the effect of dietary vitamin E, selenium (Se) and their different combination on antibody production was determined in broilers. Chicks were fed on maize-soyabean based diets with concentrations of supplemental vitamin E varying from 0 to 300 IU/Kg and selenium concentrations varying from 0 to 1mg/Kg either alone or in combination from 1 to 42 d of age. The chicks were immunized for Newcastle Disease Virus (NDV) vaccine at 21d. Antibodies to NDV in serum were determined at 10 and 21 d post immunization (PI). Chicks receiving Se (1 mg/Kg) and vitamin E (300 IU/Kg) had significantly higher cellular immune

responses in terms of percent LMI. Significantly higher antibody titres (HI and ELISA) at 10d PI were attributed to 0.06 mg/Kg and 150 IU/Kg Se and vitamin E, respectively. These data suggested that optimum growth and immune response might be achieved at supplemental level of Se of 0.06mg/Kg and vitamin E at 150IU/Kg. The vitamin E level was higher than that recommended by NRC (1984, 1994).

Singh *et al.* (2006) investigated the effects of selenium and vitamin E supplementation on some immune parameters in broilers. Broiler chicks were fed on maize-soybean diets with different concentrations of vitamin E (0-200mg/Kg) and selenium (0-0.2mg/Kg diet) either alone or in combinations from 1 to 42d of age. Chicks were immunised against Newcastle disease virus (NDV) vaccine at 21d of age and haemagglutination inhibition (HI) titres were determined after 10d. Chicks receiving supplements of 200mg vitamin E/Kg and 0.2 mg selenium/Kg produced significantly higher HI antibody titres. The chicks given 200mg vitamin E/Kg and 0.2mg selenium/Kg had significantly heavier spleen and bursa. The results suggested that vitamin E and selenium have synergistic effects on immune responses.

Habibian *et al.* (2013) conducted study to evaluate the effects of dietary vitamin E (0, 125 and 250 mg/Kg), selenium (Se, 0, 0.5 and 1 mg/Kg), or their different combinations on immune response and blood biological parameters of broilers raised under either thermoneutral (23.9 °C) or heat stress (23.9 to 37 °C) conditions. Humoral immunity was assessed by intravenous injection of 7 % sheep red blood cell (SRBC) followed by evaluation of serum for antibody titers in primary and secondary responses. The lymphoid organ weights as well as IgM and IgG, antibody titers for primary and secondary antibody responses to SRBC were reduced significantly under HS ($P<0.05$). The HS birds also showed an improved antibody titer in secondary response with high concentration of Se ($P<0.05$). Vitamin E and Se had interactive effects on anti-SRBC titers.

Dhore *et al.* (2015) conducted a study to assess the effect of herbal and synthetic vitamin C supplementation on performance of broilers under

intense summer condition. The immunological studies revealed significantly higher HI titre in vitamin C supplemented groups. Histopathologically lymphoid cell population was more in herbal vitamin C group indicating positive immune response as compared to synthetic group. It was concluded that the feeding of synthetic and herbal vitamin C has beneficial effects as it improves immune status of broilers.

2.7 Economics of broiler production

Kennedy *et al.* (1992) investigated that the economic effects of increased vitamin E supplementation in 168 commercial broiler flocks incorporating over 3 million birds was assessed using a computerized data retrieval system. Approximately half of the flocks were fed on either a high (163 mg/Kg) vitamin E or on a normal (44 mg/Kg) vitamin E-containing diet throughout production. There was increase in net income which was achieved by a significantly improved food conversion ratio and average weight per bird ($P < 0.05$). The residual improvement in target income in these flocks, after the cost of the additional vitamin E was taken into account, was 2.74% and failed to reach statistical significance.

Deniz *et al.* (2005) supplemented broiler diets with organic Se improved feed conversion ratio and reduced whole carcass drip-loss. A total of 273 one-day-old male broiler chicks (Avian Farms) were randomly divided into 3 groups of 91 birds each according to the dietary Se supplementation. The first group received no supplemental Se into starter (from the 1st to the 21st days), grower (from the 22nd to the 35th days) and withdrawal (from the 36th to the 42nd days) diets. The second and third groups were fed with the different dietary regimes supplemented with 0.3 ppm mineral Se (Sodium selenite) or organic Se (Se enriched yeast) respectively. Growth performance (body weight, body weight gain, feed intake and feed conversion ratio) were recorded on the 21st and the 42nd days and carcass traits (chilled carcass weight and chilled carcass yield, whole carcass drip-loss) were measured after slaughtering on the 42nd day. Whatever the origin of Se supplementation, no significant effect on body weight, body weight gain and feed intake was

observed on days 21 and 42, and chilled carcass weights and their yield were also unaffected at the end of experiment. In contrast, total feed conversion ratio (from 1st to 42nd days) was significantly improved and whole carcass drip-loss was significantly reduced in birds receiving 0.3 ppm organic Se in comparison to the other groups (P<0.01). These results indicated that supplementation of broiler diets with organic Se improved feed conversion ratio and decreased drip-loss, leading to increase meat quality and economical gain.

Rajput *et al.* (2009) studied the effect of vitamin A, vitamin C, vitamin E and levamisole on performance of broilers. One hundred and eighty (180) unsexed day-old chicks were weighed individually and uniformly distributed as 30 chicks in each of six groups. Each group was divided into two replicates with 15 chicks in each. The birds were offered feed and water *ad-libitum*. The chicks were fed with starter mash which contained crude protein 22% and metabolizable energy 2918 Kcal / Kg up to three weeks of age. For next 3 weeks i.e. from 4 to 6 weeks of age with finisher mash which contained crude protein 20% and metabolizable energy 2966 Kcal/ Kg. Group T₀ (control group) was fed standard broiler mash. In group T₁ Vitamin A was added @ 8500 IU/ litre of Drinking water. Group T₂ was fed Broiler mash + Vitamin C @ 500 mg/ Kg of feed. Group T₃ was fed Broiler mash + Vitamin E @ 300 mg/ Kg of feed. Group T₄ was given Broiler mash + Levamisole @ 2 gm/ Kg of feed and group. T₅ was provided with the combination of all the feed supplements Vitamin A added @ 8500 IU/ litre of Drinking water, Broiler mash + Vitamin C @ 500 mg/ Kg of feed + Vitamin E @ 300 mg/ Kg of feed + Levamisole @ 2 gm /Kg of feed. The results of economics of broiler production showed that net cost of production per bird was maximum for T₅ (Rs.76.78) followed by T₄ (Rs. 67.34), T₁ (Rs. 66.56), T₀ (Rs. 59.11), T₂ (Rs. 58.16) and T₃ (Rs. 58.02). The net profit per bird for groups T₀, T₁, T₂, T₃, T₄ and T₅ was Rs. 28.44, 21.49, 30.59, 30.48, 20.41 and 11.12, respectively. The supplemented groups T₂ and T₃ had more net profit than control group. However, group T₁, T₄ and T₅ had less net profit than the control group.

Kale *et al.*(2014) conducted an experiment on two hundred forty commercial broiler chicks (Ven-Cobb strain) divided into four groups, with three replicates of 20 chicks each to study the performance of broilers supplemented with *Emblica officinalis* (amla) and vitamin E under nutritional stress during 0–6 weeks of age. Four experimental diets were prepared, viz. standard control broiler ration as per BIS standards (T₀), low energy (ME 10% lower than T₀) broiler ration without supplement (T₁), low energy (ME 10% lower than T₀) broiler ration with 250 g of *Emblica officinalis* /ton of feed (T₂) and low energy (ME 10% lower than T₀) broiler ration with 250 g of vitamin E/ton of feed (T₃). Feed intake, and feed conversion ratio was similar in all the treatment groups up to 6th week of age, however, average body weights of the birds were higher (P<0.05) in group T₀ and T₃ compared to other two groups showing positive effect of vitamin E supplementation.

Gaikwad *et al.* (2014) investigated that the effect of feeding Shatavari Root Powder (SRP) and Amla fruit Powder (AM) on broiler performance. Two hundred and fifty day old straight run commercial broilers chicks were randomly distributed into five treatment groups with four replicates and 12-13 chicks in each replicate. The dietary treatments consisted of control without SRP or AM (T₁), 0.50% SRP (T₂), 1.00% SRP (T₃), 0.50% AM (T₄) and 1.00% AM (T₅). At six weeks of age, the average weekly gain was 467.53, 528.79, 513.88, 466.3 and 502.15g in T₁ to T₅ treatment groups, respectively. The cumulative feed consumption at sixth week of age was 3965.30, 3877.91, 3794.77, 3891.22 and 3832.35g, respectively, in T₁-T₅ treatments groups. The cumulative feed conversion efficiency of various groups T₁ to T₅ was 1.85, 1.75, 1.70, 1.76 and 1.71, respectively. The average dressing percentage among the different treatment groups varied from 68.48 to 65.61percent. Numerically higher dressing percentage was recorded in treatment T₃ (68.48). The addition of Shatavari root powder and Amla fruit powder at 1% level found to reduce the feed cost in broilers. The results showed that the use of Shatavari root powder and Amla fruit powder were found to be beneficial. The overall performance and economical benefits were better at 1.00% level of inclusion of these herbs compared to 0.50% level.

Patel *et al.* (2016) conducted a study to evaluate the dietary addition of *Emblica officinalis* (Amla) fruit powder as a growth promoter in commercial broiler chickens. An experiment was conducted on 135 commercial broiler chicks (Ven-Cobb 400 strain) divided into three groups with three replicates of 15 chicks each. Three treatment groups were as follows – T₁: Basal diet as per BIS standards; T₂: Basal diet supplemented with 0.4% of *E. officinalis* fruit powder; and T₃: Basal diet supplemented with 0.8% of *E. officinalis* fruit powder. The average body weights at the end of the 6th week were significantly higher ($P < 0.05$) in groups T₂ and T₃ compared to group T₁. Feed intake, feed conversion ratio and feed cost per Kg live weight production were similar among the treatment groups. The net profit per bird was the highest in group T₂ (Rs. 19.22/bird) followed by group T₃ (Rs. 17.86/bird) and the lowest in group T₁ (Rs. 14.61/bird). It was concluded that dietary addition of *E. officinalis* (Amla) fruit powder had a positive effect on growth performance and net profit per bird in commercial broiler chickens.

Begum *et al.* (2019b) studied effect of dietary supplementation of Gooseberry/ Amla (*Emblica officinalis*) powder on the Performance of Commercial Broiler Chicken. A total of 180 broiler chicks were randomly divided into four groups consisting of 45 numbers, sub divided into 3 replicates consisting of 15 chicks in each sub group. The chicks under T₀, T₁, T₂ and T₃ groups were fed Amla powder at the rate of 0.00, 0.25, 0.50 and 0.75% in the feed, respectively. The results of the study indicated that inclusion of Amla powder as feed supplement was found to be useful in respect of feed intake, average weekly body weight gain and feed conversion ratio as well as also rewards higher gross profit in commercial broilers production. The cost of production per broiler was highest in T₃ (INR 201.32) and lowest in T₀ (INR 197.99) groups. Similarly, gross profit was found to be highest in T₃ group (INR 17.18) and lowest in T₀ (INR 13.06) groups. From the results obtained it was concluded that Amla powder could be used effectively and economically as natural feed additive at the rate of 0.75% to improve the overall performance of the broiler chicken.

CHAPTER III

MATERIALS AND METHODS

An experiment entitled “Effect of dietary antioxidants (Vitamin C, Vitamin E and Selenium) supplementation on performance and immune response in broilers” was conducted at Poultry Research Center, Post Graduate Institute of Veterinary and Animal Sciences, Akola (MAFSU, Nagpur). The response parameters studied were growth performance in terms of feed intake, body weight changes, feed conversion ratio, nutrient metabolisability, haematological parameters (Hb, PCV, DLC, TLC and H:L ratio), blood biochemical parameters (Serum total protein, albumin, globulin, A:G ratio, uric acid and glucose), erythrocytic antioxidant enzymes status (LPO, SOD and Catalase), carcass characteristics (dressing percentage, edible meat percentage, sensory evaluation, shelf life of meat, Immune response (weight of immune organs and serum antibody titre against RD virus) and economics of broiler production. The details of different methodologies and materials used during present research work are expounded in this chapter.

3.1 Location and Period of Experiment:

The experiment was conducted at Poultry Research Centre, Post Graduate Institute of Veterinary and Animal sciences, Akola during the month October and November, 2021. The present study was conducted at Latitude: 20°70' N. Longitude: 77°02' E, Altitude: 286 m, under climatic temperature of 18°C to 37°C with average relative humidity of 55%.

3.2 Procurement of Feed Ingredients

The different components of diet i.e. maize, deoiled soybean cake, di-calcium phosphate, limestone powder, toxin binder, coccidiostat, choline chloride, trace mineral mix, vitamin premix, synthetic lysine, methionine, salt and crude soy oil were acquired from local market. Whereas, the herbal Vitamin C, Vitamin E and Selenium required for the experiment was provided by M/S Hester Biosciences, Ltd. Ahmedabad. The component of

Herbal Vitamin C were Amalki, *Citrus aurantium* (orange pulp), *Citrus limon* (lemon pulp) and Tamarind where as Vitamin E and selenium were combined with biotin composition of herbal Vitamin C is given in table 3.1 and Vitamin E and selenium contains Vitamin E of 20 gm, selenium 200 mg, and Biotin 40 mg. The analysis of various response parameters was carried out at Department of Animal Nutrition, hematological parameters were analyzed at Department of Pathology and biochemical analyses were performed at Department of Biochemistry, Post Graduate Institute of Veterinary and Animal Sciences, Akola. Rations were formulated following the BIS (2007) standards for broilers.

Table 3.1 Composition of herbal Vitamin C

Herbs	Each 100 gm	Vitamin C content (mg)
Amalki	23	138
<i>Citrus aurantium</i>	16	6.8
<i>Citrus limon</i>	14	5.39
Tamarind	16	0.58
Total	69	150.77

3.3 Experimental birds

The research was carried out for a period of 42 days (6 weeks) from 5th October and 17th November, 2021. 240 day old straight run commercial broiler chicks (Vencobb-430) procured from local hatchery were randomly distributed on equal average body weight basis into T₀ (Control) and 3 treatments: T₁, T₂ and T₃ with 60 birds in each group having 4 replicates of 15 chicks in each. Details of the dietary treatments were as follows:



Plate 3.1. Herbal Vitamin C



Plate 3.2. Vitamin E and Selenium



Plate 3.3. Shed prepared before arrival of chicks



Plate 3.4. Lighting arrangement to induce heat stress

Table 3.2 Details of the dietary treatments

Sr. No.	Treatment group	No. of birds/ pen/ replication	No. of replication	Total no. of birds
1.	(T ₀) - Standard broiler diet as per BIS (2007) without enzyme	15	4	60
2.	(T ₁) - Standard broiler diet with vitamin C @ 100g /ton	15	4	60
3.	(T ₂) - Standard broiler diet with vitamin E & Se @ 200g/ton	15	4	60
4.	(T ₃) - Standard broiler diet with combination of vitamin C @ 100g /ton and vitamin E & Se @ 200g/ton	15	4	60
Total				240

3.4 Housing and Management

Before arrival of the chicks, feeders, drinkers, separators, all the control and test pens, brooders and floor were cleaned, washed, sanitized and disinfected with the aid of formaldehyde (35ml of formalin) and potassium permanganate (10g) per cubic metre of space (40% formaldehyde to potassium per- manganate (KMnO₄) in 2:1 ratio (v/w)). Poultry shed was also fumigated and closed for 24 hours. After arrival, the day old chicks were promptly weighed and distributed randomly into 4 treatments in 4 replicates of 15 chicks in each group; which were housed in individual pens for each replicate in two opposite rows. The experimental chicks were housed in separate pens and 1 sq.ft. floor space was provided to each chick. On the first day, all experimental chicks were provided with jaggery water as an instant source of energy to overcome transportation stress. Experimental birds were raised on deep litter system which was comprised of saw dust as a litter material. *Ad-libitum* clean and fresh drinking water was given to the broiler birds. For the initial two days ground maize was made available to chicks on papers. Thereafter, measured amount of feed was offered to all the treatment

groups and the residual feed was collected and weighed on the next day at 8.00 a.m. per replicate individually throughout the experimental period. Waterers and feeders were washed daily on regular basis.

The brooding temperature was maintained by using electric bulbs. Adequate temperature was provided by adjusting number and height of electric bulbs as per atmospheric temperature and distribution of birds in the compartment. Artificial temperature stress was induced in all pens by maintaining the shed temperature between 35 to 40°C (dry bulb temperature 95-104°F) and with ambient relative humidity of about 60% after completion of brooding till the end of trial using lighting arrangements. Vaccination schedule was carried out as follows:

Table 3.3 Vaccination schedule

Sr. No.	Disease	Vaccine	Age of vaccination (days)	Dose/route
1.	Ranikhet	Lasota vaccine (F ₁ Strain)	7	One drop in eye (intraocular)
2.	Gumboro (IBD)	Gumboro vaccine	14	One drop in eye (intraocular)
3.	Gumboro (IBD) Booster Dose	Gumboro vaccine	21	Through drinking water
4.	Ranikhet Booster Dose	Lasota vaccine	28	Through drinking water

3.5 Experimental Diets and Feeding Regime

The feeding of experimental broiler birds was done in three phases as per BIS (2007) i.e. pre-starter (0-9 days), starter (10-21 days) and finisher (22-42 days) for control and all the treatment groups. As per the design of experiment, the experimental rations for pre-starter, starter and finisher were formulated as given below.

Diets were computed as per BIS (2007) standards and inclusion of Vitamin C, Vitamin E and Selenium with different proportions (@100g

/ton, 200g/ton and combination of both) to the T₁, T₂ and T₃ treatment groups respectively over and above the ration at the time of feed preparation in the premix.

Table 3.4 Per cent ingredient composition of experimental rations during various phases

Sr. No.	Ingredients	Pre-Starter	Starter	Finisher
1	Maize	55.21	56.26	60.1
2	Deoiled soybean cake	39.2	36.82	31.8
3	Oil	1.6	3	4.2
5	Limestone powder	1.1	1.05	1.1
6	Dicalcium phosphate	2.1	2.1	2.1
7	Salt	0.3	0.3	0.3
8	DL-Methionine	0.17	0.18	0.15
9	L-Lysine	0.07	0.04	0
10	Trace minerals Premix	0.05	0.05	0.05
11	Vitamin Premix	0.05	0.05	0.05
12	Choline Chloride	0.05	0.05	0.05
13	Toxin binder	0.05	0.05	0.05
14	Coccidiostat	0.05	0.05	0.05
	Total	100	100	100
	Calculated nutrients in diet (%)			
	CP	23	22	20
	ME (Kcal/Kg)	3004	3100	3203
	Calcium	1.02	1	1
	Available P	0.456	0.45	0.45
	Lysine	1.30	1.21	1.03
	Methionine	0.50	0.50	0.45

3.6 Growth Performance

The following observations in terms of performance were recorded during the entire experimental period.

3.6.1 Weekly Feed Intake

Records of feed offered and residue left over were maintained daily. Feed consumption of each pen was recorded daily and average feed intake g/chick/week was calculated by dividing the total amount of feed consumed by the number of chicks in the particular pen per week. Cumulative feed consumption of particular week was calculated by adding up the average feed consumption of the previous week with the feed consumption of current week.

3.6.2 Weekly Body Weight Gain

The live body weights (g) of all birds were recorded individually, accurately and replicate wise using electronic weighing balance at weekly interval in morning hours before feeding. The average weekly body weight gain (g) was calculated from the difference in body weight attained at the end and the start of the concerned period for each replicate of group.

3.6.3 Weekly Feed Conversion Ratio (FCR)

Weekly feed conversion ratio was calculated by dividing the weekly feed consumption by weekly weight gain. The weekly cumulative feed conversion ratio was calculated by dividing total amount of feed consumed (g) up to that particular period by the body weight gain (g) recorded up to that period. Feed conversion ratio of birds for different weeks of experiment was calculated as follows:

$$\text{FCR} = \frac{\text{Feed consumption (g/chick) in week}}{\text{Weight gain (g/chick) in week}}$$



Plate 3.4. Weighing of chick (0th day) and bird (42nd day)



Plate 3.5. Feeding and watering of birds in individual pen

3.6.4 Nutrient Metabolisability

To determine the nutrient utilization, a metabolic trial of 7 days of duration was carried out at the end of experiment i.e. after 42 days. Broiler birds were shifted to cages, 3 days before actual start of trial period for acquaintance to new ambience. Daily feed offered, residues left over and faeces voided during this period were recorded daily at 8.00 a.m in order to determine the average feed intake g/bird/day. Representative samples of feed offered, residues left and faeces voided were brought to the laboratory for further aliquoting and analyses. All birds were individually weighed before and after the metabolic trial.

