STUDY ON COMPARATIVE EFFICACY OF SYNTHETIC AND HERBAL VITAMIN E SUPPLEMENTATION ON PERFORMANCE OF BROILER CHICKENS

THESIS

By

Dr. SUMAN KUMAR PRASAD

(Admission No.: BVC/M/ANN/005/2017-18)

Submitted to

BIHAR ANIMAL SCIENCES UNIVERSITY,
PATNA, BIHAR

In partial fulfillment of the requirements
forthe degree of

MASTER OF VETERINARY SCIENCE
IN
(ANIMAL NUTRITION)

DEPARTMENT OF ANIMAL NUTRITION
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DEPARTMENT OF ANIMAL NUTRITION

BIHAR VETERINARY COLLEGE, PATNA - 14

(Bihar Animal Sciences University, Patna, Bihar)

Dr. Pankaj Kumar Singh, *Ph. D.*
Assistant Professor

CERTIFICATE - I

This is to certify that the thesis entitled **"Study on Comparative Efficacy of Synthetic and Herbal Vitamin E Supplementation on Performance on Broiler Chickens"** submitted in partial fulfillment of the requirement for the award of **Master of Veterinary Science (Animal Nutrition)** of the faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is the original work carried out by **Dr. SUMAN KUMAR PRASAD, Admission No.: BVC/M/ANN/005/2017-18** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been duly acknowledged.

Endorsed

(.....) **(Pankaj Kumar Singh)**
Chairman / Head of Department Major Advisor

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(Bihar Animal Sciences University, Patna, Bihar)

CERTIFICATE - II

We, the undersigned members of the advisory committee of **Dr. SUMAN KUMAR PRASAD, Admission No.: BVC/M/ANN/005/2017-18,** a candidate for the degree of Master of Veterinary Science with major in **Animal Nutrition** have gone though the manuscript of the thesis and agree that the thesis entitled **"Study on Comparative Efficacy of Synthetic and Herbal Vitamin E Supplementation on Performance of Broiler Chickens"** may be submitted by **Dr. SUMAN KUMAR PRASAD** in partial fulfillment of the requirements for the degree.

(**Dr. Pankaj Kumar Singh**)
Chairman, Advisory Committee

Members of the Advisory committee

1. **Dr. Kaushalendra Kumar**
   Assistant Professor
   Department of Animal Nutrition
2. **Dr. S.P.Sahu**
   Assistant Professor
   Department of LPM
3. **Dr. Ajeet Kumar**
   Assistant Professor
   Department of Veterinary Biochemistry
4. **Dr. Pankaj Kumar**
   Assistant Professor
   Department of Veterinary Microbiology
5. **Dr.Kaushal Kumar**
   Asstt.Professor
   Department of Veterinary Pathology

Dean, PGS (Nominee)

6. **Dr. Archana**
   Assistant Professor
   Department of Veterinary Parmacology

DEPARTMENT OF ANIMAL NUTRITION

BIHAR VETERINARY COLLEGE, PATNA - 14

(Bihar Animal Sciences University, Patna, Bihar)

CERTIFICATE - III

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(.....)
(…..……..……..)
Chairman, Advisory Committee **External Examiner**
Members of the Advisory committee

1. **Dr. Kaushalendra Kumar**
   Assistant Professor
   Department of Animal Nutrition
2. **Dr. S.P.Sahu**
   Assistant Professor
   Department of LPM
3. **Dr. Ajeet Kumar**
   Assistant Professor
   Department of Veterinary Biochemistry
4. **Dr. Pankaj Kumar**
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   Department of Veterinay Microbiology
5. **Dr. Kaushal Kumar**
   Assistant Professor
   Department of Veterinary Pathology

Dean, PGS (Nominee)

6. **Dr. Archana**
   Assistant Professor
   Department of Veterinary Pharmacology


Dedicated
To
My Respected
Parents

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Place _____

Date _____ (*Suman Kumar Prasad)*

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ABBREVIATIONS

ADF	-	Acid Detergent Fibre
ADG	-	Average Daily Gain
ALA	-	Alpha- Linoleic Acid
ALP	-	Alkaline Phosphatase
ALT	-	Alanine Transaminase
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemist
AST	-	Aspartate Transaminase
BIS	-	Bureau of Indian Standard
BUN	-	Blood Urea Nitrogen
BW	-	Body weigh
BHT	-	ButylatedHydroxytolune
Ca	-	Calcium
CF	-	Crude Fibre
CP	-	Crude Protein
DTNB	-	5,5-dithiobis-(2-Nitrobenzoic Acid
DM	-	Dry Matter
DMI	-	Dry Matter Intake
EDTA	-	Ethylene Diamine Tetra Acetate
EE	-	Ether Extract
FI	-	Feed Intake
FAO	-	Food and Agricultural Organization
FCR	-	Feed Conversion Ratio
GSH	-	Glutathione
g	-	Gram
HA	-	Haemagglutination
Hb	-	Haemoglobin
HI	-	Haemagglutination Inhibition

HDL	-	High Density Lipoprotein
HSP	-	Heat Shock Protein
IU	-	International Unit
Kcal	-	Kilocalorie
Kg	-	Kilogram
LDL	-	Low Density Lipoprotein
LDH	-	Lactate dehydrogenase
LPO	-	Lipid peroxidation
ME	-	Metabolizable Energy
ml	-	Millilitre
MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration
Na	-	Sodium
NFE	-	Nitrogen Free Extract
NDV	-	Newcastle Disease Vaccine
ND	-	Newcastle Disease
NDF	-	Neutral Detergent Fibre
OM	-	Organic Matter
P	-	Phosphorus
PCV	-	Packed Cell Volume
PBS	-	Phosphate-Buffered Saline
PI	-	Performance index
ROS	-	Reactive Oxygen Species
RBC	-	Red Blood Corpuscles
SOD	-	Superoxide dismutase
SEM	-	Standard Error of Mean
SGOT	-	Serum Glutamic Oxaloacetic Transaminase
SBM	-	Soybean Meal
SPSS	-	Statistical Packages for Social Science
SGPT	-	Serum Glutamic Pyruvic Transaminase

TA	-	Total Ash
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TEC	-	Total Erythrocyte Count
TLC	-	Total Leucocytes Count
U/L	-	Unit PerLiter
VLDL	-	Very Low Density Lipoprotein
WHO	-	World Health Organization
WBC	-	White Blood Cell

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STUDY ON COMPARATIVE EFFICACY OF SYNTHETIC AND HERBAL VITAMIN E SUPPLEMENTATION ON PERFORMANCE OF BROILER CHICKENS

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2019

Department of Animal Nutrition
Bihar Animal Sciences University, Patna, India-800014

Title of the thesis : Study on comparative efficacy of synthetic and herbal vitaminEsupplementation on performance of broiler chickens.