3.6.5 Proximate Analysis

Proximate analysis of ground feed and faeces of various groups including control was carried out as per the methods of AOAC (2000).

a) Dry Matter (DM)

A known weight of ground sample was dried out at $100\pm 5^{\circ}$ C in a hot air oven in a moisture cup overnight to a constant weight to determine the dry matter after deducting the lost weight from the weight of sample taken.

$$\text{DM (\%)} = 100 - \left(\frac{\text{Loss in sample weight}}{\text{Weight of sample before drying}} \times 100 \right)$$

b) Crude Protein (CP)

The nitrogen was determined by digesting a known quantity of ground sample in a presence of digestion mixture (copper sulphate and anhydrous sodium sulphate in the ratio of 1:10) and commercial sulphuric acid. The digested sample was made up to known volume and a suitable aliquot was distilled in the presence of excess of 40% sodium hydroxide in micro Kjeldahl's distillation apparatus and distillate was collected in 2% boric acid (w/v) containing Tashiro's indicator (10 ml of each 0.1% methyl red and 0.1% bromocresol green to 1000 ml of 2% boric acid solution); which was further titrated against standard N/10 HCl (1 ml of N/10 HCl = 3650 mg N)

and simultaneously a blank without sample in equal quantity of commercial HCl was also run to arrive at the nitrogen content.

$$\text{Nitrogen (\%)} = \frac{0.0014 \times \text{Titre volume} \times \text{Normality} \times \text{volume made}}{\text{Aliquot taken} \times \text{sample taken}} \times 100$$

The percent crude protein in feed was calculated by multiplying nitrogen percent with the factor 6.25.

c) Ether Extract

A known amount of moisture free sample was extracted with petroleum ether (BP 60-80°C) for 8 hours in a pre-weighed flask using Soxhlet extraction apparatus. The weight of extract was determined after oven drying it (100±5°C) to a constant weight and was expressed as percent ether extract (EE) in the sample on dry matter basis (DMB).

$$\text{Ether Extract (\%)} = \frac{\text{Weight of ether extractives}}{\text{Weight of sample}} \times 100$$

d) Total Ash (TA)

A known quantity of sample was carbonized and ignited in the muffle furnace at 550°C for 3 hours to arrive at the total ash (TA) content.

$$\text{Total Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

e) Crude Fibre

Moisture and fat free samples of feed were boiled with 1.25% sulphuric acid solution for 30 minutes and washed with hot distilled water to make it acid free. This was followed by boiling the residue with 1.25% sodium hydroxide solution for 30 minutes and washed with hot distilled water. Residue of sample with crucible was dried, weighed and then ignited in muffle furnace to obtain its ash content. Crude fibre was calculated by deducting ash from the weight of dried residue

$$\text{Crude fibre (\%)} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

g) Nitrogen Free Extract (NFE)

NFE was calculated by using following formula

$$\text{NFE (\%)} = 100 - (\text{CP\%} + \text{CF\%} + \text{EE\%} \times 2.25 + \text{TA \%})$$

h) Organic Matter (OM)

OM was calculated by using following formula

$$\text{OM (\%)} = 100 - \text{TA (\%)}$$

3.7 Carcass Characteristics

3.7.1 Sacrifice of Bird

On day 42 of experiment, one birds from each replicate were chosen according to the body weight near the mean for sacrifice in a humane way. Birds were starved for 12 hrs and *ad-lib* drinking water was made available. After 12 to 16 hrs birds were sacrificed by severing the jugular vein and carotid artery and permitted to bleed for 1 to 2 min. Defeathering was done by immersing bird in warm water for 2-3 minutes in the scald tank and feathers were expelled physically. The temperature of water was kept 50-51⁰C to prevent discoloration and drying. To calculate the carcass yield, it was considered the eviscerated carcass weight, without feet, head and abdominal fat, in relation to the live weight (Fernandes *et al.*, 2013). Different carcass traits, eviscerated yield, edible meat yield, and giblet weight were recorded and expressed on percent basis in relation to live weight. Experimental protocol was approved by the Institutional Animal Ethics Committee, vide meeting conducted at Post Graduate Institute of Veterinary and Animal Science, Akola on 06.08.2021.

3.7.2 Dressing Percentage

The dressing percentage was calculated as follows:

$$\text{Dressing (\%)} = \frac{\text{Dressed weight}}{\text{Live BW before slaughter}} \times 100$$

3.7.3 Edible Meat Percentage

The edible meat percentage was calculated using following formula:

$$\text{Edible meat (\%)} = \frac{\text{Edible meat weight}}{\text{Live BW before slaughter}} \times 100$$

3.7.4 Giblet Weight and Percentage

The combined weight of liver, gizzard and heart (i.e. giblet) was taken together and recorded on digital weighing balance and was expressed on percent live weight basis.

Giblet meat percentage was calculated as follows:

$$\text{Giblet meat (\%)} = \frac{\text{Giblet weight}}{\text{Live BW before slaughter}} \times 100$$

3.7.5 Sensory Evaluation

The sensory test was conducted for the evaluation of the broiler meat as influenced by the feeding of antioxidants to the broiler chickens. For this purpose, two bird from each dietary treatment was randomly selected and sacrificed as per the conventional standard slaughter technique. The ready-to cook meat pieces of breast and right thigh region of sacrificed bird under different dietary treatments were cooked simultaneously under pressure (1Kg/cm³ for 10-15 minutes), cooled to ambient temperature prior to subjecting the cooked meat for sensory evaluation. 7 point hedonic (degree of liking) test is used to decide the degree of consumer acceptance for a product.



Plate 3.6. Metabolic trial



Plate 3.7. Bleeding of bird using cone while humane scarifice



Plate 3.8. Dressed carcass



Plate 3.9. Giblet weight



Plate 3.10. Collection of blood from wing vein and jugular vein

It is almost considered as a consumer test as it is conducted by semi-trained panelists. The cooked meat was served for evaluation of its sensory quality by a panel of semi-trained judges using a 7-point hedonic scale on the standard proforma for sensory attributes of the meat and were asked to give comment on attributes such as appearance, tenderness, texture, juiciness, flavour and overall acceptability (Singh and Maharaj, 2014).

3.7.6 Shelf life of meat

In the present study breast and thigh meat samples of one bird per replicate of each treatment were stored at 4°C in LDPE (low density poly ethylene) bags and their oxidative stability i.e. thiobarbituric acid values was determined by modified method as described by Strange *et al.*(1977) with little modification in the technique.

1. Five ml of aliquot of TCA extract was mixed with 5ml of TBA reagent in a test tube.
2. TBA reagent was prepared according to the method of Pearson (1968), by dissolving 0.2883 g of Thiobarbituric acid in sufficient quantity of 90% acetic acid with slight warming, and the volume was made up to 100 ml with 90% acetic acid.
3. The test tubes containing samples were kept in a water bath at 100°C for 30 min along with control test tube containing a blank of 5ml of 10% TCA and 5 ml TBA reagent.
4. After cooling the tubes in running water for about 10 min the optical density was measured at 530 nm in a spectro- photometer.

Proforma for Sensory evaluation of meat

Sensory Evaluation sheet

Product : Cooked meat

Name :

Date :

Treatment	Apperance	Texture	Juiciness	Flavour	Tenderness	Overall Acceptability
1						
2						
3						
4						
5						
6						
7						
8						

NOTE: Guideline to allot marks

Apperance : Excellent(7); Very good(6); Good(5); Fair(4); Poor(3); Very poor(2); extremely poor(1).

Juiciness: Excellent(7); Very good(6); Good(5); Fair(4); Poor(3); Very poor(2); extremely poor(1).

Flavour: Excellent(7); Very good(6); Good(5); Fair(4); Poor(3); Very poor(2); extremely poor(1).

Tenderness: Excellent(7); Very good(6); Good(5); Fair(4); Poor(3); Very poor(2); extremely poor(1).

Overall acceptability: Like very much(7); Like moderately(6); Like slightly(5); Neither like nor dislike(4); Dislike Silghtly(3); Dislike moderately(2); Dislike very much(1).

Remarks, if any:

3.8 Blood Metabolite Concentration

3.8.1 Haematological Parameter

Haematological estimations were undertaken at the sixth week of age. The methodology and set of reagents used in respect of each parameter were as per the recommendation of the manufacturer of the analyzer system. The methods used for these estimations are given as follows.

a) Collection of Blood

At the end of sixth week, blood was collected randomly from one birds of each replicate. 3 ml blood was collected from wing vein and jugular vein as feasible using vacuum tubes containing potassium salts of ethylene di-amine tetra acetate (EDTA) as anticoagulant and the haematological estimations were carried out.

b) Haematological Estimation

i. Haemoglobin (g/dl)

Blood haemoglobin was determined using cyanmethaemoglobin method (Martha, 2012).

ii. Packed Cell Volume (%)

Packed cell volume was determined by Wintrobe microhaematocrit method (Martha, 2012).

iii. Total Leucocyte Count ($1 \times 10^3/\mu\text{l}$)

Total leucocyte count was estimated by using Natt and Herrick diluting fluid with improved Neubaure's chamber and method described by (Natt and Herrick, 1952).

iv. Differential Leucocyte Count (DLC)

Differential Leucocyte count (DLC) was carried from the thin blood smear prepared from fresh blood at the time of blood collection. The smear was air dried and stained by Leishman's stain as described by Benjamin

(2001). A total of 100 leucocytes were counted under oil immersion (100x) and were expressed in percentage.

v. H:L ratio

The H:L ratio was calculated by dividing the values of percent heterophils by percent lymphocytes.

3.8.2 Blood Biochemical Parameter

Blood biochemical estimations were undertaken at the end of sixth week of age. The estimations were performed on Clinical Analyzer 636 make Systronics. The methodology and the set of reagents used in respect of each parameter were as per the recommendations of the manufacturer of the analyzer system. The methods used for these estimations are given below

a) Collection of Blood

Blood from four birds of each treatment (one from each replicate) was collected towards the end of the experimental period. 3 ml blood was collected from wing vein and jugular vein as feasible in syringe without any anticoagulant and serum was separated by centrifugation at 3000 RPM for 10 minutes and kept at - 20⁰C till further investigation was done. Biochemical parameters included estimation of glucose, total protein, and albumin, utilizing biochemical kits produced by AGD Biomedicals pvt. ltd. with the assistance of AGD biochemistry auto-analyzer by following strategy.

b) Serum Total Protein

Serum total protein was estimated by AGD auto analyzer using standard kit of AGD Biochemicals (P) Ltd. Mumbai. The total protein reagent, 1ml was taken in a clean and dry mini test tube, to it, 0.01 ml of sample was added and mixed well and then incubated at room temperature for 5 minutes and read the absorbance (A) at 546 nm. The total protein was estimated as per method by Doumas *et al.* (1981).

$$\text{Total Protein (g/dL)} = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times (\text{concentration of standard g/dL})$$

c) Serum uric Acid

Serum uric acid was estimated by uricase/POD(LS) (Kaneko,1980).

d) Serum Albumin

Serum albumin was estimated by AGD auto analyzer using standard kits of AGD Biochemicals (P) Ltd. Mumbai. The albumin reagent, 1.0 ml was taken in a clean and dry mini test tube, to it, 0.01 ml of sample was added and mixed well then incubated for 5 minutes at room temperature and read the absorbance (A) at 630 nm. The serum albumin was estimated as per methods of Doumas *et al.* (1981).

$$\text{Albumin (g/dL)} = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times (\text{concentration of standard g/dL})$$

d) Serum Globulin

Serum globulin was calculated by subtracting albumin from total protein with formula given below

$$\text{Serum Globulin} = \text{Serum Total Protein} - \text{Serum Albumin}$$

e) Serum A:G ratio

The serum A:G ratio was calculated by dividing the values of serum albumin by serum globulin.

f) Blood Glucose

Blood glucose was estimated in the blood samples by GOD-POD, glucose (GOD-POD) LS kit. Glucose gets oxidized to gluconic acid and hydrogen peroxide. In the presence of enzyme peroxidase, released hydrogen peroxide is coupled with phenol and 4- aminoantipyrine (4-AAP) to form coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.

10 µl of serum was added in 1000 µl of working glucose reagent and incubated for 10 minutes at 37⁰ C. After incubation period the absorbance was measured at 505 nm under UV Spectrophotometer and the

values were expressed in mg/dl. Prior to analysis internal calibration with 1000 µl of working reagent as blank and 1000µl of working reagent + 10 µl reagent 3 standard as standard were carried out (Trinder, 1969).

g) Serum glutamate oxaloacetate transaminase (SGOT) (IU/dl)

Enzyme serum glutamate oxaloacetate transaminase level was estimated by Acta method (1976).

h) Serum glutamate pyruvate transaminase (SGPT) (IU/dl)

Enzyme serum glutamate pyruvate transaminase level was estimated by Acta method (1976).

i) Antioxidant Enzymes Status

Estimations of different oxidative stress related biochemical parameters in RBCs were carried out. The parameters were

Catalase (CAT)

Superoxide dismutase (SOD)

Lipid peroxidation (LPO)

Lipid peroxidation (LPO) in erythrocytes was measured on the day of blood collection. Absorbance of all the blood biochemical estimations were read using Systronics 118, UV-Vis spectrophotometer.

Separation of erythrocytes

The blood samples were collected from each group per replicate in heparinized anticoagulant tubes. They were centrifuged at 2000 rpm for 15 minutes. Plasma and buffy coat were removed. The resulting erythrocyte pellet was washed thrice with 0.15M NaCl solution. Dilution of the packed RBCs was made to 33% in phosphate buffer saline. PBS was prepared by dissolving NaCl (8g), KCl (0.2g), KH₂PO₄(0.2g) and N₂HPO₄(0.94g) in 800ml distilled water. The pH was adjusted to 7.4 and the volume was made to 1 liter with distilled water. The washed erythrocyte pellets were suspended in PBS; pH 7.4 and kept at 4⁰C until further analysis. These 33% packed RBCs were used for the estimation of LPO. The 1:10



Plate 3.1. Preparation of Sensory Evaluation and Shelf life

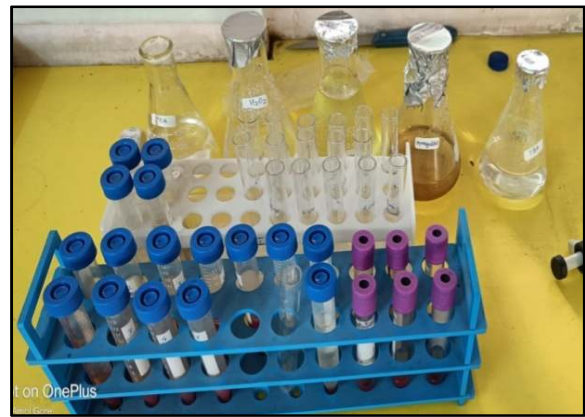
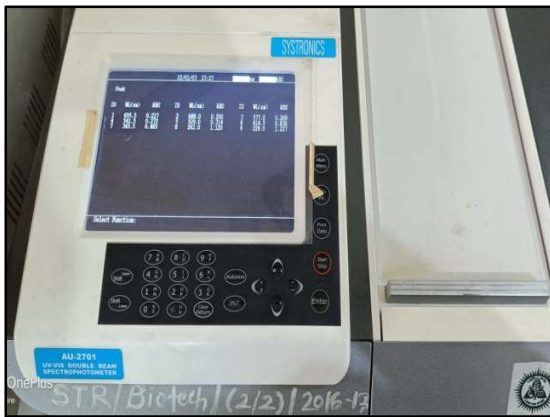


Plate 3.1. Antioxidant Estimation using Spectrophotometer

dilutions of packed RBCs (10%) in PBS were used for the estimation of CAT and SOD.

Catalase (CAT)

Catalase was assayed in erythrocytes by spectrophotometric method as described by Bergmeyer (1983). Diluted (1:10) haemolysates were used for estimation of catalase.

Reagents:

1- Phosphate buffer (50 mM, ph 7.0)

(a) 50 mM KH_2PO_4 - 1.37g/200 ml

(b) 50 mM Na_2HPO_2 - 1.42g/200ml

The solutions (a) and (b) were mixed in 1:1.5 (v/v) and the ph adjusted to 7.0

2- H_2O_2 (10mM) – 0.1 ml of 30% H_2O_2 was diluted to 100 ml in water

Procedure:

In a test tube 2 ml of phosphate buffer and 10 μl hemolysate (1:10 dilution) were added and the content was transferred to the cuvette. Adding 1ml H_2O_2 directly into the cuvette started the reaction and the optical density was recorded at every 10 seconds for 1minute at 240 nm against water blank.

Calculations:

The activity of catalase was expressed as mM of H_2O_2 utilized/min/mg of hemoglobin and calculated using the formula

$$\frac{(\Delta)\text{OD}/\text{time}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Volume of sample taken}} \times 10^9 \times \text{DF} \times \text{Time of Incubation (2hrs)}$$

OD = Mean difference between ODs at 10 seconds interval

EC = 43.6/M/cm (Shintani, 2013).

Superoxide dismutase (SOD)

SOD was estimated as per the method described by Madesh and Balasubramanian (1998). It involves generation of superoxide by

pyrogallol tetrazolium dye MTT [3-(4, 5 dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] to its formazan, measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazan formed. The color evolved is stable for many hours.

Reagents:

1. 100 µM pyrogallol- 6.3 mg of pyrogallol was dissolved in 5 ml of distilled water. One ml from this solution was added to 100 ml of distilled water.
2. 1.25mM MTT- 2.58 mg MTT was dissolved in 5 ml of distilled water.
3. Phosphate buffer saline (PBS), prepared as described for separation of erythrocytes

Procedure:

The reagents were added in the sample, control and the blank as shown below. The absorbance was read at 570 nm against distilled water.

REAGENTS	CONTROL	BLANK TEST	SAMPLE
PBS	0.65ml	0.65ml	0.65ml
MTT	30µl	30µl	30µl
Haemolysate	10µl	---	---
Pyrogallol	75µl	75µl	75µl
DMSO	0.75ml	0.75ml	0.75ml
Haemolysate	---	10µl	---

After addition of pyrogallol and before adding DMSO each sample was incubated for 5 minutes at room temperature.

Calculation:

Enzyme activity has been expressed as SOD units / mg of Hb [one unit of SOD is the amount (µg) of haemoglobin required to inhibit the MTT reduction by 50%].

$$Y\% = \frac{\text{OD of Control} - \text{OD of Test}}{\text{OD of Control}} \times 100$$

$$\text{SOD(U)} = \frac{\text{Y}\%}{50} \times \frac{1}{\text{mg of Hb}} \times \text{DF}$$

Y% = % inhibition of MTT reduction by SOD protein

DF = dilution factor

Lipid peroxidation (LPO)

Membrane peroxidative damage in erythrocytes was measured in terms of malondialdehyde (MDA) production by the method of Rehman (1984).

Reagents:

- 1- Trichloroacetic acid (TCA) 10% solution: 10g of TCA was dissolved in distilled water and the volume was made up to 100 ml with distilled water.
- 2- Thiobarbituric acid (TBA) 0.67% solution: 0.67g of TBA was added to 100 ml distilled water and warmed up for dissolving TBA.

Procedure:

One ml of packed erythrocytes was incubated at $37 \pm 0.5^\circ\text{C}$ for 2 hours. To each sample 1ml of 10% w/v TCA was added. After thorough mixing, the reaction mixture was centrifuged at 2000 rpm for 10 min. To 1 ml of supernatant 1ml of 0.67% w/v of TBA was added and kept in boiling water bath for 10 minutes, cooled and diluted with 1ml of distilled water. Blank was made by adding all the reagents except the packed RBCs. The absorbance was read at 535 nm.

Calculation:

Calculation was done using the molar extinction coefficient (EC) of MDA-TBA complex at 535nm, i.e. $1.56 \times 10^5/\text{M}/\text{cm}$ (Rehman, 1984). The amount of LPO is expressed as nanomoles (nM) of MDA formed per ml of packed RBCs.

$$\frac{\text{OD of test}}{\text{EC}} \times \frac{\text{Total volume of reaction}}{\text{Volume of sample taken}} \times 10^9 \times \text{DF} \times \text{Time of Incubation (2hrs)}$$

Here, DF= Dilution factor

$$1\text{M} = 10^9 \text{ nM}$$

3.9 Immune Response

3.9.1 Weight of Immune Organs

At the time of sacrifice bursa of fabricius and spleen of sacrificed bird were collected, weighed accurately on digital weighing balance and recorded for further analyses.

3.9.2 Serum Antibody Titre against RD Virus

The birds from the experimental trial were assessed for the antibody titre against the New Castle Disease (ND) virus antigen. One birds from each replication making a total of four birds from each treatment were randomly selected for the blood collection.

Blood samples were collected on 21st and 42nd day of trial RD vaccination, for estimation of antibody titre against RD virus. The blood samples were collected via wing vein from each bird. The collected blood samples were identified treatment wise and kept for two hours in slanting position at room temperature. The serum was separated by centrifugation at 3000 RPM for 20 minutes and decanted into clean, sterile plastic vials and stored under deep freeze at -20°C . These serum samples were used for HA and HI test to detect the antibody titre against Ranikhet Disease virus.

a) Haemagglutination (HA) Test

Procedure – HA test was performed in U bottom micro-titre plate as per O.I.E. (1992) procedure.

- 40 μl of 0.1 M phosphate buffer saline (PBS) pH 7.2 was added in first well of U bottom micro-titre plate.
- 25 μl of PBS was distributed in subsequent well (11 well).
- 10 μl of antigen was added in first well.
- Two-fold serial dilution was made up to 11th well and last well was kept as control.
- 25 μl chicken RBC (1%v/v) added to each well.
- After gentle mixing the plate were covered and then kept at 37°C for 45 minutes for incubation.

- The micro titre plates were read under bright light.
- The reciprocal of highest dilution of the antigen showing 50% haemagglutination was considered as HA titre.
- The original virus was diluted to contain 4 HA units and was used as HA antigen in the HI test

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
NSS (µl)	25	25	25	25	25	25	25	25	25	25	25	-
Antigen (µl)	25	25	25	25	25	25	25	25	25	25	-	25
Mix well and Discard 25 µl												
Dilution of virus	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024	RBC control	Ag Control
1% cRBC (µl)	25	25	25	25	25	25	25	25	25	25	25	25
Mix well and Incubate at room temperature for 45 minutes												
Results	#	#	#	#	#	#	#	#	•	•	•	#

The RBC control would show button formation and antigen control would show mat formation. The haemagglutination pattern in the remaining wells would depend on the concentration of virus present in the antigen suspension under test.

HA Titre: The reciprocal of highest dilution showing mat formation is taken as the HA titre (HAU) of virus.

b) Haemagglutination Inhibition (HI) Test

The test was carried out as per the procedure of O.I.E. (1992) with minor modification.

- 40 µl of 0.1 M PBS pH 7.2 was added in first well and 25 µl in subsequent wells (11 wells) of 'U' bottom Micro titre plate.

- 10 µl of antigen was added in first and serial two- fold dilution of serum was done.
- 25 µl of 4 HA units of antigen was added in each well.
- After this 25 µl of c-RBC 1% (v/v) was added to each well and mixed gently. The plate was incubated at room temperature for 45 minutes.

The results were recorded. The HI titre was expressed as the highest dilution of serum causing complete inhibition of antigen. The \log_{10} values for antibody titre were calculated and recorded for subsequent statistical analysis.

3.10 Economics of Broiler Production

The economics as influenced by dietary antioxidants (Vitamin C, Vitamin E and Selenium) supplementation was worked out at the end of the experimental period. All other segments of production i.e. cost of chicks, cost of total feed consumed by bird, enzymes, medicines and other miscellaneous items were considered to be uniform for all the treatment groups. However, the labour cost was not considered; as the experiment was conducted by student. Net profit per bird was estimated by subtracting the cost of production from the price fetched per bird after selling it in the local market on live body weight basis.

3.11 Statistical Analysis

The data collected during this investigation were subjected to One way analysis of variance following Completely Randomized Design (CRD) with treatment as a factor as per Snedecor and Cochran (1994). Means were compared as per Tukey's test as well as Duncan's multiple range test for certain parameters and data were processed for statistical analysis using SPSS Software package (22.0).

CHAPTER IV

RESULTS

The results of experiment entitled “Effect of dietary antioxidants (Vitamin C, Vitamin E and Selenium) supplementation on performance and immune response in broilers” are elucidated in this chapter.

4.1 Percent Proximate Analysis of Experimental Diets

The proximate composition of standard broiler diets formulated as per BIS (2007) standards for experimental broiler chickens in three phases i.e. pre-starter, starter, and finisher is showed in table 4.1. The estimated crude protein (%) was 23.06 (pre-starter), 21.98 (starter) and 19.94 (finisher) respectively.

Table: 4.1. Percent proximate analysis and calculated metabolic energy of experimental diets

Proximate Principles	Control Diet		
	Pre-starter	Starter	Finisher
Moisture	10.57	10.72	10.90
Dry Matter (DM)	89.43	89.28	89.10
Organic Matter (OM)	94.95	94.72	94.81
Crude Protein (CP)	23.06	21.98	19.94
Ether Extract (EE)	4.01	4.54	5.51
Crude Fibre (CF)	3.91	3.89	3.70
Total Ash (TA)	5.05	5.28	5.19
Nitrogen Free Extract (NFE)	63.97	64.31	65.66
Metabolic Energy (Kcal/Kg)*	3004.1	3100.4	3203.8

*Calculated

The ether extract (%) ranged from 4.01 in pre-starter to 5.51 in finisher ration. The percent crude fibre remained below 4.00% in all experimental rations. The calculated metabolisable energy (Kcal/Kg) conformed well to BIS (2007) standards for various rations formulated (Table 4.1)

4.2 Feed Intake (g) as influenced by Dietary Antioxidants Supplementation in Experimental Birds

4.2.1 Weekly feed intake (g) as influenced by dietary antioxidants supplementation in experimental birds

The mean weekly feed intake (g) for 6 weeks period is demonstrated in table 4.2.1.

Table: 4.2.1. Weekly feed intake (g) as influenced by dietary antioxidants supplementation in experimental birds

Age in Weeks	Treatments				SEM	P Value
	T ₀	T ₁	T ₂	T ₃		
I*	108.40 ^b ± 1.96	100.74 ^a ± 1.42	95.48 ^a ± 1.30	96.21 ^a ± 1.32	1.492	0.000
II	326.19 ± 13.95	327.80 ± 9.77	317.35 ± 8.47	299.48 ± 9.45	5.568	0.266
III	578.62 ± 4.80	550.78 ± 13.56	574.56 ± 18.76	556.20 ± 11.08	6.585	0.391
IV*	748.14 ^a ± 4.52	752.78 ^a ± 5.24	763.78 ^a ± 4.27	784.56 ^b ± 5.28	4.225	0.001
V	928.63 ± 14.58	902.55 ± 15.47	932.55 ± 15.10	927.92 ± 9.13	6.896	0.431
VI	1016.75 ± 25.38	976.75 ± 2.84	964.75 ± 6.98	969.75 ± 18.30	8.935	0.145

* Means bearing different superscripts in a row differ significantly (P<0.05) (n=60)

The mean weekly feed intake (g) was significantly higher (P<0.05) in control (T₀) than all treatment groups (T₁, T₂ and T₃) at the end of first week. The mean weekly feed intake (g) however, did not differ

significantly ($P>0.05$) during 2nd and 3rd week. The 4th week witnessed significantly higher ($P<0.05$) mean weekly feed intake (g) in T₃ (antioxidants combination group) as compared to T₁ (herbal Vitamin C) and T₂ (Vitamin E and Selenium) and T₀ (control) respectively. The mean weekly feed intake (g) was comparable ($P>0.05$) among all treatment groups during 5th and 6th weeks.

4.2.2 Cumulative feed intake (g) as influenced by dietary antioxidants supplementation in experimental birds

The mean cumulative feed intake (g) as recorded between 0 to 21 days, 22 to 42 days and for overall period (0 to 42 days) in experimental birds as affected by dietary antioxidants supplementation is furnished in table 4.2.2. The mean cumulative feed intake (g) during starter (0 to 21 days), finisher (22 to 42 days) and for overall period (0 to 42 days) remained comparable ($P>0.05$) among the treatment groups.

Table: 4.2.2. Cumulative feed intake (g) as influenced by dietary antioxidants supplementation in experimental birds

Days	Treatments				SEM	P Value
	T ₀	T ₁	T ₂	T ₃		
0-21	1013.22 ± 12.56	979.31 ± 18.25	987.38 ± 26.16	951.89 ± 6.63	9.635	0.155
22-42	2693.52 ± 33.92	2631.87 ± 15.82	2661.45 ± 13.20	2682.19 ± 15.25	11.270	0.236
0-42	3706.73 ± 38.94	3611.18 ± 14.70	3648.83 ± 29.00	3634.10 ± 13.88	14.876	0.119

* Means bearing different superscripts in a row differ significantly ($P<0.05$) (n=60)

4.3 Growth Performance as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

4.3.1 Weekly body weight gain (g) as influenced by dietary antioxidants supplementation in experimental birds

The body weight gain (g) during six weeks period as affected by dietary antioxidants supplementation in experimental broiler chicken is

depicted in table 4.3.1. The mean weekly body weight gain (g) did not differ significantly ($P>0.05$) among treatment groups from 1st to 3rd weeks. In 4th week the mean weekly body weight gain (g) was significantly higher ($P<0.05$) in T₀ (control) than T₂ (Vitamin E and Selenium), whereas the gain in T₁ (herbal Vitamin C) and T₃ (antioxidants combination group) remained comparable.

Table: 4.3.1. Weekly body weight gain (g) as influenced by dietary antioxidants supplementation in experimental birds

Age in Weeks	Treatments				SEM	P Value
	T ₀	T ₁	T ₂	T ₃		
I	102.20 ± 1.41	100.95 ± 1.16	96.65 ± 2.29	100.26 ± 2.07	0.962	0.207
II	206.32 ± 4.17	218.09 ± 15.67	214.05 ± 5.87	231.82 ± 5.66	4.708	0.295
III	413.28 ± 15.23	425.32 ± 8.75	421.17 ± 11.92	408.17 ± 12.33	5.753	0.759
IV*	541.50 ^b ± 9.38	501.27 ^{ab} ± 14.37	472.83 ^a ± 12.93	488.30 ^{ab} ± 19.86	9.277	0.034
V	436.69 ± 19.32	465.60 ± 27.42	493.82 ± 24.93	534.60 ± 24.49	14.298	0.074
VI	500.90 ± 31.34	537.82 ± 22.58	552.15 ± 37.92	528.57 ± 31.23	14.785	0.703

* Means bearing different superscripts in a row differ significantly ($P<0.05$) (n=60)

However, the mean weekly body weight gain (g) in 5th and 6th week did not differ significantly ($P>0.05$) among the treatment groups.

4.3.2 Cumulative body weights (g) as influenced by dietary antioxidants supplementation in experimental birds

The mean cumulative body weights (g) recorded during 0 to 21 days, 22 to 42 days and 0 to 42 days (for overall period) of experimental broiler chickens are given in table 4.3.2. The mean cumulative body weight (g) remained comparable ($P>0.05$) among treatment groups throughout the

experimental period. The overall mean cumulative body weight (g) was recorded lowest in control (2200.88 ± 40.56) and highest in T₃ (2291.71 ± 23.33). The antioxidants supplemented groups recorded numerically higher ($P>0.05$) cumulative body weights than control during overall period (0-42 d).

Table: 4.3.2. Cumulative body weights (g) as influenced by dietary antioxidants supplementation in experimental birds

Days	Treatments				SEM	P Value
	T ₀	T ₁	T ₂	T ₃		
0-21	721.80 ± 18.52	744.35 ± 11.55	731.87 ± 16.48	740.24 ± 7.21	6.705	0.691
22-42	1479.09 ± 51.55	1504.69 ± 39.70	1518.80 ± 30.89	1551.48 ± 30.15	18.719	0.629
0-42	2200.88 ± 40.56	2249.04 ± 35.03	2250.67 ± 22.43	2291.71 ± 23.33	16.278	0.288

* Means bearing different superscripts in a row differ significantly ($P<0.05$) (n=60)

4.4 Feed Conversion Ratio (FCR) as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

4.4.1 Weekly feed conversion ratio (FCR) as influenced by dietary antioxidants supplementation in experimental birds

The mean weekly feed conversion ratio in experimental broiler chickens as affected by dietary antioxidant supplementation is delineated in table 4.4.1. During 1st week the FCR was significantly improved ($P<0.05$) in T₃ (antioxidants combination group) than T₀ (control), while T₁ (herbal Vitamin C) and T₂ (Vitamin E and Selenium) ranged in between. Conversely, in 4th week the FCR was significantly improved ($P<0.05$) in T₀ than T₂ and T₃, while T₁ remained comparable. During 2nd, 3rd, 5th and 6th weeks the FCR remained comparable ($P>0.05$) among the treatment groups.

Table: 4.4.1. Weekly feed conversion ratio (FCR) as influenced by dietary antioxidants supplementation in experimental birds

Age in Weeks	Treatments				SEM	P Value
	T ₀	T ₁	T ₂	T ₃		
I*	1.06 ^b ± 0.02	1.00 ^{ab} ± 0.01	0.99 ^{ab} ± 0.02	0.96 ^a ± 0.01	0.013	0.019
II	1.59 ± 0.08	1.54 ± 0.17	1.49 ± 0.07	1.30 ± 0.07	0.055	0.281
III	1.41 ± 0.04	1.30 ± 0.01	1.37 ± 0.09	1.37 ± 0.06	0.027	0.580
IV*	1.38 ^a ± 0.03	1.51 ^{ab} ± 0.05	1.62 ^b ± 0.05	1.62 ^b ± 0.08	0.035	0.031
V	2.14 ± 0.13	1.96 ± 0.10	1.90 ± 0.11	1.75 ± 0.07	0.059	0.112
VI	2.05 ± 0.10	1.83 ± 0.07	1.77 ± 0.11	1.85 ± 0.08	0.049	0.208

* Means bearing different superscripts in a row differ significantly (P<0.05) (n=60)

4.4.2 Cumulative feed conversion ratio (FCR) as influenced by dietary antioxidants supplementation in experimental birds

The mean cumulative feed conversion ratio (FCR) as affected by dietary supplementation of antioxidants in experimental broiler chickens is presented in table 4.4.2.

Table: 4.4.2. Cumulative feed conversion ratio (FCR) as influenced by dietary antioxidants supplementation in experimental birds

Days	Treatments				SEM	P Value
	T ₀	T ₁	T ₂	T ₃		
0-21	1.41 ± 0.03	1.32 ± 0.04	1.36 ± 0.07	1.29 ± 0.01	0.022	0.268
22-42	1.83 ± 0.06	1.75 ± 0.04	1.76 ± 0.04	1.73 ± 0.03	0.022	0.504
0-42	1.69 ± 0.03	1.61 ± 0.02	1.62 ± 0.02	1.59 ± 0.01	0.015	0.056

* Means bearing different superscripts in a row differ significantly (P<0.05) (n=60)

The mean cumulative FCR from 0 to 21 days and 22 to 42 days did not differ significantly (P>0.05) among the treatment groups. However, it

was noteworthy to record numerically improved ($P=0.056$) overall FCR (0 to 42 days) in T_3 (antioxidants combination group) as compared to T_0 (control); whereas FCR in T_1 (herbal Vitamin C) and T_2 (Vitamin E and Selenium) remained comparable.

4.5 Nutrient Metabolisability (%) as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The percent nutrient metabolisability in experimental broiler chickens as affected by dietary antioxidants supplementation is given in table 4.5. The percent digestibility of dry matter (DM) and crude fibre (CF) was significantly higher ($P<0.05$) in T_1 (herbal Vitamin C) and T_3 (antioxidants combination group) when compared to T_0 (control), whereas T_2 (Vitamin E and Selenium) remained comparable. The percent digestibility of organic matter (OM) and nitrogen free extract (NFE) was significantly higher ($P<0.05$) in T_3 than T_0 ; whereas T_1 and T_2 remained comparable.

Table: 4.5. Nutrient metabolisability (%) as influenced by dietary antioxidants supplementation in experimental birds

Parameters	Treatments				SEM	P value
	T ₀	T ₁	T ₂	T ₃		
DM*	61.41 ^a ± 0.93	64.65 ^b ± 0.88	62.73 ^{ab} ± 0.51	65.72 ^b ± 0.51	0.542	0.006
OM*	64.88 ^a ± 0.96	68.09 ^{ab} ± 1.13	67.13 ^{ab} ± 0.67	69.34 ^b ± 0.26	0.559	0.015
CP*	71.18 ^a ± 0.87	73.25 ^{ab} ± 0.72	71.08 ^a ± 0.64	75.25 ^b ± 0.42	0.539	0.003
EE	69.98 ± 1.11	70.98 ± 0.88	69.88 ± 1.01	73.49 ± 1.09	0.594	0.094
CF*	45.18 ^a ± 1.84	51.15 ^b ± 1.30	49.51 ^{ab} ± 0.72	52.87 ^b ± 1.50	0.967	0.013
NFE*	66.87 ^a ± 0.91	70.00 ^{ab} ± 1.05	68.44 ^{ab} ± 0.56	70.60 ^b ± 0.49	0.514	0.024

* Means bearing different superscripts in a row differ significantly ($P<0.05$)

DM- Dry Matter, OM- Organic Matter, CP- Crude Protein, EE- Ether Extract, CF- Crude Fibre and NFE- Nitrogen Free Extract

The percent crude protein (CP) digestibility was significantly higher ($P<0.05$) in T_3 as compared to T_0 and T_2 , while T_1 remained comparable. The percent ether extract (EE) digestibility did not differ significantly ($P>0.05$) among the treatment groups.

4.6 Carcass Characteristics and Shelf life of meat as Influenced by dietary Antioxidants Supplementation in Experimental Birds

The live weight, carcass characteristics and shelf life of meat as affected by dietary antioxidants supplementation in experimental broiler chickens is illustrated in table 4.6.

Table: 4.6. Carcass characteristics and Shelf life of meat as influenced by dietary antioxidants supplementation in experimental birds

Parameters	Treatments				SEM	P value
	T ₀	T ₁	T ₂	T ₃		
Live weight (g)	2347.50 ± 40.75	2295.00 ± 46.07	2346.00 ± 37.07	2286.50 ± 68.46	23.347	0.735
Edible meat weight^s (g)	1652.50 ± 26.07	1625.00 ± 35.14	1650.75 ± 21.48	1632.25 ± 49.74	15.875	0.923
Edible meat (%)	70.41 ± 0.76	70.82 ± 1.07	70.38 ± 0.23	71.35 ± 0.54	0.337	0.758
Giblet weight[#](g)	99.75 ± 1.75	98.75 ± 3.07	99.00 ± 3.14	100.00 ± 1.08	1.091	0.980
Giblet (%)	4.25 ± 0.05	4.30 ± 0.06	4.22 ± 0.07	4.38 ± 0.10	0.036	0.467
Carcass weight* (g)	1752.25 ± 27.73	1723.75 ± 37.27	1749.75 ± 24.54	1731.25 ± 50.65	16.614	0.930
Dressing (%)	74.66 ± 0.81	75.12 ± 1.05	74.60 ± 0.21	75.73 ± 0.59	0.348	0.686
Shelf life						
0th Day (mg MDA/Kg)	0.024 ± 0.001	0.020 ± 0.003	0.020 ± 0.002	0.018 ± 0.002	0.001	0.399
4th Day (mg MDA/Kg)	0.041 ± 0.003	0.036 ± 0.002	0.038 ± 0.003	0.036 ± 0.002	0.001	0.451
8th Day (mg MDA/Kg)	0.043 ± 0.004	0.037 ± 0.006	0.042 ± 0.007	0.039 ± 0.005	0.002	0.881

*Carcass weight (weight of eviscerated carcass with skin), (n=4)

[#]Edible offal (heart, liver and gizzard),

^sEdible meat (carcass with giblet).

The Live weight (g) and Carcass characteristics namely edible meat weight (g) and percentage, giblet weight (g) and percentage, carcass weight (g) and dressing percentage did not differ significantly ($P>0.05$) among the treatment groups. The shelf life of meat (mg MDA/Kg) assessed on 0th, 4th and 8th day remained comparable ($P>0.05$) among the treatment groups.

4.7 Sensory Evaluation as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The results of sensory evaluation of meat as influenced by dietary antioxidants supplementation in experimental birds and performed by semi-trained panelist are demonstrated in table 4.7.

Table: 4.7. Sensory Evaluation as influenced by dietary antioxidants supplementation in experimental birds

Observations	Treatments				SEM	P value
	T ₀	T ₁	T ₂	T ₃		
Appearance	5.78 ± 0.22	5.78 ± 0.22	6.11 ± 0.20	6.33 ± 0.17	0.105	0.171
Texture	6.00 ± 0.37	5.89 ± 0.20	6.44 ± 0.24	5.89 ± 0.20	0.132	0.402
Juiciness	6.11 ± 0.31	6.00 ± 0.29	6.33 ± 0.24	6.78 ± 0.15	0.131	0.158
Flavor	5.67 ± 0.24	6.34 ± 0.24	6.11 ± 0.26	6.34 ± 0.24	0.125	0.190
Tenderness	5.78 ± 0.28	6.22 ± 0.22	6.56 ± 0.24	6.44 ± 0.24	0.128	0.143
Overall Acceptability*[#]	6.00 ^a ± 0.17	6.00 ^a ± 0.24	6.22 ^{ab} ± 0.28	6.78 ^b ± 0.15	0.115	0.047

* Means bearing different superscripts in a row differ significantly ($P<0.05$) (n=9)

[#] Duncan multiple range test used for superscripts

The mean score of meat qualities assessed as Appearance, Texture, Juiciness, Flavor and Tenderness remained comparable ($P>0.05$)

among different treatment groups on 7-point Hedonic scale. Moreover, the score for overall acceptability of meat was significantly higher ($P < 0.05$) in T_3 (antioxidants combination group) than T_0 (control) and T_1 (herbal Vitamin C); while T_2 (Vitamin E and Selenium) ranged in between.

4.8 Hematological Parameters, Erythrocytic Antioxidant Status and Serum Metabolite Concentration as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

4.8.1 Hematological parameters and erythrocytic antioxidant status as influenced by dietary antioxidants supplementation in experimental birds

The hematological parameters and erythrocytic antioxidant status in experimental birds as affected by dietary antioxidants supplementation are furnished in table 4.8.1. The hemoglobin (g/dl) was significantly higher ($P < 0.05$) in T_2 (Vitamin E and Selenium) than T_0 (control), while the hemoglobin (g/dl) in T_1 (herbal Vitamin C) and T_3 (antioxidants combination group) ranged in between.

The percent PCV, eosinophil, monocytes and basophils did not differ significantly ($P > 0.05$) among the treatment groups. The TLC ($\times 10^3/\mu\text{l}$) and percent lymphocyte was significantly lower ($P < 0.05$) in T_0 (control) when compared to all the treatment groups. Conversely the percent heterophils increased significantly ($P < 0.05$) in control over the treatment groups. While H:L ratio declined significantly ($P < 0.05$) in antioxidants supplemented groups than control. Among the erythrocytic antioxidant enzymes estimated, Catalase (mM $\text{H}_2\text{O}_2/\text{min}/\text{mg}$) did not differ significantly ($P > 0.05$) among the treatment groups. The SOD (nm MDA/ml) and LPO ($\times 10^2$ nM MDA/ml) activity was significantly higher ($P < 0.05$) in treatment groups (T_3 , T_2 and T_1) over the control (T_0) in the said order.