Name of the student **:** Dr. Suman Kumar Prasad

Admission number **:** BVC/M/ANN-005/2017-18

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ABSTRACT

This study aimed to compare the effects of supplementation of synthetic source of vitamin E (DL-α-tocopherol acetate) and herbal source of vitamin E (D-α-tocopherol) on the growth performance, nutrients utilization, haemato-biochemical profile, meat quality, antioxidant capacity, immunity and carcass quality in broiler chickens during a 35-day feeding period. A total of 180, day-old Cobb 400Y strain broiler chicks were procured, weighed individually and divided into four treatment groups (T_1: Basal diet,T_2: Basal diet with synthetic vit E @) 100g/quintal feed, T_3:Basal diet with herbal vit E @) 100g/quintal feed, T_4: Basal diet with synthetic vit E @) 50g/quintal feed and herbal vit E @) 50g/quintal feed) each with three replicates of 15 chicks.Growth performance in terms of weight gain, feed intake, feed conversion ratio and performance index enhanced($P<0.05$) ingroup supplemented with herbal vit E. Nitrogen retention was found to be best in T_3 group. Haemato-biochemical parameters *viz.* Hb, PCV,TEC increased significantly ($P<0.05$)and there is minimum ($P<0.05$) cholesterol level was recorded in the group supplemented with herbal vit E. However, MCV and MCH, serum glucose, total protein, albumin and globulin levels and serum enzymes (ALP, ALT and AST) remained unaffected. There was marked increase ($P<0.05$) in the antibody titre against Newcastle Disease vaccine recorded in group receiving herbal vit E. Anti-oxidant status revealed increase ($P<0.05$) in GSH but SOD and CAT activity remained unaffected in serum. In meat there was marked reduction ($P<0.05$) in the accumulation of MDA but GSH-Pxand SOD level were unaffected. Carcass characters *viz*., dressing yield, eviscerated yield, blood loss, feather loss, meat to bone ratio and giblet weight were found to be statistically similar. There was minimum ($P<0.05$) abdominal fat percent and meat cholesterol level recorded in group supplemented with herbal vit E. Also dietary treatments significantly ($P<0.05$) enhanced the tenderness, juciness and overall acceptability of broiler chicken.

It was concluded that dietary supplementation of synthetic or herbal vitamin E and their combination @ 100 gm/quintal feed improved the growth performance, antioxidant status, carcass quality and immune status of broiler chickens. Herbal vitamin E was economical and more effective as compared to the synthetic vitamin E in improving the overall performance of broiler chickens.

Dr. Suman Kumar Prasad
(Student)

Dr. Pankaj Kumar Singh
(Major Advisor)

Chairman of the Department

1 INTRODUCTION

Poultry production is one of the most dynamic and fastest segment in animal husbandry sector in India. The poultry sector in India has undergone a paradigm shift in structure and operation, which has been its transformation from a mere backyard activity into a major commercial industry over a period of four decades. Poultry farming in the country has gained momentum during the last four decades. Consequently, it has taken over the shape of full-fledged industry. India has emerged on the world poultry map as the 3rdlargest egg and 5thlargest poultry meat producer. As per the Indian economic survey, 2015-16, egg production was around 78.48 billion eggs in 2014-15, while poultry meat production was estimated at 3.04 MT. However, per capita consumption of meat and eggs are 2.15 kg and 53 eggs, respectively, which are much less than the ICMR recommendation of 11 kg of poultry meat and 180 eggs. Therefore, there is a vast scope for growth of poultry industry in India.

Economical poultry production largely depends on optimum nutrition, improved growth performance, absence of diseases, minimum morbidity and mortality. Feed accounts for 65- 70% of broiler production cost, thus, feed cost deserves befitting attention. The key nutrients that need to be supplied by the dietary ingredients are amino acids contained in proteins, vitamins and minerals. Vitamins are a group of organic compounds that poultry require in small quantities, but they are essential for normal body functions, growth, and reproduction.Vitamins help to regulate the biological processes in the body. There are 13 essential vitamins categorised into fat-soluble (vitamin A, D, E, K) and water soluble vitamins (vitamin B-complex and vitamin C). Each vitamin has specific function in animal body. A deficiency of one or more vitaminscan lead to a number of diseases or syndromes and ultimately, uneconomical poultry production.

Vitamin E is a fat-soluble vitamin, which is essential for the integrity of the reproductive, muscular, circulatory, nervous and immune systems (Leshchinsky and Klasing, 2001). Although vitamin E has multiple functions in humans and animals, its key function is protecting cells from oxidative damage. Since its discovery, several studies have demonstrated that vitamin E deficiency causes impaired fertility in humans and lab animals. There is proven role of vitamin E dietary supplementation in broilers for proper metabolism, growth performance, immunity and quality of animal

products. Potential antioxidant effect of vitamin E is able to modulate inflammatory responses and physiological adjustments to mitigate the undesirable effects of exposure of broilers to high temperatures.

Global economic pressures have driven a tendency of the poultry production to produce more products per unit of feed intake. This has led to stress to the poultry and environment. Intensive poultry production also leads to metabolic stress which ultimately affecting the performance of poultry. Under normal cellular metabolism, reactive oxygenspecies (ROS) are continuously produced and degraded by aerobic organisms, and contribute to intracellularsignaling cascades (Nordberg and Arber, 2001) andimmune responses (Abimannan*et al*., 2016) at low tomoderate concentrations. The imbalance of oxidantsand antioxidants in favor of oxidants leads to oxidativestress (Ahmad *et al*., 2012; Birben*et al*., 2012).The excessiveproduction of ROS can damage cell structures suchaslipids, proteins, DNA, and carbohydrates, and leadsto loss of cell function, and ultimately apoptosis ornecrosis (Nordberg and Arber, 2001). All of these resultsadversely affect the productive performance of thepoultry. Therefore**,**the increasing antioxidants level in the body is beneficialfor counter**a**cting oxidative stress(Panda and Cherian, 2014).

Vitamin E as a lipid component of biological membranes is known to be a major chain-breaking antioxidant (Sahin*et al*., 2002). This antioxidant vitamin has been shown to improve both cell-mediated and humoral immunity in broiler chicks. However, the effect of this vitamin depends upon dose, age and genetics of the broiler chicks (Khan *et al*., 2016).

Lipid oxidation is one of the major causes of quality deterioration in meat and meat products. This oxidation can lead to serious consequences for meat quality, including the production of off-flavors and odors, reduction of polyunsaturated fatty acids, fat-soluble vitamins and pigments, lower consumer acceptability, and the generation of compounds such as peroxides and aldehydes which may be toxic (Morrissey *et al*., 1994). Vitamin E is also known for its role as an antioxidant, protecting unsaturated bonds of cellular membrane phospholipids against free radical attack (Tappel, 1972). Various studies have demonstrated that vitamin E can improve meat quality and inhibit lipid oxidation of muscles in broilers (O'neill*et al*., 1998; Voljč*et al*., 2011; Dalolio*et al*., 2015; Rey *et al*., 2015).

Vitamin E is very essential for optimum poultry production. Part of the vitamin E that poultry need comes from the ingredients in their diet. These are tocopherols and tocotrienols in the genuinely natural form. Butthey contain a high degree of biological activity and are naturally stable (due to the protection of vegetation cell structures), but they are also associated with other natural antioxidants that recycle tocopherols and tocotrienols. Concentration of vitamin E greatly varies due to several factors, including the quality of the ingredients and their state of preservation. This is why poultry nutritionists choose to ensure suitable levels using specific sources of Vitamin E that are added to the feed.