Table: 4.8.1. Hematological parameters and erythrocytic antioxidant status as influenced by dietary antioxidants supplementation in experimental birds

Parameters	Treatments				SEM	P value
	T ₀	T ₁	T ₂	T ₃		
Hb (g/dl)*	8.63 ^a ± 0.50	10.45 ^{ab} ± 0.65	11.25 ^b ± 0.55	11.05 ^{ab} ± 0.63	0.375	0.031
PCV (%)	27.0 ± 1.08	29.5 ± 1.26	31.0 ± 1.68	31.8 ± 1.44	0.776	0.130
TLC (×10³/μl)*	11.09 ^a ± 1.11	12.57 ^{ab} ± 1.49	16.63 ^{bc} ± 0.90	18.31 ^c ± 0.84	0.901	0.002
Heterophils (%)*	30.25 ^b ± 1.11	18.75 ^a ± 0.48	20.75 ^a ± 1.44	18.25 ^a ± 1.65	1.372	0.000
Lymphocyte (%)*	62.25 ^a ± 1.11	72.00 ^b ± 0.91	70.00 ^b ± 1.08	73.25 ^b ± 1.38	1.214	0.000
Eosinophil (%)	2.25 ± 0.48	3.50 ± 0.29	4.50 ± 0.96	4.00 ± 0.71	0.365	0.146
Monocytes (%)	2.75 ± 0.63	4.00 ± 0.41	3.25 ± 1.03	3.25 ± 0.48	0.326	0.643
Basophiles (%)	2.50 ± 0.65	1.75 ± 0.48	1.50 ± 0.29	1.25 ± 0.48	0.250	0.346
H:L ratio	0.49 ^b ± 0.02	0.26 ^a ± 0.01	0.30 ^a ± 0.02	0.25 ^a ± 0.03	0.027	0.000
SOD (nM MDA/ml)*	65.30 ^a ± 0.74	67.99 ^{ab} ± 0.58	69.88 ^{bc} ± 0.62	72.47 ^c ± 0.84	0.746	0.000
Catalase (mMH₂O₂/min/ mg)	8.38 ± 0.14	8.59 ± 0.16	8.44 ± 0.15	8.62 ± 0.22	0.081	0.704
LPO (×10² nM MDA/ml)*	33.84 ^a ± 0.74	42.12 ^c ± 0.65	38.18 ^b ± 0.91	45.82 ^d ± 0.81	1.204	0.000

* Means bearing different superscripts in a row differ significantly (P<0.05) (n=4)
Hb- Hemoglobin, **PCV**- Packed Cell Volume and **TLC**- Total Leucocyte count
SOD- Super-oxidase Dismutase, **LPO**- Lipid peroxidase and **CAT**- Catalase

4.8.2 Serum metabolite concentration as influenced by dietary supplementation of antioxidants in experimental birds

The serum metabolite and enzyme concentration in experimental broiler chickens as affected by dietary supplementation of antioxidant is given in table 4.8.2.

Table: 4.8.2. Serum metabolite concentration as influenced by dietary antioxidants supplementation in experimental birds

Parameters	Treatments				SEM	P value
	T ₀	T ₁	T ₂	T ₃		
Glucose (mg/dl)	179.88± 8.05	174.70 ± 5.53	162.88 ± 7.30	176.25± 5.86	3.445	0.357
Total protein (g/dl)*	3.17 ^a ± 0.06	3.48 ^{ab} ± 0.11	3.22 ^a ± 0.08	3.70 ^b ± 0.05	0.065	0.001
Albumin (g/dl)*	1.21 ^a ± 0.06	1.50 ^{ab} ± 0.15	1.36 ^{ab} ± 0.06	1.63 ^b ± 0.08	0.058	0.043
Globulin (g/dl)	1.96 ± 0.10	1.98 ± 0.19	1.86 ± 0.06	2.07 ± 0.08	0.056	0.685
A:G Ratio	0.62 ± 0.06	0.79 ± 0.12	0.74 ± 0.04	0.79 ± 0.07	0.040	0.513
Uric Acid (mg/dl)	8.10 ± 0.57	6.27 ± 0.68	7.01 ± 0.67	7.22 ± 0.47	0.317	0.250
SGOT (IU/L)	238.00 ± 12.99	231.50 ± 9.97	204.00 ± 9.17	218.25 ± 11.10	5.927	0.182
SGPT (IU/L)	24.50 ± 3.28	26.50 ± 2.10	22.75 ± 1.93	24.00 ± 2.89	1.218	0.785

* Means bearing different superscripts in a row differ significantly (P<0.05) (n=4)

A:G - Albumin: Globulin, **SGOT**- Serum Glutamic-oxalacetic transaminase and **SGPT**- Serum glutamic-pyruvic transaminase

The glucose (mg/dl) was comparable (P>0.05) among the treatment groups and ranged from 162.88 ± 7.30 in T₂ to 179.88 ± 8.05 in T₀.

The globulin (g/dl) and A:G ratio did not differ significantly ($P>0.05$) among the treatment groups. Total protein (g/dl) was significantly higher ($P<0.05$) in T_3 than T_0 and T_2 , while T_1 remained comparable. The Albumin (g/dl) was significantly higher ($P<0.05$) in T_3 as compared to T_0 (control); whereas T_1 and T_2 remained comparable. The Uric acid (mg/dl) reduced numerically ($P>0.05$) in antioxidants supplemented groups than control. The mean values of SGOT and SGPT (IU/L) did not differ significantly ($P>0.05$) among the treatment groups.

4.9 Immune Response as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The weight of immune organs (g), immune response in terms of serum antibody titre against RD virus in experimental broiler chickens as affected by dietary antioxidants supplementation are depicted in table 4.9.

Table: 4.9. Weights of immune organs, serum antibody titre against RD virus (\log_{10}) as influenced by dietary antioxidants supplementation in experimental birds

Parameters	Treatments				SEM	P value
	T ₀	T ₁	T ₂	T ₃		
Weight of bursa	4.15 ± 0.15	4.33 ± 0.08	4.11 ± 0.09	4.25 ± 0.05	0.050	0.414
Weight of spleen	2.17 ± 0.04	2.13 ± 0.04	2.15 ± 0.05	2.12 ± 0.05	0.021	0.883
HI titre day 21 (\log_{10})*#	1.43 ^a ± 0.14	1.51 ^a ± 0.08	1.73 ^{ab} ± 0.09	1.88 ^b ± 0.07	0.067	0.045
HI titre day 42 (\log_{10})*	2.03 ^a ± 0.14	2.41 ^{ab} ± 0.12	2.33 ^{ab} ± 0.08	2.56 ^b ± 0.09	0.070	0.035

* Means bearing different superscripts in a row differ significantly ($P<0.05$) (n=4)

Duncan multiple range tests used for superscripts

The weight of immune organs i.e. bursa and spleen remained comparable ($P>0.05$) among treatment groups. The serum antibody titre against RD virus was significantly higher ($P<0.05$) in T_3 (antioxidants

combination group) over T₀ (control) at both 21st and 42nd day of experiment; whereas the titres of T₁ and T₂ ranged in between.

4.10 Cost Economics of Broiler Production as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The economics of experimental broiler chicken production as affected by dietary antioxidant supplementation is illustrated in table 4.10. The cost of day old chicks was Rs.35 and cost of feed was Rs.45/Kg. The cost of supplements like herbal vitamin C, vitamin E and selenium was added in total feed cost as per their respective inclusion levels. The total feed cost (Rs./Kg) was highest in T₃ (45.29) and lowest in T₀ (45.00). The average total feed consumption (Kg) ranged from 3.61 in T₁ to 3.71 in T₀. The cost of feed consumed per bird in treatment groups remained below the control (Rs.166.95).

The cost of feed per Kg live weight (Rs.) and total cost of production remained higher in control than the treatment groups. The average price realized by selling the birds at Rs.75 per Kg live weight was Rs.168.00 (control), Rs.171.75 (herbal Vitamin C), Rs.171.75 (Vitamin E and Selenium) and Rs.174.75 (antioxidants combination group) respectively in the said order.

The profit/loss statement indicated that the loss in broiler production was reduced in treatment groups than the control (T₀) and was minimized by 20.30% in T₁, 13.87% in T₂ and 23.63% in T₃ respectively over the control group.



Plate 4.1. Plates showing Haemagglutination Inhibition titres (21st and 42nd Day)

Table: 4.10. Cost economics of broiler production in experimental birds fed antioxidants supplemented diets

Sr. No.	Particulars	T₀	T₁	T₂	T₃
1	Cost of day old chick (Rs.)	35.00	35.00	35.00	35.00
2	Cost of Feed (Rs/Kg)	45.00	45.00	45.00	45.00
3	Cost of Vitamin C (Rs.)	-	0.04	-	0.04
4	Cost of Vitamin E and Selenium (Rs.)	-	-	0.25	0.25
5	Total cost of feed (Rs/Kg) (2+3+4)	45.00	45.04	45.25	45.29
6	Average total feed consumed per bird (Kg)	3.71	3.61	3.65	3.63
7	Cost of feed consumed per bird (Rs.) (5×6)	166.95	162.59	165.16	164.26
8	Average body weight at the end of 6 th week (Kg)	2.24	2.29	2.29	2.33
9	Feed consumption per live body weight(Kg) (6÷8)	1.66	1.58	1.59	1.56
10	Cost of feed per Kg live weight (Rs.) (7÷8)	74.53	71.00	72.13	70.50
11	Cost of medicines, vaccine, litter material etc per bird (Rs.)	6.00	6.00	6.00	6.00
12	Total cost of production (Rs.) (1+7+11)	207.95	203.59	206.16	205.26
13	Average price realized @ Rs.75 per Kg live weight (Rs.)	168.00	171.75	171.75	174.75
14	Net profit/loss per bird (Rs.) (12-13)	-39.95	-31.84	-34.41	-30.51
15	Abatement of Loss (%)	-	20.30	13.87	23.63

CHAPTER V

DISCUSSION

The performance and immune response of experimental broiler chickens under production stress as influenced by dietary supplementation of antioxidants (Vitamin C, Vitamin E and Selenium) is explicated in this chapter. The details thereof are given in succeeding paragraphs.

5.1 Percent Proximate Analysis of Experimental Diets

The estimated proximate composition and calculated metabolisable energy (Kcal/Kg) of experimental diets is presented in table 4.1. The percent crude protein in experimental diets were 23.06 (pre-starter), 21.98 (starter) and 19.94 (finisher) respectively. The percent crude fibre remained well below 5.00 and percent total ash ranged from 5.05 (pre-starter) to 5.28 (starter). The proximate composition and calculated metabolisable energy (Kcal/Kg) conformed well to BIS (2007) standards. Lagana *et al.* (2007) reported 21.5 and 19.5 percent crude protein in starter (1 to 21 days) and grower (22 to 35 days) diets respectively in broilers under heat stress. In agreement with present findings, the ME (Kcal/Kg) values reported by Lagana *et al.* (2007) were 3000 (starter) and 3100 (grower) respectively.

Moreover chick starter mash (22.84 % CP and 2852.5 Kcal/Kg ME) and finisher mash (20.5% CP and 2966 Kcal/Kg ME) were used to assess the performance of broiler during hot weather supplemented with Ashwagandha and commercial synthetic compounds (Shisodiya *et al.*, 2008). In consonance with present findings Aikpitanyi and Imasuen (2019) also reported CP percent and ME Kcal/Kg 23.01 and 3240.04 in starter and 20.62 and 3088.15 in finisher diet of broiler chickens supplemented with oral administration of lime fruit (*Citrus aurentifolia*). On other hand Adeyemi *et al.* (2015) reported slightly lower nutritional composition for starter (22.55 % CP and 11.48 MJ/Kg ME) and slightly higher composition for finisher (20.32% CP and 11.69 MJ/Kg ME) as compared to the diets during similar phases in current experiment.

5.2 Feed Intake (g) as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

5.2.1 Weekly feed intake (g) as influenced by dietary antioxidants supplementation in experimental birds

The weekly feed intake (g) as influenced by dietary supplementation of antioxidants in experimental birds is given in table 4.2.1, and Fig 5.1. The weekly feed intake (g) was significantly higher ($P < 0.05$) in control than all other treatment groups during 1st week. Conversely during 4th week, the mean weekly feed intake (g) was significantly higher ($P < 0.05$) in T₃ (antioxidant combination) when compared to control and other treatment groups. However, during 2nd, 3rd, 5th and 6th week the mean weekly feed intake (g) was comparable ($P > 0.05$) among the treatment groups. It was observed that the effect of dietary antioxidants supplementation on weekly feed intake was not conspicuous.

In agreement with present findings Rindhe *et al.* (2016) observed comparable ($P > 0.05$) weekly feed consumption in control and herbal anti-stress product supplemented groups under physiological heat stress. Suk and Washburn (1995) opined that the consumption and efficiency of feed in broilers declined during stressful condition owing to increased heat increments. Khajali *et al.* (2010) found no significant ($P > 0.05$) effect on feed consumption of broilers supplemented with dietary organic selenium and fed with diets containing different protein levels. Similarly the effect of dietary supplementation of herbal vitamin C, vitamin E and selenium or their combination on mean weekly feed consumption of experimental broiler chickens in current experiment seemed to be confounding.

5.2.2 Cumulative feed intake (g) as influenced by dietary antioxidants supplementation in experimental birds

The cumulative feed intake (g) as affected by dietary supplementation of antioxidants in experimental birds is showed in table 4.2.2. The cumulative feed intake (g) was not influenced ($P > 0.05$) due to dietary antioxidants supplementation to experimental birds during 0 to 21 (starter), 22

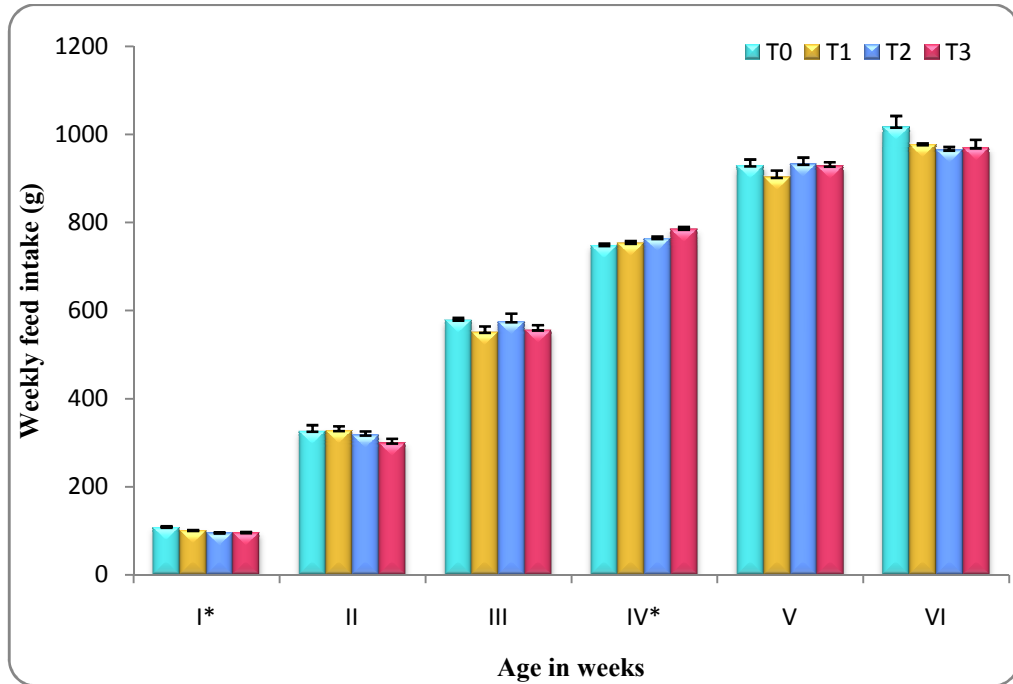


Fig5.1. Weekly feed intake (g) as influenced by dietary antioxidants supplementation in experimental birds

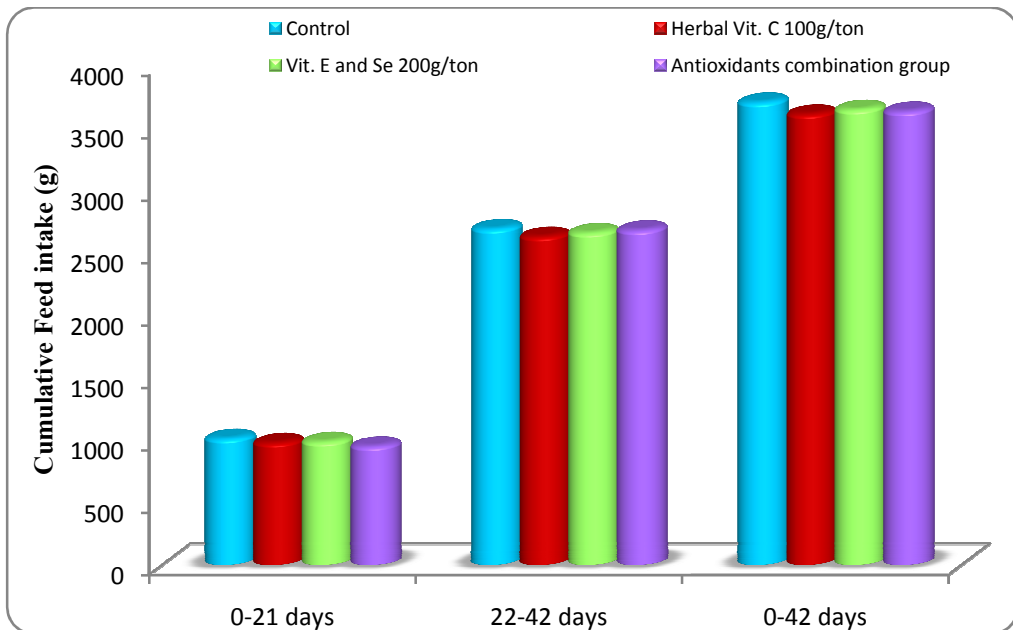


Fig5.2. Cumulative feed intake (g) as influenced by dietary antioxidants supplementation in experimental birds

to 42 (finisher) and 0 to 42 (overall) period. In congruence with present finding Tawfeek *et al.* (2014) observed no significant influence ($P>0.05$) of dietary antioxidant nutrients supplementation to broiler chicken during first 3 weeks of experiment. They further noted that during next three weeks (22 to 42 days) the feed consumption (g) was reduced by about 210 and 231 g in birds supplemented with Vitamins and Zinc + Selenium. The chromium and Zinc + Selenium supplemented group consumed least feed during 0 to 42 days. Deniz *et al.* (2005) found that mineral and organic form of selenium supplementation did not affect ($P>0.05$) the feed intake and mortality.

Moreover, Habibi *et al.* (2014) recorded comparable feed intake among bird receiving different concentration of ginger root powder and its essential oil. Khajali *et al.* (2010) noted that dietary CP reduction significantly reduced ($P>0.05$) the feed consumption in experimental bird except during 21 to 42 days; however Selenium supplementation had no significant ($P>0.05$) effect on feed intake from 7 to 49 days. Conversely, Kutlu and Forbes (1993) recorded significant depression ($P<0.05$) in feed consumption due to heating; however there was no influence ($P>0.05$) of ascorbic acid supplementation on intake. Most of the studies reviewed, indicated no significant effect of dietary antioxidant supplementation on feed intake of stressed birds (Tuleun and Njoku, 2000). The cumulative feed intake (g) as affected by dietary supplementation of antioxidants in experimental birds is showed in Fig 5.2.

5.3 Growth Performance as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

5.3.1 Weekly body weight gain (g) as influenced by dietary antioxidants supplementation in experimental birds

The weekly gain in body weight (g) in experimental birds as affected by dietary antioxidants supplementation is depicted in table 4.3.1. There was no significant influence ($P>0.05$) of dietary antioxidants supplementation in experimental birds during starter period (0 to 21 days).

However during 4th week control group recorded significantly higher ($P<0.05$) weight gain than in T_2 ; whereas T_1 and T_3 remained comparable. In 5th and 6th week the mean weekly body weight gain (g) remained comparable ($P>0.05$) among the treatment groups. Tuleun and Njoku (2000) observed comparable ($P>0.05$) feed intake and weight gain between control and ascorbic acid supplemented broilers. Pardue *et al.* (1985) recorded comparable ($P>0.05$) body weights of ascorbic acid supplemented males; they however reported that females receiving 1000 ppm of dietary ascorbic acid revealed significantly higher ($P<0.05$) body weights during 2nd and 4th week of age. Niu *et al.* (2009a) observed that feeding different dietary levels of vitamin E had no significant ($P>0.05$) effects on body weight changes as well as feed intake. The present study seemed to reaffirm aforesaid findings of comparable body weights in dietary antioxidants supplemented broiler chickens. The weekly gain in body weight (g) in experimental birds as affected by dietary antioxidants supplementation is depicted in Fig 5.3.