Vitamin E is found in high quantities in vegetable oils. Eight naturally occurring substances have been found tohave vitamin E activity: α-, β-, γ-, and δ-tocopherols and α-,β-, γ-, and δ-tocotrienols (Voljč*et al*., 2011). Of these, the α-tocopherol is the most biologically active and most widely distributed form (Halliwell and Gutteridge, 2000). This molecule possesses three centres where stereoisomers canoccur, and the naturally occurring molecule is the D-α-tocopherol (RRR-α-tocopherol) configuration that has the highest vitamin activity (McDonald *et al*., 2011). Synthetic DL-α-tocopherol acetate (all racemic α-tocopherol acetate)consisting of all eight possible stereoisomers.Vitamin E is usually added to poultry feeding the synthetic form of DL-α-tocopherol acetate.It has been reported that D-α-tocopherol exhibited a superior bioavailability than the synthetic DL-α-tocopherol acetate in retaining in tissues (Lauridsen*et al*., 2002), alleviating lipopolysaccharide-induced inflammatory response (Kaiser *et al*., 2012), and improving meat quality and muscular antioxidant capacity (Boler*et al*., 2009; Voljč*et al*., 2011;Rey *et al*., 2015).Analternate dietary source of vitamin E is the naturalsource, D-α-tocopherol. It was reported that herbal vitamin E got quickly absorbed, slowly excreted and remains more bioactive in animal system as compared to synthetic vitamin E.Herbal vitamin E assimilates far better in the body than synthetic vitamin E as specific binding, transport proteins in the liver have selective affinity for natural vitamin E, and they largely ignore all other forms (Hosomi*et al*., 1997; Kiyose*et al.,* 1997).

Previous studies have reportedthat natural (D-α-tocopherol) vitamin E has a superiorbioavailability than synthetic (DL-α-tocopherol acetate)vitamin E, which is observed in turkeys (Rey *et al*., 2015), cows (Weiss *et al*., 2009), and pigs (Lauridsen*et al*., 2002) in improving meat quality and muscularantioxidant capacity

(Voljč*et al*., 2011; Rey *et al*., 2015).In addition, Kaiser et al. (2012) reported that broilersfed natural-type vitamin E had asignificantly lower lipopolysaccharide-induced inflammatoryresponse in comparison to synthetic vitamin E. However, there is not much informationthat shows the effects of replacing syntheticvitamin E with natural vitamin E (D-α-tocopherol) ongrowth performance, antioxidant capacity, immunity and carcass characteristics of broiler chickens.

Keeping these facts in view, the present study was therefore, proposed to compare the effects of supplementation of DL-α-tocopherol acetate (Synthetic source of vitamin E) and D-α-tocopherol (Herbal source of vitamin E) on the growthperformance, nutrients utilization, haemato-biochemical profile meat quality, antioxidant capacity, immunityand carcass quality of broiler chickens.

Objectives of investigation:

1. To study the effect of synthetic and herbal vitamin E supplementation on growth performance in broilers
2. To study the effect of synthetic and vitamin E supplementation on hemato-biochemical parameters and oxidative status in broilers
3. To study the effect of synthetic and herbal vitamin E supplementation on nutrients utilization in broilers
4. To study the effect of synthetic and vitamin E supplementation on carcass characteristics in broilers

2 REVIEW AND LITERATURE

Sustainable poultry production largely depends on optimum nutrition, improved growth performance, absence of diseases, minimum morbidity and mortality. Feed accounts for 65- 70% of broiler production cost, thus, feed cost deserves befitting attention. The key nutrients that need to be supplied by the dietary ingredients are amino acids contained in proteins, vitamins and minerals. Vitamins are a group of organic compounds that poultry require in small quantities, but they are essential for normal body functions, growth, and reproduction. Vitamins help to regulate the biological processes in the body. There are 13 essential vitamins categorized into fat-soluble (vitamin A, D, E, K) and water soluble vitamins (vitamin B-complex and vitamin C). Each vitamin has specific function in animal body. A deficiency of one or more vitamins can lead to a number of diseases or syndromes and ultimately, uneconomical poultry production.

2.1 Chemistry of vitamin E:

Vitamin E (α-tocopherol) is a fat soluble vitamin which is essentially required in poultry diet. The term vitamin E, according to the International Union of Pure and Applied Chemistry-International Union of Biochemistry Commission on Biochemical Nomenclature is used as a generic descriptor for all tocopherols and tocotrienols derivatives that qualitatively exhibit the biological activity of alpha-tocopherol (IUPAC-IUB 1973). Both the tocopherols and tocotrienols consist of a hydroquinone nucleus and an isoprenoid side chain. Characteristically, tocopherols have a saturated side chain, whereas the tocotrienols have an unsaturated side chain containing three double bonds. There are four principal compounds of each of these two sources of vitamin E activity (alpha, beta, gamma, delta), differentiated by the presence of methyl ($-CH_3$) groups at positions 5, 7 or 8 of the chroman. Alpha-tocopherol, the most biologically active of these compounds, is the predominant vitamin E active compound in feedstuffs and the form used commercially for supplementation of animal diets (Schaffer *et al*. 2005; Freiser and Jiang. 2009).

Alpha-tocopherol is a yellow oil that is insoluble in water but soluble in organic solvents. Tocopherols are extremely resistant to heat but readily oxidized. Natural vitamin E is subject to destruction by oxidation, which is accelerated by heat,

moisture, rancid fat, copper, and iron. Alpha-tocopherol is an excellent naturally occurring antioxidant that protects carotene and other oxidizable materials in the feed and in the body. However, in the process of acting as an anti-oxidant, it is oxidized and becomes biologically inactive. Much of the acetate is readily split off in the intestinal wall and the alcohol is absorbed, thereby permitting the vitamin to function as a biological antioxidant. Any acetate form absorbed into the body is converted to the alcohol form. Vitamin E absorption is related to fat digestion and is facilitated by bile and pancreatic lipase (Sitrin *et al*. 1987). The efficiency of digestion and absorption of vitamin E varies with dietary inclusion level. Vitamin E-adequate animals absorb 20% to 30% and the animals receiving excess dietary vitamin E only 1% to 5%. However, Hidiroglou *et al.* (1988) reported no correlation between vitamin E status and tocopherol absorption. Vitamin E absorption may be impaired by a variety of disorders associated with fat malabsorption.

Vitamin E activity in foods and feedstuffs is derived from a series of compounds of plant origin. Natural vitamin E is the mixture of two classes of compounds, tocopherols and tocotrienols.

2.2. Functions of vitamin E

Vitamin E (alpha-tocopherol) is recognised as a powerful antioxidant, essential for the preservation and integrity of cell membranes and an effective compound for preventing and treating oxidative stress. Vitamin E acts as an efficient biological antioxidant in protecting cells from the adverse effects of reactive oxygen species or free radical initiators. Vitamin E is part of the body's intracellular defense against the adverse effects of reactive oxygen and free radicals that initiate oxidation of unsaturated phospholipids (Chow. 1979) and critical sulfhydryl groups (Brownlee *et al.* 1977). Vitamin E functions as a membrane-bound antioxidant, trapping lipid peroxyl free radicals produced from unsaturated fatty acids under conditions of "oxidative stress." Lipids, especially phospholipids present in cell membranes are particularly susceptible to oxidative damage, being positively correlated with the degree of unsaturation of its fatty acids. Vitamin E functions as a quenching agent for free radical molecules with single, highly reactive electrons in their outer shells. Free radicals attract a hydrogen atom, along with its electron, away moisture, rancid fat, copper, and iron. Alpha-tocopherol is an excellent naturally moisture, rancid fat, copper, and iron. Alpha-tocopherol is an excellent naturally occurring antioxidant that

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unit from yet another PUFA. This reaction can continue in a chain, resulting in the destruction of thousands of PUFA molecules (Gardner. 1989; Herdt and Stowe. 1991). Free radicals can be extremely damaging to biological systems (Padh. 1991). Highly reactive oxygen species such as the superoxide anion radical (O_2-), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2) and singlet oxygen (O_2) are continuously produced in the course of normal aerobic cellular metabolism. Also, phagocytic granulocytes undergo respiratory burst to produce oxygen radicals to destroy the intracellular pathogens. However, these oxidative products can, in turn, damage healthy cells if they are not eliminated. Antioxidants serve to stabilize these highly reactive free radicals, thereby maintaining the structural and functional integrity of cells (Chew. 1995). Therefore, antioxidants are very important to immune defense and health of humans and animals.