5.3.2 Cumulative body weights (g) as influenced by dietary antioxidants supplementation in experimental birds

The cumulative body weights (g) of experimental birds as influenced by dietary supplementation of antioxidants are given in table 4.3.2. The cumulative body weights (g) were not affected ($P>0.05$) due to dietary antioxidants supplementation in experimental broiler chickens during starter (0 to 21days), finisher (22 to 42 days) and overall period (0 to 42 days). However, it was noted that throughout the experimental period the cumulative body (g) of all treatment groups (T_1 , T_2 and T_3) were numerically higher ($P>0.05$) than control (T_0). Lagana *et al.* (2007) in support of present findings reported comparable weight gain and feed intake in broilers supplemented with vitamin E and Organic minerals like zinc and selenium. In contrast with present finding significantly higher ($P<0.05$) weight gain in broilers supplemented with ascorbic acid were reported earlier (Pardue *et al.*, 1985; Edrise *et al.*, 1986 and Adeyemi *et al.*, 2015). Kutlu and Forbes (1993) reported greater body weights of 250 mg/Kg ascorbic acid supplemented heat stressed broiler when compared to those receiving no supplementation.

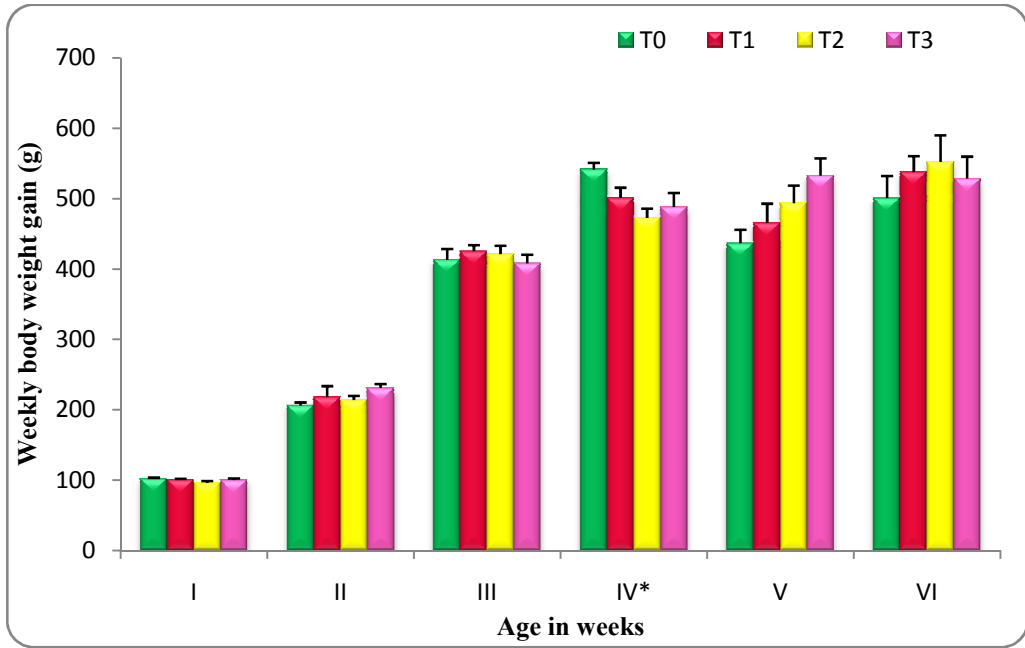


Fig5.3. Weekly body weight gain (g) as influenced by dietary antioxidants supplementation in experimental birds

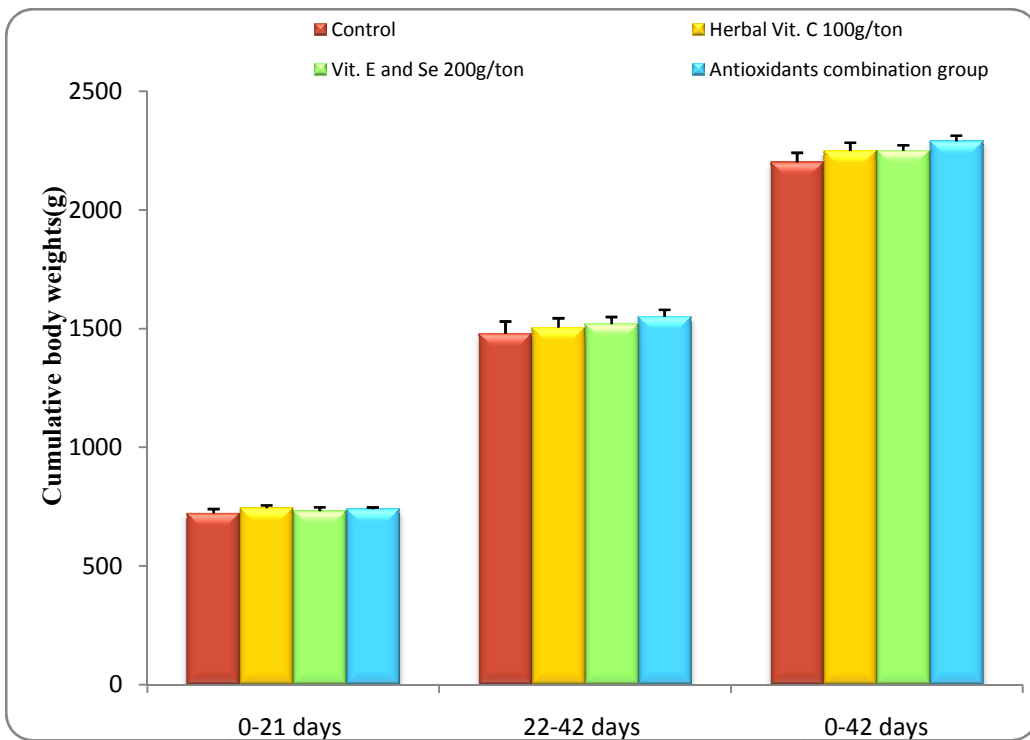


Fig5.4. Cumulative body weights (g) as influenced by dietary antioxidants supplementation in experimental birds

Marković *et al.* (2018) also noted increased body weights and weight gain in 0.9 mg/Kg dietary selenium supplemented group than those supplemented selenium at lower doses. However, Khajali *et al.* (2010) in agreement with present finding recorded no significant effect of dietary organic selenium supplementation on growth performance. Similar to numerical increment in cumulative body weight (g) of antioxidants supplemented groups over control in present experiment, Habibi *et al.* (2014) also recorded numerical improvement in body weight of ginger herb or essential oil supplemented group. They attributed this to improved enzymatic activity, intestinal microflora and gut function due to intrinsic antioxidant compounds present in ginger root powder and its essential oils. The cumulative body weights of experimental birds as influenced by dietary supplementation of antioxidants are given in Fig 5.4.

5.4 Feed Conversion Ratio (FCR) as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

5.4.1 Weekly feed conversion ratio (FCR) as influenced by dietary antioxidants supplementation in experimental birds

The weekly feed conversion ratio in experimental birds as affected by dietary antioxidants supplementation is presented in table 4.4.1. During 1st week the FCR was significantly improved ($P < 0.05$) in T₃ than T₀, while T₁ and T₂ ranged in between. Conversely in 4th week the FCR was significantly improved ($P < 0.05$) in T₀ than T₂ and T₃, while T₁ remained comparable. During 2nd, 3rd, 5th and 6th weeks the FCR remained comparable ($P > 0.05$) among the treatment groups. In contrast with present finding Marković *et al.* (2018) recorded improved body weights, weight gain and feed conversion efficiency in organic selenium supplemented broilers. They deduced that selenium being integral part of iodo-thyronine deiodinases enzyme groups instrumental in thyroid hormone metabolism and essential for normal growth and development (Arthur, 1991), could have improved weight gain and feed conversion efficiency (Choct *et al.*, 2004).

Kutlu and Forbes (1993) recorded improved feed conversion efficiency due to ascorbic acid supplementation in broilers under heat stress than non-supplemented groups. Adeyemi *et al.* (2010) observed improvement in feed:gain ratio on ascorbic acid supplementation in broilers fed restricted diets. Lagana *et al.* (2007) recorded that though there was no significant ($P>0.05$) effect of vitamin E and organic mineral supplementation on weight gain and feed intake in heat stressed broilers, the feed conversion ratio revealed significant improvement ($P<0.05$) on vitamin E or organic mineral supplementation. Similarly, Niu *et al.* (2009a) also reported comparable body weights and feed intake in vitamin E supplemented heat stressed broilers, however the feed conversion ratio were significantly ($P<0.05$) improved due to vitamin E supplementation. They however concluded that vitamin E supplementation could not alter the growth performance of six week aged broilers maintained under heat stress. The weekly feed conversion ratio in experimental birds as affected by dietary antioxidant supplementation is presented in Fig 5.5.

5.4.2 Cumulative feed conversion ratio (FCR) as influenced by dietary antioxidants supplementation in experimental birds

The cumulative FCR as affected by dietary supplementation of antioxidants in experimental birds is showed in table 4.4.2. The cumulative FCR during starter (0 to 21 days) and finisher (22 to 42 days) period did not differ significantly ($P>0.05$) among treatment groups than the control. Moreover when estimated for overall period (0 to 42 days) the FCR was numerically improved ($P=0.056$) in treatment groups over the control. Tuleun and Njoku (2000) observed that though ascorbic acid supplementation could not affect ($P>0.05$) feed intake and weight gain, the FCR of 250 mg/Kg ascorbic acid supplemented broilers was significantly improved ($P<0.05$) during 0 to 4 weeks over un-supplemented groups. They however recorded comparable FCR during 5th to 8th week period. Niu *et al.* (2009a) deduced that vitamin E supplementation at 100 mg/Kg though did not affect ($P>0.05$) body weight and feed intake; significantly improved ($P<0.05$) the feed conversion ratio. Some reports in past have recorded no significant effect of selenium

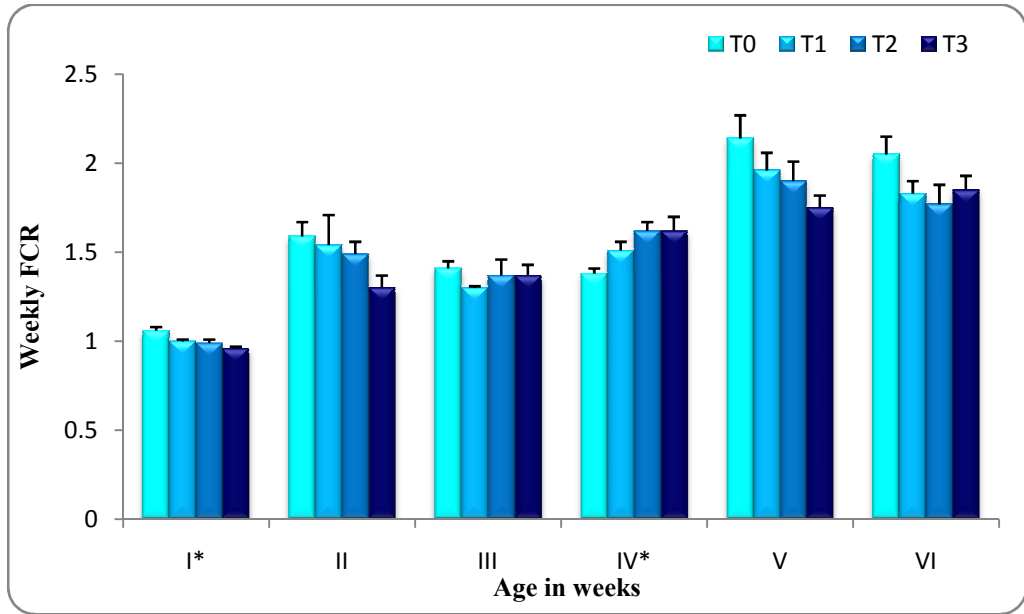


Fig5.5. Weekly feed conversion ratio (FCR) as influenced by dietary antioxidants supplementation in experimental birds

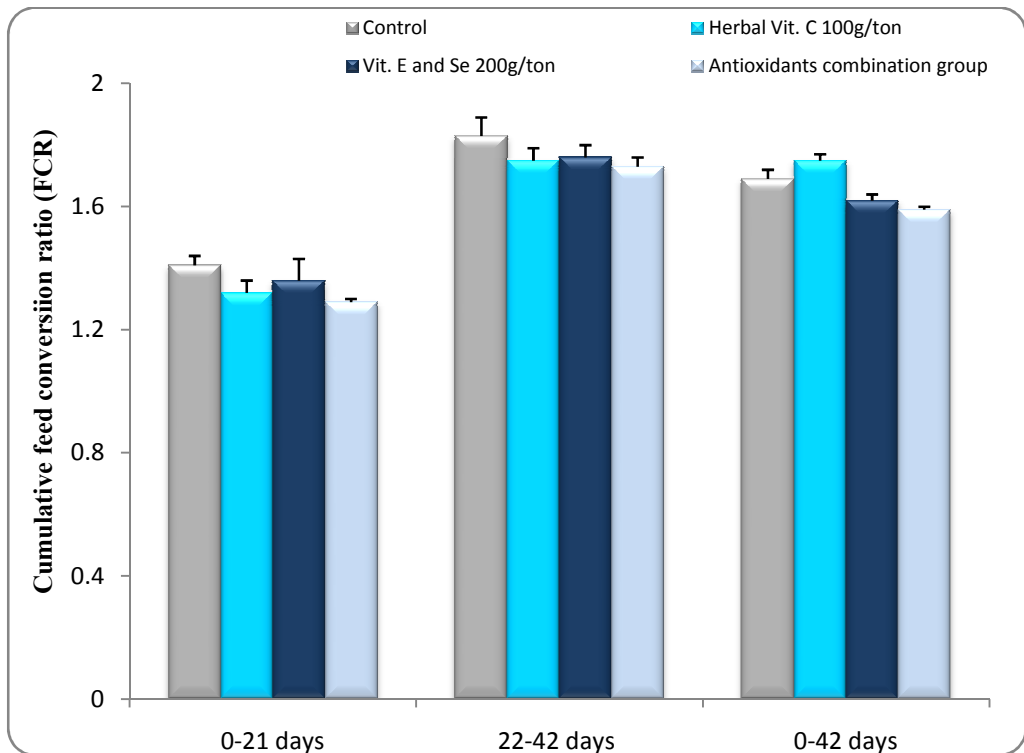


Fig5.6. Cumulative feed conversion ratio (FCR) as influenced by dietary antioxidants supplementation in experimental birds

supplementation on growth performance in broilers (Payne and Southern, 2005; Ryu *et al.*, 2005; Peri'c *et al.*, 2009; Cai *et al.*, 2012; Chen *et al.*, 2013; Briens *et al.*, 2014; Boostani *et al.*, 2015; Chadio *et al.*, 2015).

Similarly 0.2 ppm selenium addition as selenium selenite or selenium yeast could not affect FCR in broiler birds (Upton *et al.*, 2009). Khajali *et al.* (2010) recorded no significant ($P>0.05$) effect of either dietary CP content or Selenium supplementation and even their interactions on feed conversion ratio in broilers reared under heat stress. The present findings indicate numerical, though not significant ($P>0.05$) improvement in feed conversion ratio throughout the experimental period in antioxidants supplemented groups over the control. Özkan *et al.* (2007) noted comparable feed conversion ratio among broilers receiving 0.15 and 0.35 mg/Kg of selenium during normal or cold conditions. The cumulative FCR as affected by dietary supplementation of antioxidants in experimental birds is showed in Fig 5.6.

5.5 Nutrient Metabolisability (%) as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The percent nutrient metabolisability as affected by dietary supplementation of antioxidants in experimental birds is given in table 4.5 and Fig 5.7. The digestibility of dry matter and crude fibre increased significantly ($P<0.05$) in T₃ and T₁ as compared to T₀, while T₂ remained comparable. The organic matter and NFE digestibility (%) was significantly higher ($P<0.05$) in T₃ than T₀; whereas T₁ and T₂ ranged in between. The percent crude protein digestibility was significantly higher ($P<0.05$) in T₃ when compared to T₀ and T₂, while T₁ remained intermediate. The percent EE digestibility was comparable ($P>0.05$) among treatment groups. In agreement with present findings Goñi *et al.* (2007) observed significantly higher ($P<0.05$) amino acid digestibility in Vitamin E supplemented broilers than those supplemented with grape pomace. The numerically improved cumulative body weight and FCR in present study could be understood from parallel improvement in nutrient

digestibility of antioxidant supplemented groups over control (Tables 4.3.2, 4.4.2 and 4.5).

The present results are supported by the fact that though apparent digestibility of nutrients might get depressed by oxidative stress, the antioxidant supplementation could probably elevate the oxidative protein denaturation and thereby facilitate the nutrient utilization and consequently improve upon weight gain and FCR (Tatli Seven, 2008 and Seven *et al.*, 2012). Seven *et al.* (2012) reported significant improvement in protein and total ash digestibility in propolis and vitamin C supplemented broilers exposed to lead. Similarly various authors in past had reported positive effect of vitamin C and propolis on broilers subjected to oxidative stress (Ichikawa *et al.*, 2002; Sun *et al.*, 2000 and Tatli Seven, 2008). In contrast with present findings Rezvani and Shojaee (2021) observed no significant effect of watermelon juice, vitamin E, selenium and vitamin C supplementation in drinking water in broilers under heat stress; however, a significant improvement ($P < 0.05$) in prececal digestibility of dry matter was observed in birds supplemented with 60% watermelon juice than vitamin C and control. They attributed this improvement in prececal dry matter digestibility in water melon juice supplemented groups to the increased efficiency of GI tract along with improvement in length of intestine and proventriculus.

5.6 Carcass Characteristics and Shelf life of Meat as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The carcass characteristics and shelf life of meat in experimental birds as affected dietary antioxidants supplementation is showed in table 4.6. The live weight and edible meat weight (g), edible meat (%), giblet weight (g) and (%), carcass weight (g), dressing (%) and shelf life of meat estimated as mg MDA/Kg concentration on 0th, 4th and 8th day in breast and thigh muscle did not differ significantly ($P > 0.05$) among the treatment groups. In contrast with present finding of comparable carcass characteristics and MDA concentration in breast muscle, Leskovec *et al.* (2018) recorded

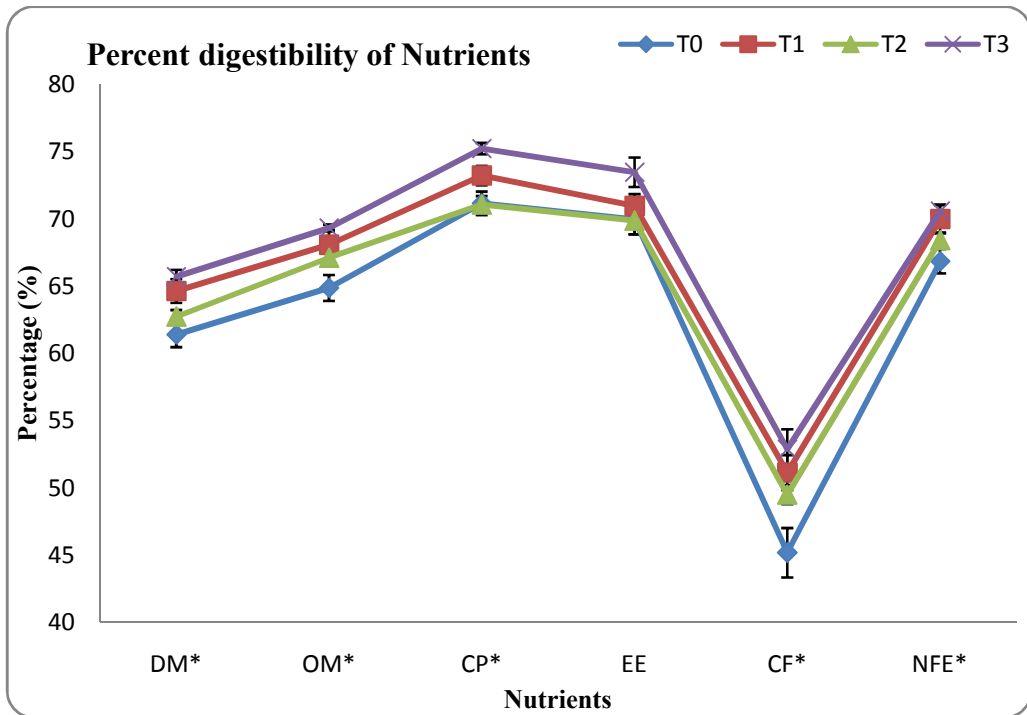


Fig5.7. Nutrient Metabolisability as influenced by dietary antioxidants supplementation in experimental birds

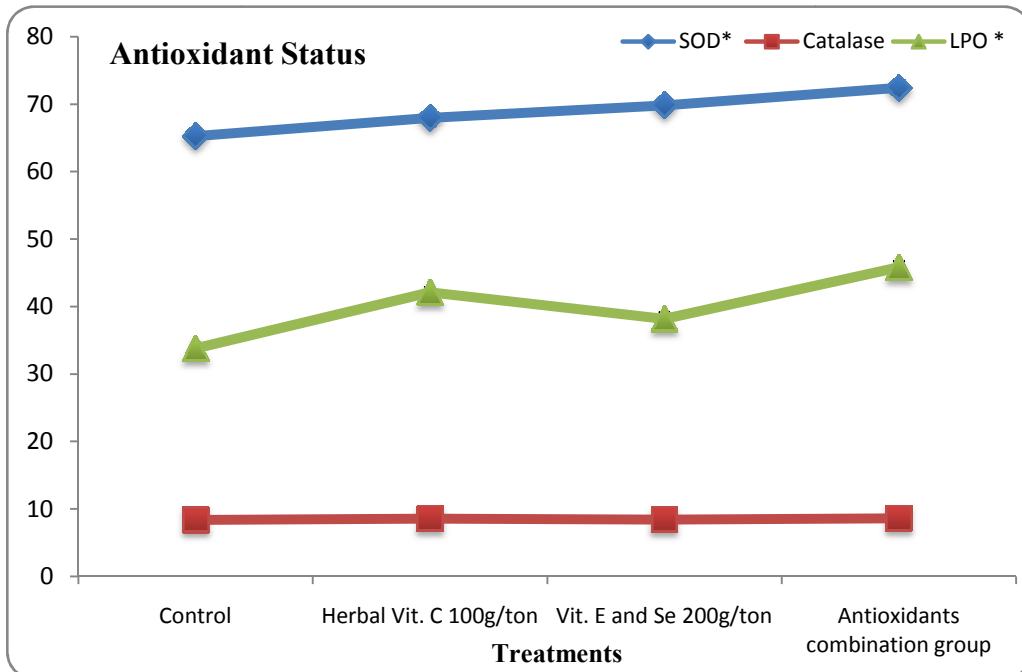


Fig 5.8. Antioxidant status of Chickens RBCs (Erythrocytes) as influenced by dietary antioxidants supplementation in experimental birds