Eight naturally occurring substances have been found to have vitamin E activity: α-, β-, γ-, and δ-tocopherols and α-,β-, γ-, and δ-tocotrienols (Voljč *et al*. 2011). These compounds are antioxidants, which means they protect the plant from the toxicity of oxygen. Tocopherols and tocotrienols scavenge the peroxy radicals from lipids, thereby preventing the spread of lipid peroxidation in membranes, while the subsequent products of tocopheroxyl and tocotrienoxyl radicals, respectively, are recycled into tocopherols and tocotrienols. Moreover, tocopherols and tocotrienols protect lipids and other components of the membrane by physically suppressing and chemically reacting with the singlet oxygen (Munné-Bosch & Alegre. 2010).

Vitamin E is essential for integrity and optimum function of reproductive, muscular, circulatory, nervous, and immune systems (Hoekstra. 1975; Sheffy and Schultz. 1979; Bendich. 1987; McDowell. 2000). Vitamin E activates specific and nonspecific immune responses toward infectious diseases (Machlin. 1991). Vitamin E has vital role in egg production, egg fertility, hatchability, and sperm motility. Vitamin E deficiency has several disadvantages including crazy chick disease, muscular weakness, enlarged hocks, the disorder in nervous system, circulatory system, muscular system, skeletal system, immune system, and reproductive system (Mahajan *et al.* 2001). It is required for the regulation of heme biosynthesis, apparently by controlling induction and repression of aminolevulinic acid synthase and porphobilinogen synthase. Vitamin E plays a specific role in the essential transport of amino acids and possibly lipids in the intestine. Vitamin E is also

involved in iron metabolism and steroidogenesis, and it stimulates humoral and cellular immune responses against infectious diseases.

Vitamin E is perhaps the most studied nutrient related to the immune response (Meydani and Han, 2006). Vitamin E is an essential nutrient for the normal function of the immune system. Higher concentrations of d-tocopherol (50 and 100 mg per kg or 22.7 and 45.5 mg per lb) in feed reduced lipid peroxidation activity and enhanced activities of anti-oxidative enzymes and also improved cell-mediated immune response in commercial broilers (Ram Rao *et al.* 2011). Vitamin E and selenium play in protecting leukocytes and macrophages during phagocytosis, the mechanism whereby animals immunologically kill invading bacteria. Both vitamin E and selenium may help these cells to survive the toxic products that are produced in order to effectively kill ingested bacteria (Badwey and Karnovsky, 1980). Macrophages and neutrophils from vitamin E-deficient animals have decreased phagocytic activity (Burkholder and Swecker, 1990).Since vitamin E acts as a tissue antioxidant and aids in quenching free radicals produced in the body, any infection or other stress factor may exacerbate depletion of the limited vitamin E stores from various tissues. During stress and disease, there is an increase in production of glucocorticoids, epinephrine, eicosanoids and phagocytic activity. Eicosanoid and corticoid synthesis and phagocytic respiratory bursts are prominent producers of free radicals, which challenge an animals antioxidant systems. The protective effects of vitamin E on animal health may be involved with its role in reduction of glucocorticoids, which are known to be immunosuppressive (Golub and Gershwin, 1985).

The effects of vitamin E and selenium supplementation on protection against infection by several types of pathogenic organisms, as well as antibody titers and phagocytosis of the pathogens, have been reported for calves (Cipriano *et al.* 1982; Reddy *et al*. 1985a, 1987b) and lambs (Reffett *et al.* 1988; Finch and Turner, 1989; Turner and Finch, 1990). Vitamin E at high supplementation levels has a strong immune response with enhance resistance of poultry to infectious diseases (Silva *et al.* 2009). Vitamin E affects both cellular and humoral immune function; T lympocytes were increased (Abdukalykova *et al.* 2008). Previously, Moriguchi and Muraga (2000) observed that vitamin E improved the immune system by enhancing host antiviral activity and the production of the antiviral cytokine interferon, which is produced by activated T cells. Vitamin E and selenium appear to enhance host defenses against infections by improving phagocytic cell function. Both vitamin E and

GSH-Px are antioxidants that protect phagocytic cells and surrounding tissues from oxidative attack by free radicals produced by the respiratory burst of neutrophils and macrophages during phagocytosis (Baboir, 1984; Baker and Cohen, 1983). Hogan *et al.* (1990, 1992) reported that vitamin E supplementation of diets increased intracellular kill of Staphylococcus aureus and Esherichia coli by neutrophils.

Additional functions of vitamin E that have been reported (Scott *et al.* 1982) include (1) normal phosphorylation reactions, especially of high-energy phosphate compounds, such as creatine phosphate and adenosine triphosphate (ATP); (2) a role in synthesis of vitamin C (ascorbic acid); (3) a role in synthesis of ubiquinone; and (4) a role in sulfur amino acid metabolism. Pappu *et al.* (1978) have reported vitamin E to play a role in vitamin B_{12} metabolism. A deficiency of vitamin E interfered with conversion of vitamin B_{12} to its coenzyme 5′ deoxyadenosylcobalamin and concomitantly, metabolism of methylmalonyl-CoA to succinyl-CoA. In rats, vitamin E deficiency has been reported to inhibit vitamin D metabolism in the liver and kidneys with the formation of active metabolites and decreases in the concentration of the hormone-receptor complexes in the target tissue. Liver vitamin D hydroxylase activity decreased by 39%, 25-(OH)D3 1-alpha hydroxylase activity in the kidneys decreased by 22% and 24-hydroxylase activity by 52% (Sergeev *et al.* 1990).

2.3. Natural vitamin E Vs synthetic vitamin E

The term Vitamin E refers to a group of 8 fat-soluble compounds in their natural form, with antioxidant activities. Eight naturally occurring substances have been found to have vitamin E activity: α-, β-, γ-, and δ-tocopherols and α-,β-, γ-, and δ-tocotrienols (Voljč *et al*., 2011). Of these, the α-tocopherol is the most biologically active and most widely distributed form (Halliwell and Gutteridge, 2000). α-tocopherol is known to comprise the greatest biological activity and maintain the highest concentrations in animal and human plasma and, while tocotrienols are chiefly found in seeds. The α-tocopherol naturally produced by plants is presented in a single form: the stereoisomer RRR. Since this is the form produced in nature, as expected, itis fully recognised and usable by organisms; i.e., α-tocopherol RRR shows the isfully recognised and usable by organisms; i.e., α-tocopherol RRR shows the greatest biological activity as Vitamin E.

However, the most common form of Vitamin E used for supplementing animal feed is the synthetic form (all rac-α-tocopherol). This form is chemically synthesised

from a catalysed reaction between trimethylhydroquinone and isophytol, which is subjected to high-vacuum molecular distillation, resulting in purified dl-α-tocopherol. DL-α-tocopherol, obtained through chemical synthesis, is composed of a blend of 8 stereoisomers of α-tocopherol in equivalent amounts, which are distinguished by three-chiral-carbon formations in the positions 2, 4' and 8': RRR, RSR, RRS, RSS, SRR, SSR, SRS and SSS-α-tocopherol. While all of them show in vitro antioxidant activity, only the forms with the R formation in position 2 present biological activity as Vitamin E in organisms. The dl-α-tocopherol contains just 12.5% RRR-α-tocopherol (similar to the natural version). Due to its instability, to be usable in diets, purified dl-α-tocopherol has to be subjected to esterification, generally carried out using acetic acid (another chemical process), resulting in alpha-tocopherol acetate, the most common commercial form of synthetic Vitamin E supplements and additive.

Natural α-tocopherol, while contained in plants, is naturally protected by their cell structures, which endows it with natural stability. Furthermore, it comes in a form that is readily absorbed upon reaching the intestine, since it is not esterified and is 100% in the RRR form. On the other hand, upon arriving at the small intestine, α-tocopherol acetate (synthetic Vitamin E) needs to be hydrolysed by a pancreatic esterase in order to release the α-tocopherol, which can then be included in a lipid phase and absorbed. This additional step, required for intestinal absorption, can generate problems for certain types of animals, such as weanling piglets and very young poultry chicks, due to a longer absorption time compared to natural Vitamin E (Wilburn *et al.* 2008). Both forms of Vitamin E (natural and synthetic) are absorbed by the intestine and transported by chylomicrons to the liver, and there is basically no distinction between the different isomers. However, once it reaches the liver, the α-Tocopherol Transfer Protein (α-TTP), which is responsible for transferring the α-tocopherol from the liver and distributing it to the body tissues, is specific to the natural RRR form of α-tocopherol. The α-TTP can transport the RRR form, and little to nothing of the other forms. These other forms are subsequently metabolised in the liver and ultimately excreted without performing any function similar to that of Vitamin E in the body (Chung *et al.* 2015; Hosomi *et al.* 1997; Lim & Traber, 2007; Traber & Arai, 1999). Thus, a small part of the dl-α-tocopherol is transferred from the liver to the rest of the body to act as Vitamin E, but a large part is simply metabolised and excreted.

Vitamin E is usually added to poultry feeding the synthetic form of DL-α-tocopherol acetate .It has been reported that D-α-tocopherol exhibited a superior bioavailability than the synthetic DL-α-tocopherol acetate in retaining in tissues (Lauridsen *et al*. 2002), alleviating lipopolysaccharide-induced inflammatory response (Kaiser *et al*., 2012), and improving meat quality and muscular antioxidant capacity (Boler *et al*. 2009; Voljč *et al*. 2011;Rey *et al*. 2015). An alternate dietary source of vitamin E is the natural source, D-α-tocopherol. It was reported that herbal vitamin E got quickly absorbed, slowly excreted and remains more bioactive in animal system as compared to synthetic vitamin E. Herbal vitamin E assimilates far better in the body than synthetic vitamin E as specific binding, transport proteins in the liver have selective affinity for natural vitamin E, and they largely ignore all other forms (Hosomi *et al*., 1997; Kiyose*et al.*, 1997). Previous studies have reported that natural (D-α-tocopherol) vitamin E has a superior bioavailability than synthetic (DL-α-tocopherol acetate)vitamin E, which is observed in turkeys (Rey *et al*., 2015), cows (Weiss *et al*., 2009), and pigs (Lauridsen *et al*., 2002) in improving meat quality and muscular antioxidant capacity (Voljč*et al*., 2011; Rey *et al*., 2015). In addition, Kaiser *et al.* (2012) reported that broilers fed natural-type vitamin E had a significantly lower lipopolysaccharide-induced inflammatory response in comparison to synthetic vitamin E. However, there is not much information that shows the effects of replacing synthetic vitamin E with natural vitamin E (D-α-tocopherol) on growth performance, antioxidant capacity, immunity and carcass characteristics of broiler chickens.

2.4 Growth performance

2.4.1 Body weight gain/growth rate

Colnago *et al.* (1984) conducted an experiment to study the effect of selenium and vitamin E supplementation (100 IU per kg diet) of broiler ration on the body weight and immune response to coccidiosis challenged chickens. Vitamin E supplementation @ 100 IU vitamin E of non-immunized infected chicken resulted in significantly highest body weight gain during 25-31 days of age in broilers with reduction in mortality. Feed intake of chickens fed diets with either Selenium or vitamin E was higher than that of chickens fed basal diet.

Lin *et al.* (1989) conducted an experiment, in which each group was raised on standard mash starter (first four weeks) and finisher (last three weeks) broiler diets.

After three weeks, the diets of the first group was supplemented with 55g/kg oxidized sunflower oil (peroxide value of 22meq/kg). the diet of second group contained 55g/kg sunflower oil (unoxidised oil), which was supplemented with α-tocopherol(200mg/kg diet) in the form of α-tocopheryl acetate for the last 10 days of dietary regimen (short term supplementation). The third group received the α-tocopheryl acetate (200mg/kg diet) supplemented diet at the age of one week for 6 weeks of age (long term supplementation). The third group received a diet containing the same amount of unoxidised sunflower oil supplemented with butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) (12.5mg/bird/day). Broilers fed on the BHA/BHT- supplemented diet had significantly higher carcass weight than those of control group. Similarly short term and long term α-tocopherol supplementation also significantly increased carcass weight of broilers. Short term supplementation produced increase of 1.8% and 2.5% in body and carcass weights, while long term supplementation resulted in increase of 1.6% and 3.7% respectively, when compared to corresponding weights of control.

Bartov and Frigg (1992) determined the effect of high concentration of vitamin E (α-tocopheryl acetate) fed during various age periods on the performance and plasma vitamin E. The basal diet (for the age period 0 to 3, 3 to 6 and 6 to 7 weeks) contained 60g soyabean oil and 24mg vitamin E/kg. The following five treatment were evaluated: (1) the basal diet from 0 to 7 weeks of age (control); (2) vitamin E, 100 mg/kg diet from 0 to 7 weeks of age; (3) vitamin E, 150 mg/kg of diet from 0 to 3 weeks of age; (4) vitamin E, 150 mg/kg diet from 0 to 3 weeks of age and 100 mg/kg diet from 6 to 7 weeks of age; (5) vitamin E, 150 mg/kg diet from 5 to 7 weeks of age. Feed intake, weight gain and FCR were not significantly affected by vitamin E treatments.

Concentration of vitamin E, vitamin C, and glutathione (GSH) were depressed in birds with PHS (pulmonary hypertension syndrome), suggesting that antioxidents could be important in the etiology of PHS. Antioxidents play a major role in protecting cells from the action of reactive oxygen species by reducing chemical radicals and breaking the process of lipid peroxidation (Enkvetchakul et. al.,1993 and Botjee *et al.*, 1995)

Mcllory *et al.* (1993) studied the effect of increasing tocopherol supplementation in broilers with sub clinical IBD and found improved performance

and reduction in mortality. Bottje *et al.* (1995) studied the effect of vitamin E on PHS-induced mortality and found low mortality in broilers.

Vitamin E supplementation at a level 300IU/kg showed beneficial effect on weight gain, feed intake and FCR (Raza *et al.* 1996). Scheideler and Froning (1996) observed that egg production was significantly improved in hens fed with various flaxseed diets with high level of vitamin E (50 IU/kg diet) compared to hens fed with the same diets containing low level of vitamin E (27 IU/kg diet).

Friedman *et al.* (1998) studied the effect of high dietary intakes of vitamin E on the growth performance and antibody production was investigated in chicks with 0, 10, 30 and 150 mg/kg added vitamin E. Added dietary vitamin E did not affect weight gain and feed efficiency in chicks, but improved immune status and reduced in mortality.Maximum body weight gain was obtained in chicks fed diets containing 0.50mg/kg Se and 300IU/kg vitamin E. (Surai *et al.* 2000)

Swain *et al.* (2000) studied the effect of supplementation of different vitamin as vitamin E @ 14.5 IU/kg, vitamin E and selenium (150 IU/kg, 0.10mg/kg and 300 IU/kg, 0.0mg/kg) on the performance and immune response of broilers. They found no significant effect on body weight gain.