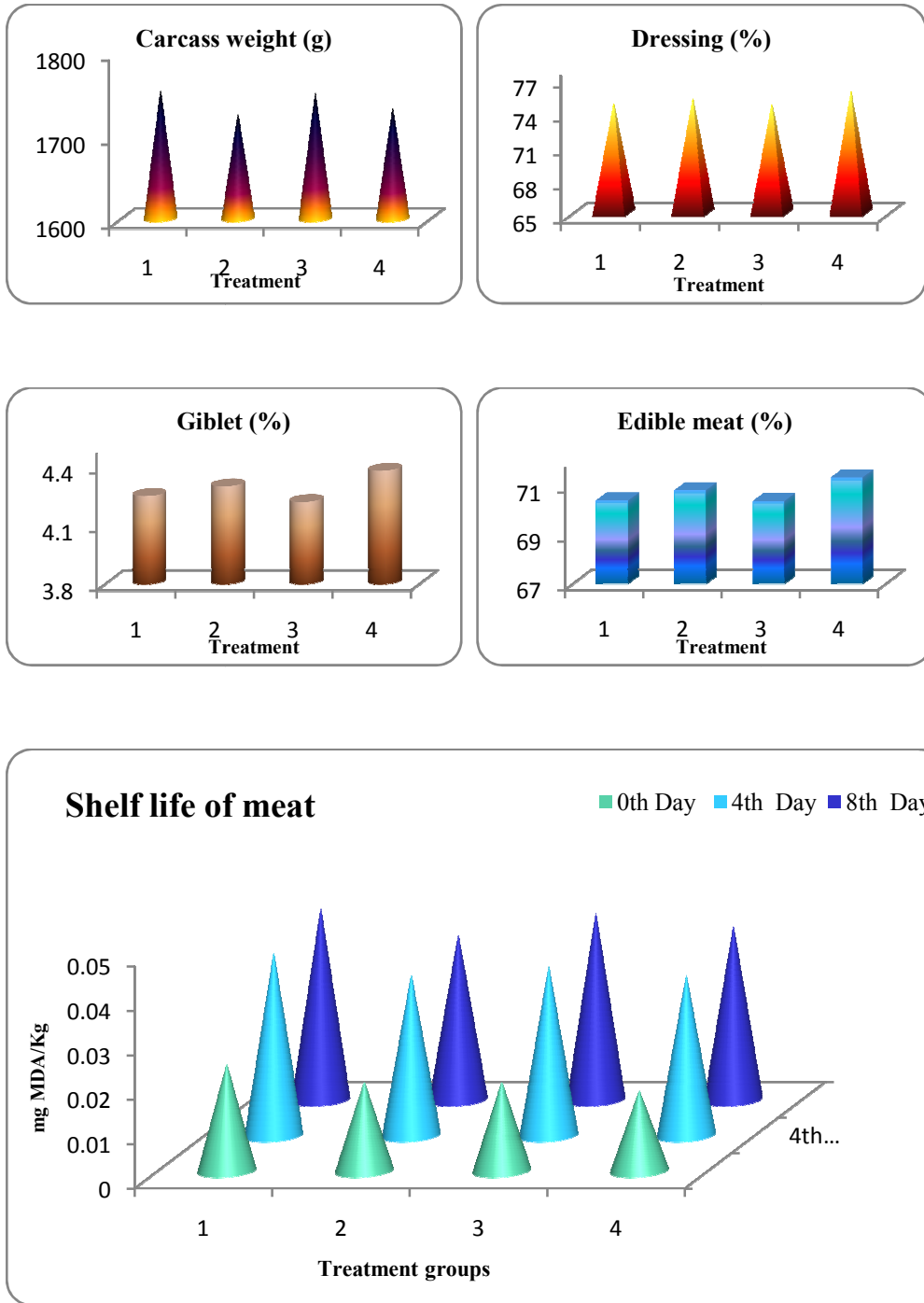


Fig 5.9. Carcass Characteristics and Shelf life of meat as influenced by dietary antioxidants supplementation in experimental birds

increased α -tocopherol concentration in breast muscle of broilers supplemented with vitamin E. They further observed that the vitamin E and multiple antioxidants (Vitamin E, Vitamin C and Selenium) supplemented broilers showed diminished MDA concentration by 49 and 44 percent respectively than control. Leskovec *et al.* (2018) deduced inverse correlation between plasma MDA concentration and dietary inclusion of vitamin E. They further elucidated that though relatively un-specific marker; the MDA is the best indicator of oxidative stress (Lykkesfeldt and Svendsen, 2007). In agreement with present findings Seven *et al.* (2012) recorded comparable carcass characteristics except the breast yield in vitamin C and Propolis supplemented broilers exposed to lead and control.

The numerical improvement in dressing percentage of dietary antioxidants supplemented groups over control in present study might be due to the improved nutrient utilization (Table 4.5). Similarly Seven *et al.* (2012) justified that the comparable carcass yield of lead exposed but Propolis and vitamin C supplemented broilers might be due to probable improvement in protein synthesis and reduction in cortisone synthesis during heat stress. Moreover the antioxidants could have improved the nutrient utilization and had helped to overcome the stress (Tatli Seven *et al.*, 2008 and Hayashi *et al.*, 1994). Goñi *et al.* (2007) recorded depression in lipid oxidation of meat during refrigerated storage in grape pomace and vitamin E supplemented broilers. The present findings of comparable carcass characteristics along with numerically increased dressing percentage in dietary antioxidants supplemented broilers are supported by Choct *et al.* (2004). They observed comparable eviscerated weight, dressed weight and breast or Maryland weight (piece of thigh muscle along with drumstick) in broilers supplemented with different levels of selenium; however when the source was organic selenium, the improvement in eviscerated and Maryland weight as well as in dressed weight was significant ($P < 0.05$). Sahin *et al.* (2003) recorded improvement in hot and chilled carcass weights and yields and weight of liver, heart, spleen and gizzard in broilers supplemented with chromium and/or vitamin C. However, in present study the weight and percentage of various organs were

not affected ($P>0.05$) due to dietary antioxidants supplementation. The carcass characteristics and Shelf life of meat in experimental birds as affected dietary antioxidant supplementation is presented in Fig 5.9.

5.7 Sensory Evaluation as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The sensory evaluation of meat of experimental birds as affected by dietary antioxidants supplementation is portrayed in table 4.7. The sensory evaluation performed by semi trained panelist in terms of appearance, texture, juiciness, flavour and tenderness of meat did not differ significantly ($P>0.05$) among treatment groups. However the overall acceptability was significantly improved ($P<0.05$) in antioxidants combination supplemented groups (T_3) when compared to control (T_0) and herbal Vitamin C (T_1), while T_2 (Vitamin E and Selenium) remained comparable. In consonance with present findings, Skřivan *et al.* (2012) recorded comparable appearance and odour of breast and thigh meat and flavour and overall acceptability for cooked meat in 475 mg/Kg Vitamin C supplemented broilers.

However in present study the overall acceptability of cooked meat was significantly improved ($P<0.05$) in Vitamin C+ Vitamin E and Selenium supplemented group over control and sole Vitamin C supplemented group. On other hand an improvement in sensory quality of dark chicken meat was noted by Bou *et al.* (2001) in vitamin E supplemented broilers fed different sources of fat. Shirzadegan and Falahpour (2014) reported higher overall acceptability and juiciness of thigh meat in medicinal herb extract mixture supplemented broilers. The sensory evaluation of meat of experimental birds as affected by dietary antioxidant supplementation is shown in Fig 5.10.

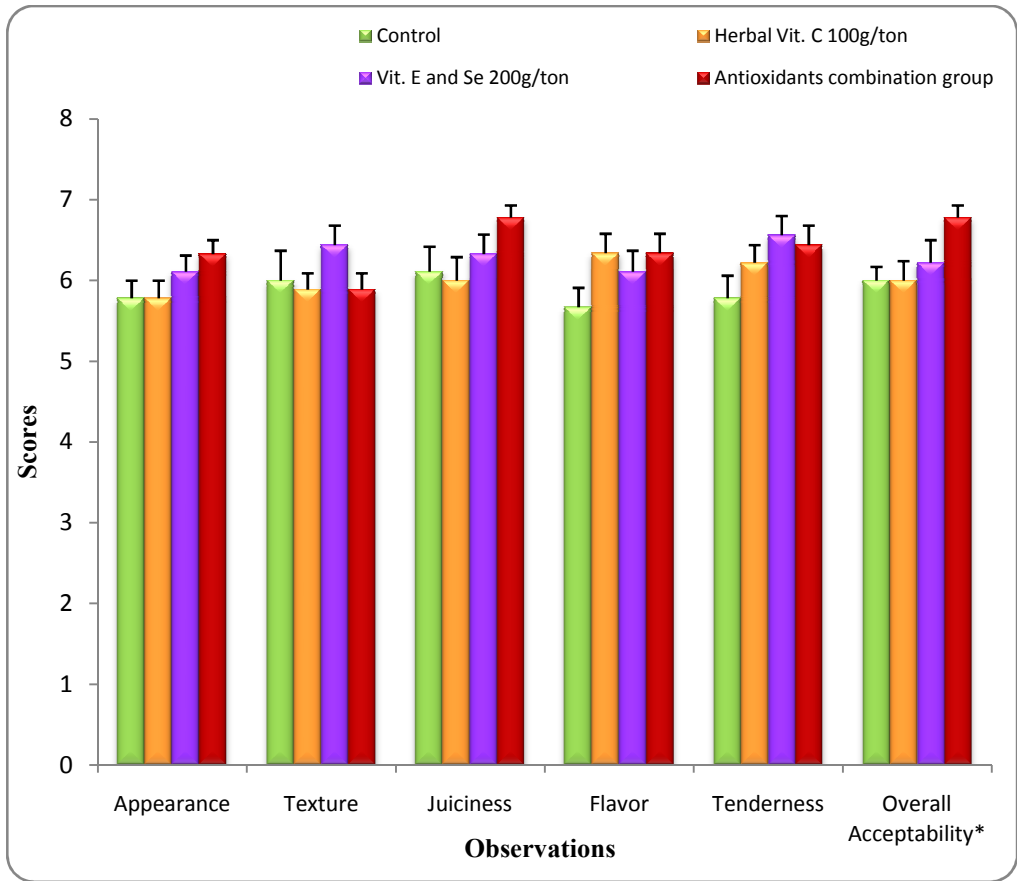


Fig 5.10. Sensory Evaluation as influenced by dietary antioxidants supplementation in experimental birds

5.8 Hematological Parameters, Erythrocytic Antioxidant Enzyme Status and Serum Metabolite Concentration as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

5.8.1 Hematological parameters and erythrocytic antioxidant enzyme status as influenced by dietary antioxidants supplementation in experimental birds

The hematological parameters and erythrocytic antioxidant enzymes status as affected by dietary antioxidant supplementation in experimental birds is presented in table 4.8.1; Fig 5.8 and Fig 5.11. The Hemoglobin (g/dl) was significantly higher ($P<0.05$) in T_2 when compared to T_0 , while T_1 and T_3 remained comparable. The percent PCV, eosinophils, monocytes, basophils remained comparable ($P>0.05$) among treatment groups. TLC ($\times 10^3/\mu\text{l}$) and percent lymphocytes were significantly higher ($P<0.05$) in treatment groups over the control. Conversely, the percent heterophils were significantly reduced ($P<0.05$) in treatment groups than control. The H:L ratio was significantly reduced ($P<0.05$) in antioxidants supplemented groups than control. In agreement with current findings, Kadam *et al.* (2009) also reported significantly reduced ($P<0.05$) percent heterophils along with significantly increased ($P<0.05$) percent lymphocytes in natural, herbal and synthetic vitamin C supplemented broiler chickens under summer stress. They justified the depression in heterophils percent in vitamin C supplemented groups than control might be due to anti-stress activity of vitamin C. Similar to present findings, a reduction in H:L ratio in stressed broilers supplemented with 0.03% vitamin C was reported by Karthiyaini and Philomina (2009). Authors in past have asserted that H:L ratio is reliable indicator of stress in broilers. The present results of significantly reduced ($P<0.05$) heterophils and elevation of lymphocytes is supported by Abd El-Hack *et al.* (2017) who recorded increased lymphocyte count and decreased heterophils, monocytes and eosinophils along with increase in level of selenium supplementation. They also observed significant effect of vitamin E and selenium combination on various hematological parameters excluding basophil count.

The significant increase ($P < 0.05$) in TLC ($\times 10^3/\mu\text{l}$) in antioxidants supplemented groups over the control is in congruence with findings of El-Sebai (2000) and Abd El-Hack *et al.* (2017); who reported 4.65 % rise in WBC count due to Vitamin E supplementation in broilers. It can be understood that Vitamin E acted as major antioxidant and enhanced immunity in chickens by increasing macrophage phagocytic function (Abdukalykova and Ruiz-Ferria, 2006). The improved hemoglobin (g/dl) and total leucocyte count ($\times 10^3/\mu\text{l}$) in present study is supported by earlier reports (Elaroussi *et al.*, 2007; Hanafy *et al.*, 2009 and El-sheikh and Salama, 2010). Selenium supplementation significantly improved ($P < 0.05$) WBC count and Hemoglobin (g/dl) in heat stressed broiler chickens (Hanafy *et al.*, 2009; El-sheikh and Salama, 2010). The present finding of improved hematological parameters due to dietary antioxidant supplementation are in accordance with Abd El-Hack *et al.* (2017), who recorded similar observations in heat stressed laying hens fed vitamin E and selenium either individually or in combination.

The erythrocytic antioxidant indices in present study revealed significantly increased ($P < 0.05$) SOD and LPO concentration in antioxidant supplemented groups than control; whereas a comparable ($P > 0.05$) Catalase concentration was recorded among the treatment groups. Aydemir *et al.* (2000) reported consonant findings of significantly increased ($P < 0.05$) erythrocyte SOD activity and comparable Catalase activity in broilers under stress and supplemented with vitamin A, C, E and trace minerals like copper and selenium; However the LPO activity decreased significantly in antioxidant vitamins and minerals supplemented groups. The improved antioxidant activity in erythrocytes of broilers in treatment groups indicated enhanced free radical phagocytic activity and savior of biological membranes from attack of free radicals (Aydemir *et al.*, 2000). Aydemir *et al.* (2000) opined that the improved glutathione peroxidase activity in their study might be due to the fact that Selenium is an integral part of GSH-Px, catalyzing the phagocytosis of free radicals. The significantly improved ($P < 0.05$) SOD and LPO activity and numerically increased ($P > 0.05$) Catalase activity in erythrocytes of antioxidants supplemented broiler in present study corroborated well with

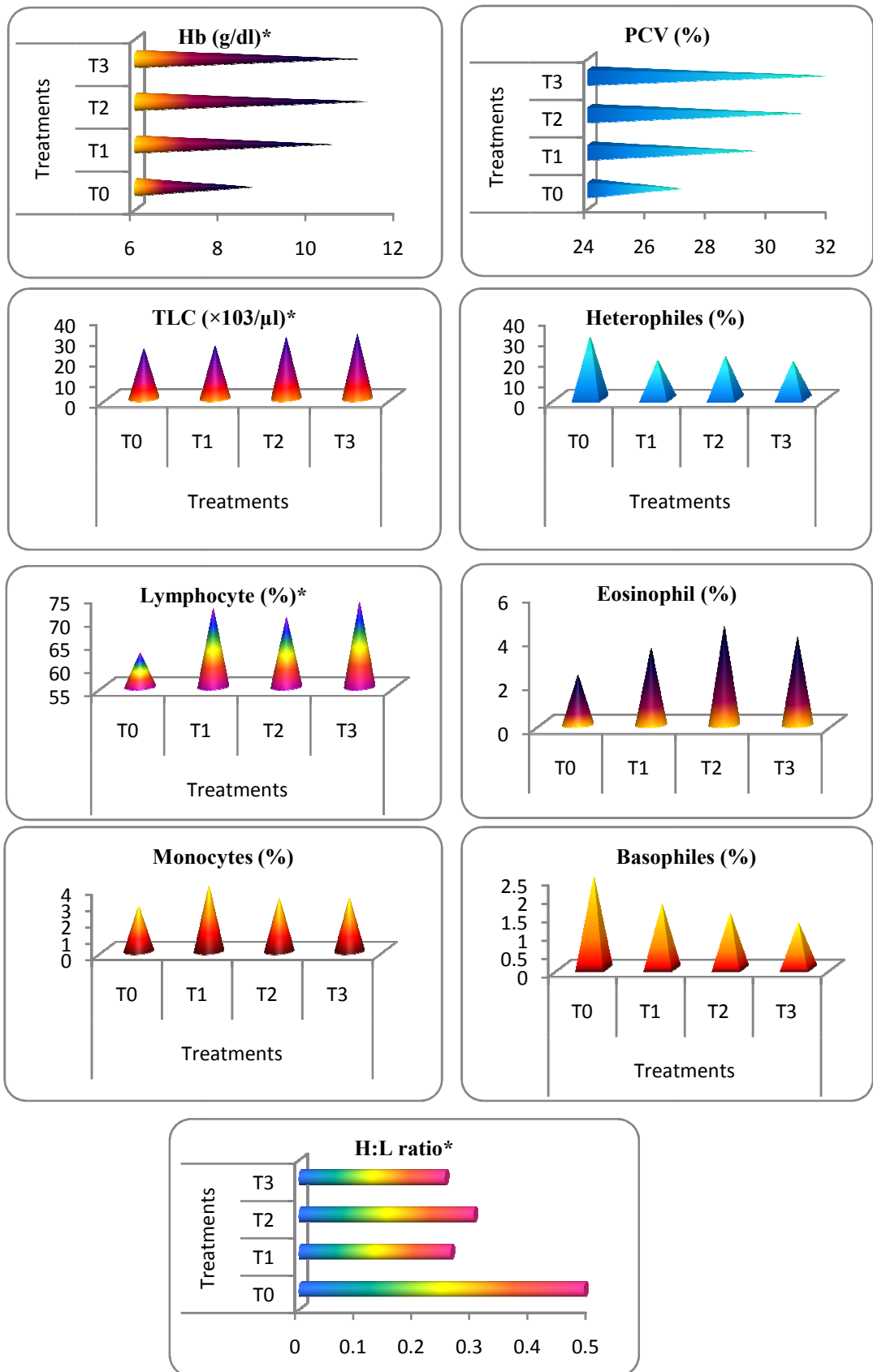


Fig 5.11. Hematological parameters as influenced by dietary antioxidants supplementation in experimental birds

earlier finding of improved nutrient utilization (Table 4.5) and numerically improved FCR (Table 4.4.2) in said groups. Maini *et al.* (2007) also concluded that oxidative stress in broilers could be ameliorated through dietary supplementation of vitamin E, Amla electrolyte and mint; which was evident by increased GSH, catalase, SOD and glutathione reductase activity in antioxidant supplemented broilers. Ghazi Harsini *et al.* (2012) recorded minimum MDA concentration and maximum SOD activity in skeletal muscles in heat stressed broilers supplemented with selenium, vitamin E and their combinations in diet.

5.8.2 Serum metabolite concentration as influenced by dietary antioxidants supplementation in experimental birds

The serum metabolite concentration in experimental birds as affected by dietary antioxidants supplementation is presented in table 4.8.2 and Fig 5.12. The glucose (mg/dl), globulin (g/dl), A:G ratio, SGOT and SGPT (IU/L) did not differ significantly ($P>0.05$) among the treatment groups. The total protein (g/dl) was significantly higher ($P<0.05$) in T_3 than T_0 and T_2 , while T_1 remained comparable. The albumin (g/dl) was significantly higher ($P<0.05$) in T_3 as compared to control; whereas T_1 and T_2 ranged in between. There was a numerical reduction ($P>0.05$) in uric acid (mg/dl) in antioxidant supplemented groups than control. Similar to the present findings significant elevation ($P<0.05$) in serum total protein, albumin and total cholesterol concentration on selenium supplementation was noted by Abd El-Hack *et al.* (2017). They further noted significant reduction in serum AST and gradual elevation in total lipids, cholesterol and calcium levels along with increasing level of vitamin E supplementation. In present study the values of SGOT and SGPT were comparable ($P>0.05$). Further in congruence with present findings, Abd El-Hack *et al.* (2017) recorded comparable blood constituents except serum globulin, ALT and calcium concentration due to interaction of vitamin E and selenium supplementation levels. They attributed the rise in serum total protein and albumin levels to augmented transport and synthesis of sex hormones in birds maintained in comfortable conditions (Ranaweera and Wise, 2008).