Guo *et al.* (2001) fed 0, 5, 10, 50 and 100mg/kg tocopherol to broiler chicks and did not find any effect on feed intake at 0-3 weeks measurement, but tocopherol supplementation tended to improve growth and feed utilization. They did not find any effect during 0-6 weeks period.Sodhi *et al.* (2001) reported that the toxic effects of melathionare ameliorated to a certain extent by vitamin E and selenium supplementation in chicks by diminution of oxidative stress.

Sijben *et al.* (2002) conducted an experiment using 5, 20, 40 and 80mg/kg vitamin E in diet. The concentration of vitamin E did not affect body weight gain.Han Rui *et al.* (2004) Luxi yellow broilers (AnaK×Guangxi Yellow native stain) randomly divided into three groups were fed on three diets supplemented with α-tocopherol acetate of 0,50,100mg/kg respectively from6-week old to10-week old. The basal diet contains 18mg/kg α-tocopherol acetate. The results showed that there was no significant difference in body weight and average daily gain among the three diets ($P>0.05$).

Panda *et al.* (2004) reported that supplementation of vitamin E @ 100 mg/kg in poultry diet showed better feed conversion ratio (1.99) as compared to control group (2.05) at five week of age. Lohkare *et al.* (2005) studied the effect of vitamin E @ 10mg/kg and 20mg/kg in broiler ration. The gross energy digestibility was higher in supplemented diet as compared to non supplemented diet.

As per Chatterjee and Agrawal (2005) Herbal E-50 is a unique product that contains vitamin E in natural and highly bioavailable form, which gets quickly absorbed, slowly excreted and is more active as compared to synthetic vitamin E. The antioxidant activity of herbal E-50 is nearly three times more compared to synthetic vitamin E.

The effect of vitamin E and vitamin C supplementation in diets were investigated by Ciftci *et al.* (2005), on feed consumption, egg production, and egg quality in laying hens exposed to chronic heat stress. One group was fed with basal diet (control group) and treatment groups were fed with the basal diet supplemented with either 125mg of Vitamin E groups), and 200mg of Vitamin C or 125mg of Vitamin E plus 200mg of Vitamin C. Although feed intake among groups did not significantly differ, mortality was significantly decreased in supplemented birds compared to the controls.

Kumar and Singh (2005) conducted feeding trial in broiler with diet containing vitamin E @ 125 mg/kg and found better growth in vitamin E supplemented group. Singh *et al.* (2005) fed *ad libitum* basal diet T_0 (control) with the supplementation in different groups viz. T_1 (Se 0.1mg/kg), T_2 (Se 0.2mg/kg), T_3 (vitamin E 100 IU/kg), T_4 (Se 0.1mg/kg and vitamin E 100 IU/kg) T_5 (Se 0.2mg/kg and vitamin E 100 IU/kg), T_6 (vitamin E 200 IU/kg), T_7 (Se 0.1 mg/kg and vitamin e 200 IU/kg) and T_8 (Se 0.2mg/kg and vitamin E 100 IU/kg). The results indicated that supplementation of vitamin E or selenium both in different combinations helped in improving the body weight gains.

Chae *et al.* (2006) studied the effect of incremental levels of α-tocopherol acetate (AT) on performance, nutrient digestibility and chicken meat stability in broilers. The dietary tocopherol levels used were 0, 10, 50, 100 and 200mg/kg, respectively. They found significantly higher weight gains at higher levels of dietary AT especially at higher levels between 100 and 200mg/kg in broilers diet.

In a study by Shaik *et al.* (2005), assessed the effect of supplementation of vitamin E, selenium and their combination on growth and immune response in broiler. Supplementation of vitamin E or selenium (organic or inorganic) did not influence the body weight. However, vitamin E supplemented diet @ 150 300 IU/kg showed highest body weight as 1903 and 1899g respectively at 42 days of age.

Siegal *et al.* (2006) conducted an experiment to measure the effect of supplementation of vitamin E @ 10 and 300 IU/kg on the growth and immunocompetence of poults. There was little effect of vitamin E level on body weight.

Supplementation of Herbal vitamin E in commercial broilers showed significantly better growth, over un-supplemented control birds and the same was comparable to supplementation of synthetic vitamin E (Dani, 2007)

Basmacioglu *et al.* (2009) reported that higher dietary vitamin E level in broiler ration delayed the lipid peroxidation and thereby 300 mg/kg vitamin E supplementation in broiler diet during last 10 days before marketing improved the keeping quality of meat. The results indicated that supplementation of vitamin E in different combination help in improving the body weight gain.

Liu *et al.* (2009) conducted an experiment to evaluate the effect of dietary vitamin E on growth performance and immune response of broilers under heat stress. Basal diet was supplemented with vitamin E at 0, 100, 200mg/kg, respectively. They found that body weight was not significantly influenced by dietary vitamin E ($P > 0.05$).

Martinez Benavides *et al.* (2010)Carried out an investigation to evaluate the effect of the application of early thermal conditioning (ETC) and the dietary addition of vitamin E and zinc on broiler chicken performance. No significant differences among the main effects was found for body weight.

Niu *et al.* (2009) conducted an experiment to evaluate the effect of dietary vitamin E on growth performance and immune response of broilersunder heat stress (HS). 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-oldmale 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 was not significantly influenced by dietary vitamin E ($P > 0.05$).

Panda *et al.* (2009) conducted an experiment to study the effect of higher levels of vitamin E supplementation on growth, immune competence and antioxidant status in broiler chickens. A basal diet based on maize-soybean meal was formulated. Subsequently, another four test diets were prepared by supplementing vitamin E to contain 10, 30, 150 and 300 IU vitamin E per kg diet. Each diet was fed *ad-libitum* to 6 replicates of 6 chicks each during the experimental period of 0–6 weeks. The body weight gain was significantly lower in the chicks fed diet containing 10IU vitamin E per kg diet. Increasing the vitamin E supplementation to 30IU per kg diet significantly increased the weight gain; however no further improvement in weight gain could be noticed on higher levels of supplementation (150 or 300 IU per kg diet).

In an experiment conducted by Rama Rao *et al.* (2009) to study the effect of supplementing different concentrations (10, 50 an 100mg/kg diet) of vitamin E (Vitamin E) on performance, immunity in broiler starter chicks (1 to 21 days of age) fed three sources of supplemental oil (sunflower- SFO, palm - PMO, safflower - SAO). Three concentrations of Vitamin E and three oil sources were supplemented in maize - soybean meal based diet in 3 × 3 factorial experiment. Each diet was fed *ad-libitum* to eight replicates containing six birds in each housed in stainless steel battery brooders. Interaction between source of oil and concentration of Vitamin E did not influence body weight gain.

Zhang *et al.* (2009) conducted an experiment to compare the effect of 2 esters of α-tocopherol, all-*rac*- α-tocopherol acetate and RRR-α-tocopherol succinate (D-α-TOS) on growth and immunity in broiler chicks. Three hundred twenty 1-d-old commercial Arbor Acres broilers were randomly distributed to 4 treatments, each of which had8 pens of 10 chicks per pen. Birds in the control group were fed with the diets supplemented with 30 mg/kg of all-*rac* α-tocopherolacetate or the basal diet with D-α-TOS supplementation at 10mg/kg (TOS1 group), 30 mg/kg (TOS2 group), and 50 mg/ kg (TOS3group), respectively, for 42 d. The results showed that therewas no significant difference ($P > 0.05$) in body weight gain.