Ghazi Harsini *et al.* (2012) recorded interesting observation that the serum glucose and uric acid concentration were significantly higher ($P < 0.05$) in birds under heat stress; which however were not affected on dietary vitamin E or Selenium supplementation in birds maintained in thermo-neutral zone. However, they further noted that the uric acid concentration was significantly reduced ($P < 0.05$) in broilers supplemented in 125 mg/Kg vitamin E as compared to control (T_0). Similarly in present study though not significant ($P > 0.05$) a numerical decline in uric acid concentration was recorded in antioxidant supplemented groups. Ghazi Harsini *et al.* (2012) opined that the significant reduction in serum uric acid and glucose concentration in selenium and vitamin E supplemented groups was recorded, plausibly due to alleviation of muscle catabolism and reduction in effect of corticosterone due to antioxidant nutrient supplementation. They further elucidated that the protein catabolism in body gets inhibited due to enhanced antioxidant capacity through selenium and vitamin E supplementation; which consequently is recorded as decreased uric acid and glucose concentration. Adeyemi *et al.* (2015) observed no significant influence ($P > 0.05$) of quantitative feed restriction and ascorbic acid supplementation on hematological and serum biochemical parameters. In agreement with present findings Rindhe *et al.* (2016) reported improvement in total protein and albumin concentration in herbal anti-stress premix supplemented broilers than control.

5.9 Immune Response as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The immune response in terms of weight of immune organs and serum antibody titres against RD virus in experimental birds as affected by dietary antioxidants supplementation is projected in table 4.9. The weight of bursa and spleen (g) did not differ significantly ($P > 0.05$) among treatment groups. The mean serum antibody titres at days 21 and 42 was significantly improved ($P < 0.05$) in T_3 (antioxidants combination) when compared to T_0 (control), whereas T_1 and T_2 ranged in between. The raised antibody titres in antioxidant supplemented groups are indicative of better health of broiler

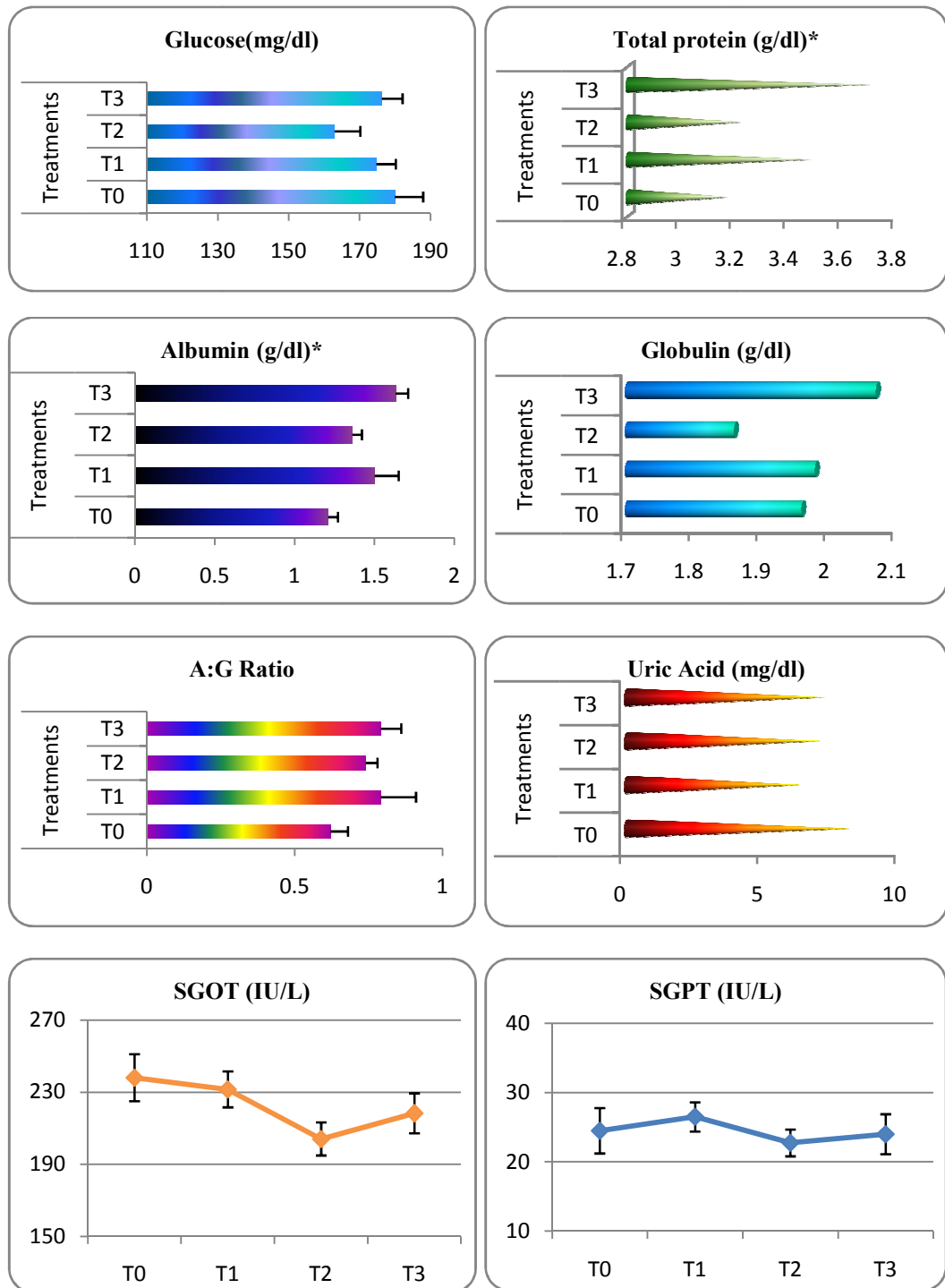


Fig 5.12. Serum metabolite concentration as influenced by dietary antioxidants supplementation in experimental birds

chickens in said groups. Singh *et al.* (2006) also reported similar findings of higher HI titres ($P < 0.05$) in broilers supplemented with selenium and vitamin E in combination. They observed higher bursa and spleen weight and lower thymus weight due to selenium supplementation. The authors attributed improvement in immune response to synergistic effect of vitamin E and selenium leading to increase lymphoid organ weight as well as higher HI titres (Siddique, 1991; Swain *et al.*, 2000). Kadam *et al.* (2009) noted higher spleen, bursa and thymus weights in vitamin C supplemented broilers under summer stress. They elucidated higher ambient temperature elicit lymphoid organ depression through increased corticosterone levels (Gross *et al.*, 1980); which might be alleviated by supplementation of antioxidants like vitamin C.

Habibian *et al.* (2013) noted that the weight of liver and lymphoid organs get reduced in birds exposed to heat stress, which could not be revived through selenium supplementation alone (Bartlett and Smith, 2003; Ghazi *et al.*, 2012); however vitamin E supplementation increased the relative weights of liver and lymphoid organs significantly ($P < 0.05$) in their study. The comparable immune organ weight in present study are supported by findings of Niu *et al.* (2009 a, b) who observed similar lymphoid organ weight in control and vitamin E and selenium supplemented broilers reared in thermoneutral as well as heat stressed ambience. Swain *et al.* (2000) reported significantly increased HI titres against NDV in 150 IU/Kg vitamin E supplemented broilers than control. They concluded that supplementation of vitamin E and selenium at 150 IU/Kg and 0.1 mg/Kg respectively in combination improved health, immunity and performance of broilers. The immune response in terms of weight of immune organs and serum antibody titres against RD virus in experimental birds as affected by dietary antioxidant supplementation is projected in Fig 5.13.

5.10 Cost Economics of Broiler Production as Influenced by Dietary Antioxidants Supplementation in Experimental Birds

The economics of broiler production as affected by dietary antioxidants supplementation is illustrated in table 4.10. After working out

cost economics of experimental broiler production it was noted that the total cost of production (Rs.) was higher in control (207.95) followed by T₂ (206.16), T₃ (205.26) and T₁ (203.59) respectively. The average price realized (Rs.) by selling broilers at Rs.75/Kg was 168.00 (T₀), 171.75(T₁ and T₂) and 174.75 (T₃) respectively. Though a loss was incurred, the percent abatement in loss in treatment groups over control was recorded 20.30% in T₁, 13.87% in T₂ and 23.63 % in T₃ respectively. Hence it was noted that the loss could be minimized due to dietary antioxidants supplementation. In agreement with present finding Begum *et al.* (2019) recorded higher gross profit in Amla (*Emblica officinalis*) powder supplemented broiler chickens. Similarly, Gaikwad *et al.* (2016) also recorded improved performance and economics in Amla supplemented broilers. Gaikwad *et al.* (2014) concluded that inclusion of Shatawari root and Amla fruit powder at 1% dietary level was economical and also improved performance of broilers. Kennedy *et al.* (1992) reported increment in net income due to enhanced average weight per bird and FCR due to increase in vitamin E supplementation in broiler diet. Patel *et al.* (2016) also reported higher net profit in broilers supplemented with Amla fruit powder at 0.4 and 0.8 percent of diet. Abatement of Loss of Broiler Production as influenced by dietary antioxidants supplementation in experimental birds is illustrated in Fig 5.14.

On rumination of results obtained for various response parameters in present experiments, it is concluded that dietary supplementation of Herbal vitamin C in combination with vitamin E and Selenium could improve overall performance, hematological and blood biochemical parameters, erythrocyte antioxidant enzyme status, overall acceptability of broiler meat, immune response and economics of broiler production without any adverse effect on carcass characteristics and Shelf life of meat.

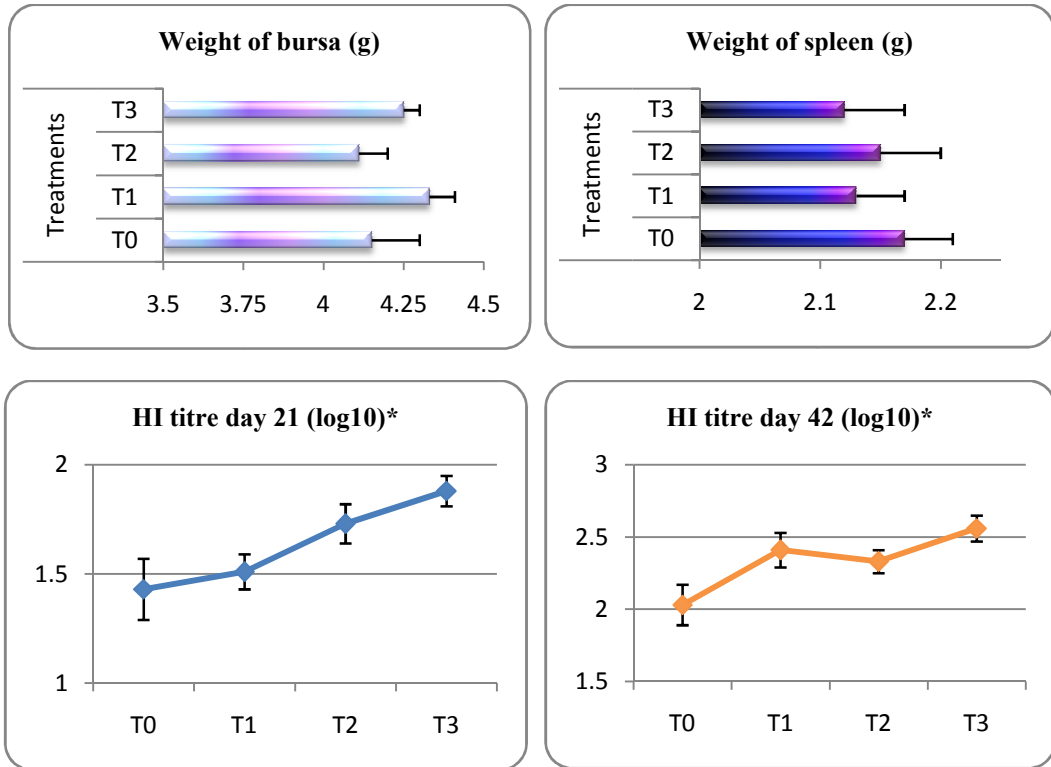


Fig 5.13. Immune Response as influenced by dietary antioxidants supplementation in experimental birds

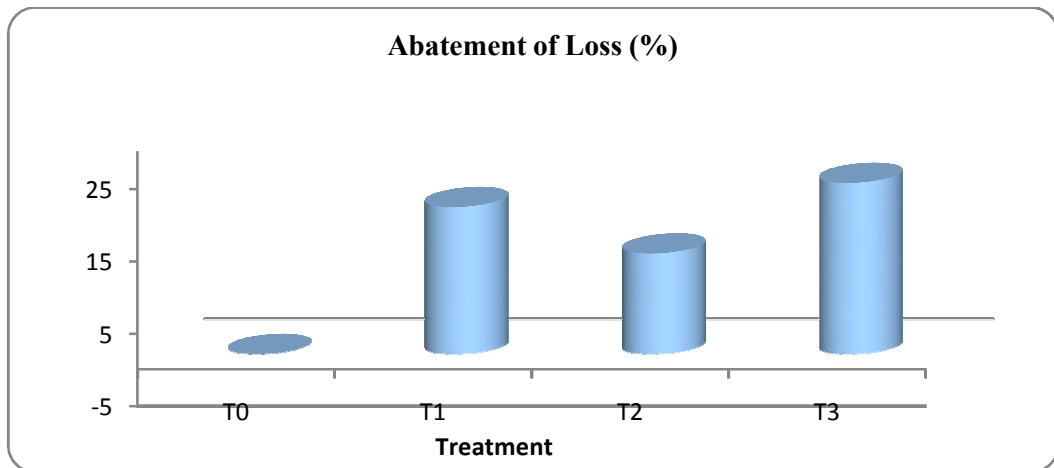


Fig 5.14. Abatement of Loss of Broiler Production as influenced by dietary antioxidants supplementation in experimental birds

CHAPTER VI

SUMMARY AND CONCLUSIONS

An experiment entitled “Effect of dietary antioxidants (Vitamin C, Vitamin E and Selenium) supplementation on performance and immune response in broilers” was conducted to assess the growth performance, feed consumption, weight gain, feed conversion ratio, nutrient metabolisability, carcass parameters, shelf life of meat, sensory evaluation, hematological parameters, serum metabolite concentrations, immune response, antioxidant enzymes indices and cost economics of broilers production.

The total of two hundred and forty day old straight run chicks of vencobb-430 were weighed and distributed randomly into 4 treatments which were composed of one control and three treatment groups, also each treatment group was divided into 4 replicates with 15 chicks each which were housed in individual pens for each replicate in two opposite rows. Experimental birds were raised on deep litter system and *ad-libitum* clean and fresh drinking water was given to the broiler birds. Artificial temperature stress was induced in all pens by maintaining the shed temperature between 35 to 40⁰C (dry bulb temperature 95-104⁰F) after completion of brooding till the end of trial using lighting arrangements. The birds were provided with standard broiler diets as per BIS (2007) standards. Ground maize was made available to chicks on papers on the first day of arrival and from next subsequent day prestarter ration was offered during 1 to 8 days of age with crude protein of 23.06 percent and metabolizable energy of 3004.1 Kcal/Kg; whereas, starter diet (21.98% CP and 3100.4 Kcal/Kg of ME) was given from 9 to 21 days and finisher diet was fed during 4th to 6th week containing 19.94 percent of crude protein and 3203.8 Kcal/Kg metabolizable energy.

The diets were grouped into four treatments; the control (T₀) was without antioxidant addition while T₁ was having 100g/ton of herbal vitamin C; T₂ was added with 200g/ton of vitamin E and selenium and T₃ was composed with combination of 100g/ton herbal vitamin C and 200g/ton

vitamin E & selenium respectively. At the end of 42nd day of experiment the average cumulative body weights for groups T₀, T₁, T₂ and T₃ were 2200.88, 2249.04, 2250.67 and 2289.95 (g) respectively, indicating that the highest body weight of birds in T₃ (combination of antioxidants) group as compared to T₀, T₁ and T₂ groups.

The mean weekly gain in body weights at the end of 6th week were 500.90, 537.82, 552.15 and 528.57 (g) for treatment groups T₀, T₁, T₂ and T₃ respectively. However, the average weekly body weight in 1st to 3rd groups was not significant (P>0.05) among the all treatment groups. Average weekly body weight gain in 4th week were significantly higher (P<0.05) in T₀ than in T₂, whereas T₁ and T₃ remained comparable. However, the mean weekly body weight gain (g) in 5th and 6th week remained comparable (P>0.05) among the treatment groups.

The weekly mean feed intake (g) of 1st week was significantly higher (P<0.05) in control (T₀) than other treatment groups (T₁, T₂ and T₃). The mean weekly feed intake (g) for 2nd, 3rd, 5th and 6th week did not differ significantly (P>0.05) among the treatment groups. However, in 4th week the mean weekly feed intake (g) was significantly higher (P<0.05) in T₃ (antioxidants combination group) as compared to other treatment groups (T₁ and T₂) and T₀ (Control). Whereas, cumulative feed intake (g) remained comparable (P>0.05) throughout the experiment during 0 to 21 days (starter), 22 to 42 days (finisher) and 0 to 42 days (overall period).

During 1st week the FCR was significantly improved (P<0.05) in T₃ than T₀, while T₁ and T₂ ranged in between. Conversely in 4th week the FCR was significantly improved (P<0.05) in T₀ than T₂ and T₃ while T₁ remained comparable. During 2nd, 3rd, 5th and 6th weeks the FCR remained comparable (P>0.05) among the antioxidant treatment groups. The average cumulative feed conversion ratio per bird from 0 to 21 days and 22 to 42 days did not differ significantly (P>0.05) however, for overall period (0 to 42 days) average cumulative FCR was numerically improved (P=0.056) in T₃ (1.59) than in T₀ (1.69) whereas, T₁ (1.61) and T₂ (1.62) remained comparable.

The percent digestibility of dry matter and crude fibre was significantly improved ($P<0.05$) in T_1 (herbal vitamin C 100g/ton) and T_3 (antioxidant combination group) when compared to T_0 ; whereas T_2 remained comparable. The percent digestibility of organic matter, crude protein and nitrogen free extract was significantly higher ($P<0.05$) in T_3 (antioxidant combination group). The percent ether extract (EE) digestibility did not differ significantly ($P>0.05$) among the treatment groups.

The live weight and carcass characteristics namely edible meat weight (g) and percentage, giblet weight (g) and percentage, carcass weight (g) and dressing percentage did not differ significantly ($P>0.05$) among the treatment groups. The shelf life of meat (mg MDA/Kg) assessed on 0th, 4th and 8th day remained comparable ($P>0.05$) among the treatment groups. The sensory evaluation was carried out on 7-point hedonic scale by semi-trained panelist having scores for overall acceptability significantly higher ($P<0.05$) in T_3 (antioxidants combination group) than T_0 , whereas T_1 and T_2 remained comparable.

The haematological parameters, Hb (g/dl) at the end of sixth week was significantly higher ($P<0.05$) in T_2 (Vitamin E and Selenium) while percent PCV, eosinophils, basophils and monocytes did not differ significantly ($P>0.05$). The TLC ($\times 10^3/\mu\text{l}$) and percent lymphocyte was significantly lower ($P<0.05$) in T_0 (Control) when compared to all the treatment groups. The percent heterophils increased significantly ($P<0.05$) in Control (T_0) over the treatment groups. The H:L ratio was significantly reduced ($P<0.05$) in antioxidants supplemented groups than control.

Among the erythrocytic antioxidant enzyme assessed, the SOD (nm MDA/ml) and LPO ($\times 10^2$ nM MDA/ml) activity was significantly higher ($P<0.05$) in treatment groups (T_3 , T_2 and T_1) over the control (T_0) in the said order. However, the Catalase (mM H_2O_2 /min/mg) remained comparable ($P>0.05$) among all experimental groups. The serum metabolites, glucose, globulin (mg/dl) and A:G ratio did not differ significantly ($P>0.05$) among treatment groups. The total protein and albumin (g/dl) were significantly

higher ($P < 0.05$) in T_3 (antioxidants combination group) and was recorded equal to 3.70 and 1.63 respectively. The Uric acid (mg/dl) reduced numerically ($P > 0.05$) than control. The mean values of SGOT and SGPT (IU/L) did not differ significantly ($P > 0.05$) among the treatment groups.

The immune response in relation to weights (g) of immune organ i.e. bursa and spleen did not differ significantly ($P > 0.05$). However the serum antibody titre against RD virus was significantly higher ($P < 0.05$) in T_3 (antioxidants combination group) over T_0 (control) and T_1 and T_2 remained comparable at both 21st and 42nd day of experiment respectively.

The cost of feed in rupees per Kg live weight gain was highest for T_0 (Rs.74.53) followed by T_2 (Rs.72.13), T_1 (Rs.71.00) and the lowest for T_3 (Rs.70.50) groups. The profit/loss statement indicates that the loss in broiler production was reduced in treatment groups than the control (T_0) and was minimized by 20.30% in T_1 , 13.87% in T_2 and 23.63% in T_3 respectively over the control groups.

CONCLUSION

The research findings of present study are concluded precisely in the following points:

- 1) It is concluded that dietary supplementation antioxidants in combination (Herbal Vitamin C 100g/ton with Vitamin E and Selenium 200g/ton) improved cumulative feed conversion ratio and nutrient utilization in broilers.
- 2) It is concluded that dietary supplementation of antioxidants separately or in combination did not affect the carcass characteristics and Shelf life of meat but improved overall acceptability of meat.
- 3) It is concluded that dietary supplementation of antioxidants separately or in combination improved hemato-biochemical parameters, erythrocytic antioxidant enzyme status and immune response in broilers.
- 4) It is concluded that dietary supplementation of antioxidants in combination improved the overall performance and net profit of broiler production under stressed condition.