Skrivan *et al.* (2010) broiler chicks (Ross 308) were allocated to 3 or 5 dietary treatments comprising 300 (3 replicated pens of 100 chicks per pen), 50, 50 or 100 chickens. The experiments lasted 42, 38, 42 and 38 days and aimed at evaluating the effect of different levels of caprylic acid (CA) and vitamin E added to the basal diet. The inclusion of 0.5% CA in diets significantly reduced the body weight (BW). The diet supplemented with 0.25% CA and 30mg vitamin E (total vitamin E concentration 50mg) resulted in similar body weight like the basal diet, but the increase of supplemental vitamin E to 150mg or to 100mg significantly decreased BW and increased mortality.

Rama Rao*et al.* (2011) An investigation was carried out into the effects of dietary α-tocopherol (α-T) concentration and source of supplemental oil on performance, activity of anti-oxidative enzymes and some immune responses in broilers from day-old to 41 days of age. Three dietary concentrations of α-T (10, 50 and 100mg/kg) with three sources of supplemental oil (sunflower - SFO, palm - PMO and safflower - SAO) were provided using a 3 × 3 experimental design. Body weight gain was not affected by either interaction or Concentration of α-T and sources of oil in diet.

Selvam*et al* (2017) carried out a study to investigate the effect of dietary supplementation with vitamin E and stocking density on the mortality, body weight gain, feed conversion ratio, European production efficiency factor, heterophil/ lymphocyte (H/L) ratio and liver glutathione (GSH) and malondialdehyde (MDA) levels in broilers. The supplementation of vitamin E to the birds subjected to HSD had a positive effect on body weight gain, feed conversion ratio (FCR), European Production Efficiency Factor (EPEF), H/L ratio, and liver GSH and MDA levels with those subjected to HSD and not supplemented with vitamin E. The results of the study indicateed that the supplementation of vitamin E at 70g/ton of feed to broilers subjected to HSD could effectively reverse the negative effects of HSD, and improve broiler performance.

2.4.2 Feed intake

Colnago *et al.* (1984) conducted an experiment to study the effect of selenium and vitamin E supplementation of broiler ration on the body weight and immune response to coccidiosis immunized chickens. Feed intake of chickens fed diets with either selenium or vitamin E was higher than that of chickens fed basal diet

Bartov and Frigg (1992) determined the effect of high concentration of vitamin E (α-tocopheryl acetate) fed during various age periods on the performance and plasma vitamin E. The basal diet (for the age period 0 to 3, 3 to 6 and 6 to 7 weeks) contained 60g soyabean oil and 24mg vitamin E/kg. The following five treatment were evaluated: (1) the basal diet from 0 to 7 weeks of age (control); (2) vitamin E, 100 mg/kg diet from 0 to 7 weeks of age; (3) vitamin E, 150 mg/kg of diet from 0 to 3 weeks of age; (4) vitamin E, 150 mg/kg diet from 0 to 3 weeks of age and 100 mg/kg diet from 6 to 7 weeks of age; (5) vitamin E, 150 mg/kg diet from 5 to 7 weeks of age. Feed intake was not significantly affected by vitamin E treatments.

Vitamin E supplementation at a level 300 IU/kg showed beneficial effect on feed intake (Raza *et al.*, 1996).

The difference in bioavailability of the acetate and succinate esters of all-rac-tocopherol was investigated in a feeding experiment with 12-day-old male cob broilers and lasted for 4 weeks. The two source of vitamin E were fed to eight group of broilers at four different dietary levels (50, 100, 150, 200 mg/kg feed, including the naturally occurring tocopherol). There was no difference among the eight experimental groups with respect to animal performance or feed intake (Jensen et. al., 1999)

Swain *et al.* (2000) studied the effect of supplementation of different vitamin as vitamin E @ 14.5 IU/kg, vitamin E and selenium (150 IU/kg, 0.10mg/kg and 300 IU/kg, 0.0mg/kg) on the performance and immune response of broilers. They found no significant effect on feed intake

Guo *et al.* (2001) fed 0, 5, 10, 50 and 100mg/kg tocopherol to broiler chicks and did not find any effect on feed intake from 0-3 weeks duration, but tocopherol supplementation tended to improve growth and feed utilization. They did not find any effect during 0-6 weeks period. Sijben *et al.* (2002) conducted an experiment using 5, 20, 40 and 80mg/kg vitamin E in diet. The concentration of vitamin E did not affect feed consumption.

The effect of vitamin E and vitamin C supplementation in diets were investigated by Ciftci *et al.* (2005), on feed consumption, egg production, and egg quality in laying hens exposed to chronic heat stress. One group was fed with basal diet (control group) and treatment groups were fed with the basal diet supplemented

with either 125mg of Vitamin E groups), and 200mg of Vitamin C or 125mg of Vitamin E plus 200mg of Vitamin C. The feed intake among groups did not significantly differ.

Kim *et al.* (2007) conducted and experiment to examine the effects of dietary organic selenium and vitamin E supplementation on egg production, egg weight, daily egg mass, feed intake, feed conversion, egg quality, lipid-soluble antioxidative capacity (ACL) in egg yolk, and selenium retention of egg in laying hens, Hy-Line laying hens of 77 wk old were replaced in the individual cage for 12 week. A corn-soybean meal based diet was supplemented with 0 (control), vitamin E 100 IU/kg and the combination of 0.9 ppm Se from selenium yeast (SY) and vitamin E 50, 100 and 150 IU/kg. Egg production and daily egg mass were significantly increased (P less than 0.05) in supplemental vitamin E 100 IU, and 0.9 ppm SY + vitamin E 50 IU than control for the whole experimental period. However, feed intake was not affected by supplemental vitamin E and SY.

Martinez Benavides *et al.* (2009) carried out an investigation to evaluate the effect of the application of early thermal conditioning (ETC) and the dietary addition of vitamin E and zinc on broiler chicken performance. No significant differences among the main effects was found for feed intake .

Niu *et al.* (2009) conducted and experiment to evaluate the effect of dietary vitamin E on growth performance and immune response of broilers under heat stress (HS). 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-oldmale 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. Feed intake was not significantly influenced by dietary vitamin E ($P > 0.05$).

In an experiment conducted by Rama Rao *et al.* (2009) to study the effect of supplementing different concentrations (10, 50 an 100mg/kg diet) of vitamin E (Vitamin E) on performance, immunity in broiler starter chicks (1 to 21 days of age) fed three sources of supplemental oil (sunflower- SFO, palm - PMO, safflower -

SAO). Three concentrations of Vitamin E and three oil sources were supplemented in maize - soybean meal based diet in 3 × 3 factorial experiment. Each diet was fed *ad-libitum* to eight replicates containing six birds in each housed in stainless steel battery brooders. Interaction between source of oil and concentration of Vitamin E did not influence body weight gain, feed efficiency.

Zhang *et al.* (2009) conducted and experiment to compare the effect of 2 estersof α-tocopherol, all-*rac*-α-tocopherol acetate and RRR-α-tocopherolsuccinate (D-α-TOS) on growth and immunity in broiler chicks. Three hundred twenty 1-d-old commercial Arbor Acres broilers were randomly distributed to 4 treatments, each of which had8 pens of 10 chicks per pen. Birds in the control group were fed with the diets supplemented with 30 mg/kg of all-*rac*-α-tocopherolacetate or the basal diet with D-α-TOS supplementation at 10mg/kg (TOS1 group), 30 mg/kg (TOS2 group), and 50 mg/ kg (TOS3group), respectively, for 42 d. The results showed that there was no significant difference ($P > 0.05$) in feed intake.