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APPENDIX I

Analysis of variance of weekly feed intake (g/bird) as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
I Week				
Between Groups	3	422.919	140.973	15.182
Within Groups	12	111.423	9.285	-
Total	15	534.342	-	-
II Week				
Between Groups	3	2024.529	674.843	1.495
Within Groups	12	5415.392	451.283	-
Total	15	7439.921	-	-
III Week				
Between Groups	3	2227.015	742.338	1.089
Within Groups	12	8177.891	681.491	-
Total	15	10404.907	-	-
IV Week				
Between Groups	3	3155.999	1052.000	11.202
Within Groups	12	1126.981	93.915	-
Total	15	4282.980	-	-
V Week				
Between Groups	3	2260.804	753.601	0.988
Within Groups	12	9153.751	762.813	-
Total	15	11414.555	-	-
VI Week				
Between Groups	3	6731.000	2243.667	2.166
Within Groups	12	12429.000	1035.750	-
Total	15	19160.000	-	-

APPENDIX II

Analysis of variance of Cumulative feed intake (g) as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
0-21 Days				
Between Groups	3	7653.120	2551.040	2.093
Within Groups	12	14626.434	1218.870	-
Total	15	22279.554	-	-
21-42 Days				
Between Groups	3	8794.460	2931.487	1.622
Within Groups	12	21686.399	1807.200	-
Total	15	30480.859	-	-
0-42 Days				
Between Groups	3	19919.838	6639.946	2.401
Within Groups	12	33187.789	2765.649	-
Total	15	53107.628	-	-

APPENDIX III

Analysis of variance of weekly body weight gain (g/bird) as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
I Week				
Between Groups	3	68.117	22.706	1.769
Within Groups	12	154.066	12.839	-
Total	15	222.183	-	-
II Week				
Between Groups	3	1368.542	456.181	1.385
Within Groups	12	3951.048	329.254	-
Total	15	5319.590	-	-
III Week				
Between Groups	3	713.931	237.977	0.395
Within Groups	12	7229.827	602.486	-
Total	15	7943.758	-	-
IV Week				
Between Groups	3	10380.620	3460.207	4.041
Within Groups	12	10275.933	856.328	-
Total	15	20656.552	-	-
V Week				
Between Groups	3	20909.945	6969.982	2.971
Within Groups	12	28151.417	2345.951	-
Total	15	49061.362	-	-
VI Week				
Between Groups	3	5601.441	1867.147	0.478
Within Groups	12	46858.156	3904.846	-
Total	15	52459.598	-	-

APPENDIX IV

Analysis of variance of cumulative body weights (g/bird) as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
0-21 Days				
Between Groups	3	1192.380	397.460	0.497
Within Groups	12	9597.249	799.771	-
Total	15	10789.629	-	-
21-42 Days				
Between Groups	3	10929.729	3643.243	0.598
Within Groups	12	73164.064	6097.005	-
Total	15	84093.793	-	-
0-42 Days				
Between Groups	3	16556.024	5518.675	1.408
Within Groups	12	47033.267	3919.439	-
Total	15	63589.292	-	-

APPENDIX V

Analysis of variance of weekly feed conversion ratio (FCR) as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
I Week				
Between Groups	3	0.022	0.007	4.874
Within Groups	12	0.018	0.002	-
Total	15	0.041	-	-
II Week				
Between Groups	3	0.194	0.065	1.436
Within Groups	12	0.541	0.045	-
Total	15	0.735	-	-
III Week				
Between Groups	3	0.026	0.009	0.681
Within Groups	12	0.150	0.013	-
Total	15	0.176	-	-
IV Week				
Between Groups	3	0.151	0.050	4.150
Within Groups	12	0.145	0.012	-
Total	15	0.296	-	-
V Week				
Between Groups	3	0.322	0.107	2.466
Within Groups	12	0.522	0.043	-
Total	15	0.843	-	-
VI Week				
Between Groups	3	0.177	0.059	1.762
Within Groups	12	0.401	0.033	-
Total	15	0.577	-	-

APPENDIX VI

Analysis of variance of Cumulative feed conversion ratio (FCR) as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
0-21 Days				
Between Groups	3	0.032	0.011	1.485
Within Groups	12	0.087	0.007	-
Total	15	0.119	-	-
21-42 Days				
Between Groups	3	0.020	0.007	0.828
Within Groups	12	0.096	0.008	-
Total	15	0.116	-	-
0-42 Days				
Between Groups	3	0.023	0.008	3.333
Within Groups	12	0.028	0.002	-
Total	15	0.051	-	-

APPENDIX VII

Analysis of variance of nutrient metabolisability as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
DM				
Between Groups	3	44.588	14.863	6.904
Within Groups	12	25.831	2.153	-
Total	15	70.419	-	-
OM				
Between Groups	3	42.647	14.216	5.277
Within Groups	12	32.327	2.694	-
Total	15	74.973	-	-
CP				
Between Groups	3	46.899	15.633	8.225
Within Groups	12	22.809	1.901	-
Total	15	69.709	-	-
EE				
Between Groups	3	33.872	11.291	2.681
Within Groups	12	50.540	4.212	-
Total	15	84.413	-	-
CF				
Between Groups	3	130.519	43.506	5.550
Within Groups	12	94.061	7.838	-
Total	15	224.580	-	-
NFE				
Between Groups	3	33.636	11.212	4.505
Within Groups	12	29.868	2.489	-
Total	15	63.505	-	-

APPENDIX VIII

Analysis of variance of Carcass characteristics and shelf life of meat as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
Live weight (g)				
Between Groups	3	12693.000	4231.000	0.430
Within Groups	12	118126.000	9843.833	-
Total	15	130819.000	-	-
Edible meat weight (g)				
Between Groups	3	2293.250	764.417	0.158
Within Groups	12	58192.500	4849.375	-
Total	15	60485.750	-	-
Edible meat (%)				
Between Groups	3	2.455	0.818	0.397
Within Groups	12	24.748	2.062	-
Total	15	27.203	-	-
Giblet weight (g)				
Between Groups	3	4.250	1.417	0.060
Within Groups	12	281.500	23.458	-
Total	15	285.750	-	-
Giblet (%)				
Between Groups	3	0.058	0.019	0.907
Within Groups	12	0.257	0.021	-
Total	15	0.315	-	-
Carcass weight (g)				
Between Groups	3	2334.000	778.000	0.146
Within Groups	12	63913.000	5326.083	-
Total	15	66247.000	-	-
Dressing (%)				
Between Groups	3	3.257	1.086	0.505
Within Groups	12	25.804	2.150	-
Total	15	29.061	-	-
0th day (mg MDA/Kg)				
Between Groups	3	0.000	0.000	1.069
Within Groups	12	0.000	0.000	-
Total	15	0.000	-	-
4th day (mg MDA/Kg)				
Between Groups	3	0.000	0.000	0.943
Within Groups	12	0.000	0.000	-
Total	15	0.000	-	-
8th day (mg MDA/Kg)				
Between Groups	3	0.000	0.000	0.219
Within Groups	12	0.000	0.000	-
Total	15	0.000	-	-

APPENDIX IX

Analysis of variance of Sensory evaluation as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
Appearance				
Between Groups	3	2.000	0.667	1.778
Within Groups	12	12.000	0.375	-
Total	15	14.000	-	-
Texture				
Between Groups	3	1.889	0.630	1.007
Within Groups	12	20.000	0.625	-
Total	15	21.889	-	-
Juiciness				
Between Groups	3	3.194	1.065	1.847
Within Groups	12	18.444	0.576	-
Total	15	21.639	-	-
Flavour				
Between Groups	3	2.667	0.889	1.684
Within Groups	12	16.889	0.528	-
Total	15	19.556	-	-
Tenderness				
Between Groups	3	3.194	1.065	1.941
Within Groups	12	17.556	0.549	-
Total	15	20.750	-	-
Overall acceptability				
Between Groups	3	3.639	1.213	2.960
Within Groups	12	13.111	0.410	-
Total	15	16.750	-	-

APPENDIX X

Analysis of variance of haematological Parameters as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
Hb (g/dl)				
Between Groups	3	17.142	5.714	4.146
Within Groups	12	16.537	1.378	-
Total	15	33.679	-	-
PCV (%)				
Between Groups	3	52.688	1.700	1.073
Within Groups	12	91.750	1.583	-
Total	15	144.438	-	-
TLC ($\times 10^3/\mu\text{l}$)				
Between Groups	3	137.175	0.903	0.620
Within Groups	12	59.559	1.356	-
Total	15	196.669	-	-
Heterophiles (%)				
Between Groups	3	377.000	125.667	20.107
Within Groups	12	75.000	6.250	-
Total	15	452.000	-	-
Lymphocyte (%)				
Between Groups	3	292.200	97.417	19.008
Within Groups	12	61.500	5.125	-
Total	15	353.750	-	-
Eosinophil (%)				
Between Groups	3	11.188	3.729	2.157
Within Groups	12	20.750	1.729	-
Total	15	31.938	-	-
Monocytes (%)				
Between Groups	3	3.188	1.063	0.573
Within Groups	12	22.250	1.854	-
Total	15	25.437	-	-
Basophiles (%)				
Between Groups	3	3.500	1.167	1.217
Within Groups	12	11.500	0.958	-
Total	15	15.000	-	-
H:L ratio				
Between Groups	3	0.143	0.048	29.176
Within Groups	12	0.020	0.002	-
Total	15	0.163	-	-

APPENDIX XI

Analysis of variance of Erythrocytic antioxidant status as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
SOD (nM MDA/ml)				
Between Groups	3	109.920	36.640	18.540
Within Groups	12	23.715	1.976	-
Total	15	133.635	-	-
Catalase (mM H₂O₂/min/mg)				
Between Groups	3	0.166	0.055	0.477
Within Groups	12	1.390	0.116	-
Total	15	1.555	-	-
LPO ($\times 10^2$ nM MDA/ml)				
Between Groups	3	318.301	106.100	43.389
Within Groups	12	29.344	2.445	-
Total	15	347.645	-	-

APPENDIX XII

Analysis of variance of Serum metabolite concentration as influenced by dietary supplementation of antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
Glucose (mg/dl)				
Between Groups	3	650.045	216.682	1.183
Within Groups	12	2197.805	183.150	-
Total	15	2847.850	-	-
Total protein (g/dl)				
Between Groups	3	0.724	0.241	9.857
Within Groups	12	0.294	0.024	-
Total	15	1.018	-	-
Albumin (g/dl)				
Between Groups	3	0.394	0.131	3.692
Within Groups	12	0.427	0.036	-
Total	15	0.821	-	-
Globulin (g/dl)				
Between Groups	3	0.091	0.030	0.542
Within Groups	12	0.673	0.056	-
Total	15	0.764	-	-
A:G Ratio				
Between Groups	3	0.075	0.025	0.996
Within Groups	12	0.301	0.025	-
Total	15	0.376	-	-
Uric Acid (mg/dl)				
Between Groups	3	6.785	2.262	1.559
Within Groups	12	17.407	1.451	-
Total	15	24.192	-	-
SGOT (IU/L)				
Between Groups	3	2723.188	907.729	1.908
Within Groups	12	5707.750	475.646	-
Total	15	8430.938	-	-
SGPT (IU/L)				
Between Groups	3	29.188	9.729	0.357
Within Groups	12	326.750	27.229	-
Total	15	355.938	-	-

APPENDIX XIII

Analysis of variance of weight of Immune organs and serum antibody titer against RD virus as influenced by dietary supplementation of Antioxidants in experimental birds

Source	Degree of freedom	Sum of squares	Mean square	F
Weight of bursa				
Between Groups	3	0.122	0.041	1.031
Within Groups	12	0.475	0.040	-
Total	15	0.598	-	-
Weight of spleen				
Between Groups	3	0.006	0.002	0.217
Within Groups	12	0.105	0.009	-
Total	15	0.110	-	-
RD titre (21st day)				
Between Groups	3	0.515	0.172	3.604
Within Groups	12	0.566	0.047	-
Total	15	1.082	-	-
RD titre (42nd day)				
Between Groups	3	0.589	0.196	4.000
Within Groups	12	0.589	0.049	-
Total	15	1.178	-	-

VITA

The author **Mr. Nandanwar Chetan Bhaurao** is born on 18th December, 1996 at Chamorshi, Tal. Chamorshi, Dist.Gadchiroli, Maharashtra state. He completed secondary school certificate (SSC) examination in 2012 from Platinum Jubilee High School, Gadchiroli in first class with distinction and subsequently completed higher secondary (HSC) examination in 2014 from Dr. Ambedkar College, Deeksha Bhoomi, Nagpur in first class.

Later he joined veterinary profession due to his special aptitude and interest for animal welfare activities and has successfully completed B.V.Sc. & A.H. from Nagpur Veterinary College, Nagpur, in July, 2019 with first class. He joined post-graduate (M.V.Sc.) studies in the discipline of Animal Nutrition in the Post-graduate Institute of Veterinary and Animal Sciences, Akola in October, 2019.

He actively participated in National Cadet Corps (NCC) unit of Nagpur Veterinary College, Nagpur during graduation studies. He has been selected as Livestock Development Officer (Grade-A) during his Post Graduation studies.

THESIS ABSTRACT

- a) **Title of the thesis (in Capital letters)** : **EFFECT OF DIETARY ANTIOXIDANTS (VITAMIN C, VITAMIN E AND SELENIUM) SUPPLEMENTATION ON PERFORMANCE AND IMMUNE RESPONSE IN BROILERS**
- b) **Full name of student** : **Nandanwar Chetan Bhaurao**
- c) **Name and address of Major advisor** : **Dr. K. Y. Deshpande**
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- d) **Degree to be awarded** : **M.V.Sc. (Animal Nutrition)**
- e) **Year of award of degree** : **2022**
- f) **Major subject** : **Animal Nutrition**
- g) **Total number of pages In the thesis** : **98**
- h) **Number of words in the abstract** : **295**
- i) **Signature of Student** :
- j) **Signature, Name and address of forwarding authority** :

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ABSTRACT

An experiment was conducted to investigate the effect of dietary antioxidants (Vitamin C, Vitamin E and Selenium) supplementation on performance and immune response in broilers. 240 chicks (VenCobb-430) were distributed in 4 groups having 15 chicks in each replicate using completely randomized design. Continuous heat stress was introduced using lighting arrangement throughout the experimental period. The diet was

formulated as per BIS (2007) standards for control, while antioxidants were added in T₁ : herbal vitamin C @ 100g/ton, T₂ : vitamin E and Selenium @ 200g/ton and T₃ was combination of T₁ and T₂. The cumulative FCR for starter and finisher phase was comparable (P>0.05); whereas, FCR for overall period improved numerically (P=0.056) in T₃ (1.59) than in T₀ (1.69). The percent digestibility of DM, OM, CP, CF and NFE was significantly higher (P<0.05) in treatment groups than control, while EE remained comparable. Carcass characteristics and shelf-life were not affected (P>0.05); however the overall acceptability of meat was improved significantly (P<0.05). The Hb, TLC, heterophils, lymphocytes, H:L ratio, LPO and SOD was improved significantly (P<0.05) in antioxidants supplemented groups. The total protein and albumin improved significantly (P<0.05) in antioxidants supplemented groups; while other metabolites and enzymes like Catalase, SGOT and SGPT remained comparable (P>0.05). The serum antibody titre against RD virus was significantly higher (P<0.05) in T₃, whereas the weights of immune organs did not differ significantly (P>0.05). The net loss in broiler production was minimized by 20.30% in T₁, 13.87% in T₂ and 23.63% in T₃ respectively over the control group. Hence, it is concluded that supplementation of dietary antioxidants (Vitamin C @ 100g/ton with Vitamin E and Selenium @ 200g/ton) in combination to stressed broilers improved FCR, overall acceptability of meat, hematobiochemical parameters, erythrocytic antioxidant indices, immune response and economics of broiler production.

प्रबंध सारांश

१. प्रबंधाचे शिर्षक : आहारातील अँटिऑक्सिडंट्स (व्हिटॅमिन सी, व्हिटॅमिन ई आणि सेलेनियम) यांचा पक्षांच्या वाढीवर आणि रोगप्रतिकारक क्षमतेवर होणारा परिणाम
२. विद्यार्थ्यांचे पूर्ण नांव : नंदनवार चेतन भाऊराव
३. मुख्य मार्गदर्शकाचे नांव व पत्ता : डॉ. के. वाय. देशपांडे
सहाय्यक प्राध्यापक व विभाग प्रमुख
पशु पोषण व आहारशास्त्र विभाग
स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला
४. प्रदान केली जाणारी पदवी : एम.व्ही.एसस्सी.
५. पदवी प्रदान करण्याचे वर्ष : २०२२
६. मुख्य विषय : पशु पोषण व आहारशास्त्र
७. प्रबंधामधील एकूण पाने : ९८
८. प्रबंध सारांशामधील एकूण शब्द : २७८
९. विद्यार्थ्यांची सही :
१०. प्रबंधक कार्यवाहीस्तव :
पाठविणाऱ्या अधिकाऱ्याची
सही, नाव व पत्ता

(डॉ. के. वाय. देशपांडे)

विभाग प्रमुख

पशु पोषण व आहारशास्त्र विभाग

स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला.

सारांश

मांसल पक्षांमधील वाढीवर आणि रोगप्रतिकारक क्षमतेवर आहारातील अँटिऑक्सिडंट्स (व्हिटॅमिन सी, व्हिटॅमिन ई आणि सेलेनियम) पुरवणीचा प्रभाव तपासण्यासाठी एक प्रयोग आयोजित केला गेला. २४० पिल्ले (वेणकोब-४३०) ४ गटांमध्ये वितरीत करण्यात आली ज्यांच्या प्रत्येक प्रतिकृतीमध्ये १५

पिल्ले पूर्णपणे यादृच्छिक डिझाइन याचावापर केला गेला. संपूर्ण प्रायोगिक कालावधीत प्रकाश व्यवस्था वापरून सतत उष्णतेचा ताण आणला गेला. नियंत्रणासाठी बीआयएस (२००७) मानकांनुसार आहार तयार केला गेला, तर टी_३ मध्ये अँटिऑक्सिडंट्स जोडले गेले: हर्बल व्हिटॅमिन सी @ १०० ग्रॅम/टन, टी_३ : व्हिटॅमिन ई आणि सेलेनियम @ २०० ग्रॅम/टन आणि टी_३ हे टी_३ आणि टी_३ चे संयोजन होते. स्टार्टर आणि फिनिशर फेजसाठी एकत्रित अन्नपरिवर्तनाचे गुणोत्तर लक्षणीयरीत्या भिन्न नव्हते (पी>०.०५); तर, टी_३ (१.६९) पेक्षा टी_३ (१.५९) मध्ये एकूण कालावधीसाठी अन्नपरिवर्तनाचे गुणोत्तर संख्यात्मकदृष्ट्या (पी=०.०५६) सुधारला होता. उपचार गटांमध्ये DM, OM, CP, CF आणि NFE सारख्या पोषक घटकांची टक्केवारी पचनक्षमता नियंत्रणापेक्षा लक्षणीयरीत्या जास्त (पी<०.०५) होती, तर EE तुलना करता आली. शव वैशिष्ट्ये आणि शेल्फ-लाइफ प्रभावित झाले नाहीत (पी>०.०५); तथापि मांसाची एकूण स्वीकार्यता लक्षणीयरीत्या सुधारली गेली (पी<०.०५). हिमोग्लोबिन, टीएलसी, हेटरोफाइल्स, लिम्फोसाइट्स, एलपीओ, एसओडी आणि एच:एल गुणोत्तरामध्ये अँटिऑक्सिडंट्स पूरक गटांमध्ये लक्षणीय सुधारणा झाली (पी<०.०५). अँटिऑक्सिडंट्स पूरक गटांमध्ये एकूण प्रथिने आणि अल्ब्युमिन (ग्राम/डीएल) लक्षणीयरीत्या सुधारले (पी<०.०५); इतर चयापचय आणि, क्याटालेस, एसजीओटी व एसजीपीटी सारखी एन्झाइम्स तुलना करता आली (पी>०.०५). टी_३ मध्ये आरडी विषाणूविरूद्ध सीरम अँटीबॉडी टायटर लक्षणीयरीत्या जास्त (पी<०.०५) होते, तर रोगप्रतिकारक अवयवांचे वजन लक्षणीय भिन्न नव्हते (पी>०.०५). नियंत्रण गटापेक्षा मांसलपक्षी उत्पादनातील निव्वळ तोटा टी_३ मध्ये २०.३०%, टी_३ मध्ये १३.८७% आणि टी_३ मध्ये २३.६३% कमी झाला. त्यामुळे, प्रयोगाअंती असा निष्कर्ष काढला आहे की आहारातील अँटिऑक्सिडंट्स (व्हिटॅमिन सी @ १०० ग्रॅम/टन आणि व्हिटॅमिन ई आणि सेलेनियम @ २०० ग्रॅम/टन) तणावग्रस्त मांसलपक्षी मध्ये अन्नपरिवर्तनाचे गुणोत्तर, मांसाची एकंदर स्वीकार्यता, रक्तपेशी मधील गुणोत्तर, लालरक्त पेशी मधील अँटिऑक्सिडंट्स चे प्रमाण, रोग प्रतिकरक्षमता आणि आर्थिक फयदेशीर उत्पादनामध्ये सुधारणा झाली.