2.4.3 Feed conversion ratio

Bartov and Frigg (1992) determined the effect of high concentration of vitamin E (α-tocopheryl acetate) fed during various age periods on the performance and plasma vitamin E. The basal diet (for the age period 0 to 3, 3 to 6 and 6 to 7 weeks) contained 60g soyabean oil and 24mg vitamin E/kg. The following five treatment were evaluated: (1) the basal diet from 0 to 7 weeks of age (control); (2) vitamin E, 100 mg/kg diet from 0 to 7 weeks of age; (3) vitamin E, 150 mg/kg of diet from 0 to 3 weeks of age; (4) vitamin E, 150 mg/kg diet from 0 to 3 weeks of age and 100 mg/kg diet from 6 to 7 weeks of age; (5) vitamin E, 150 mg/kg diet from 5 to 7 weeks of age. FCR was not significantly affected by vitamin E treatments.

Vitamin E supplementation at a level 300 IU/kg showed beneficial effect on FCR (Raza *et al.*, 1996). Friedman *et al.* (1998) studied the effect of high dietary intakes of vitamin E on the growth performance and antibody production was investigated in chicks with 0, 10, 30 and 150 mg/kg added vitamin E. Added dietary vitamin E did not affect FCR in chicks.

Maximum body FCR was obtained in chicks fed diets containing 0.50mg/kg Se and 300 IU/kg vitamin E. (Surai *et al.* 2000).

Swain *et al.* (2000) studied the effect of supplementation of different vitamin as vitamin E @ 14.5 IU/kg, vitamin E and selenium (150 IU/kg, 0.10mg/kg and 300 IU/kg, 0.0mg/kg) on the performance and immune response of broilers. They found no significant effect on feed efficiency. Arvind *et al.* (2001) observed better FCR value with inclusion of vitamin E @ 75ppm alone or in combination with selenium.

Sijben *et al.* (2002) conducted an experiment using 5, 20, 40 and 80mg/kg vitamin E in diet. The concentration of vitamin E feed conversion ratio. Panda *et al.* (2004) reported that supplementation of vitamin E @ 100 mg/kg in poultry diet showed better feed conversion ratio (1.99) as compared to control group (2.05) at five week of age.

Siegal *et al.* (2006) conducted an experiment to measure the effect of supplementation of vitamin E @ 10 and 300 IU/kg on the growth and immunocompetence of poults. There was little effect of vitamin E level on feed conversion, whereas male poults had better feed conversion than female poult. Supplementation of Herbal vitamin E in commercial broilers showed significantly better FCR over un-supplemented control birds and the same was comparable to supplementation of synthetic vitamin E (Dani, 2007)

Kim *et al.* (2007) conducted and experiment to examine the effects of dietary organic selenium and vitamin E supplementation on egg production, egg weight, daily egg mass, feed intake, feed conversion, egg quality, lipid-soluble antioxidative capacity (ACL) in egg yolk, and selenium retention of egg in laying hens, Hy-Line laying hens of 77 wk old were replaced in the individual cage for 12 week. A corn-soybean meal based diet was supplemented with 0 (control), vitamin E 100 IU/kg and the combination of 0.9 ppm Se from selenium yeast (SY) and vitamin E 50, 100 and 150 IU/kg. Egg production and daily egg mass were significantly increased (P less than 0.05) in supplemental vitamin E 100 IU, and 0.9 ppm SY + vitamin E 50 IU than control for the whole experimental period. However, feed conversion was not affected by supplemental vitamin E and SY.

Liu *et al.* (2009) conducted an experiment to evaluate the effect of dietary vitamin E on growth performance and immune response of broilers under heat stress. Basal diet was supplemented with vitamin E at 0, 100, 200mg/kg, respectively. They found that 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 100mg/kg (P< 0.05).

Martinez Benavides *et al. (2009)* carried out an investigation to evaluate the effect of the application of early thermal conditioning (ETC) and the dietary addition of vitamin E and zinc on broiler chicken performance. No significant differences among the main effects was found for feed conversion ratio.

Niu *et al.* (2009) conducted and experiment to evaluate the effect of dietary vitamin E on growth performance and immune response of broilers under heat stress (HS).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-oldmale broiler chicks were randomly assigned to 6 groups; each group had 4 replicates of 10 birds. Humoral immunity was assessedby 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 macrophagephagocytic ability. Feed conversion was significantly affected by vitamin E at 100 mg/kg(P< 0.05).

An investigation was carried out into the effects of dietary α-tocopherol (α-T) concentration and source of supplemental oil on performance, activity of anti-oxidative enzymes and some immune responses in broilers from day-old to 41 days of age. Three dietary concentrations of α-T (10, 50 and 100mg/kg) with three sources of supplemental oil (sunflower - SFO, palm - PMO and safflower - SAO) were provided using a 3 × 3 experimental design. Feed conversion efficiency was not affected by either interaction or Concentration of α-T and sources of oil in diet (Rama Rao *et al.,* 2011) .

2.5 Mortality

Colnago *et al.* (1984) conducted an experiment to study the effect of selenium and vitamin E supplementation of broiler ration on the body weight and immune response to coccidiosis immunized chickens. Vitamin E supplementation @ 100 IU vitamin E of non-immunized infected chicken resulted in significantly highest body weight gain during 25-31 days of age in broilers with reduction in mortality.

Mcllory *et al.* (1993) studied the effect of increasing tocopherol supplementation in broilers with sub clinical IBD and found improved performance and reduction in mortality. Bottje *et al.* (1995) studied the effect of vitamin E on PHS-induced mortality and found low mortality in broilers. Friedman *et al.* (1998) studied the effect of high dietary intakes of vitamin E on the growth performance and antibody production was investigated in chicks with 0, 10, 30 and 150 mg/kg added vitamin E. Added dietary vitamin E improved immune status and reduced in mortality.

The effect of vitamin E and vitamin C supplementation in diets were investigated by Ciftci *et al.* (2005), on feed consumption, egg production, and egg quality in laying hens exposed to chronic heat stress. One group was fed with basal diet (control group) and treatment groups were fed with the basal diet supplemented with either 125mg of Vitamin E groups), and 200mg of Vitamin C or 125mg of Vitamin E plus 200mg of Vitamin C.Mortality was significantly decreased in supplemented birds compared to the controls.Shaik *et al.* (2005), assessed the effect of supplementation of vitamin E, selenium and their combination on growth and immune response in broiler. There was reduction in mortality (5%) in diet supplemented with vitamin E @ 150mg/kg due to the increase in immune response.

Supplementation of Herbal vitamin E in commercial broilers showed significantly better livability over un-supplemented control birds and the same was comparable to supplementation of synthetic vitamin E (Dani, 2007). Liu *et al.* (2009) conducted an experiment to evaluate the effect of dietary vitamin E on growth performance and immune response of broilers under heat stress. Basal diet was supplemented with vitamin E at 0, 100, 200mg/kg, respectively. There was improvement in the immune response of broilers by dietary supplementation of vitamin E.

Skrivan *et al.* (2010) Broiler cockerels Ross 308 were allocated to 3 or 5 dietary treatments comprising 300 (3 replicated pens of 100 chicks per pen), 50, 50 or 100 chickens. The experiments lasted 42, 38, 42 and 38 days and aimed at evaluating the effect of different levels of caprylic acid (CA) and vitamin E added to the basal diet. The inclusion of 0.5% CA in diets significantly reduced the body weight (BW). The diet supplemented with 0.25% CA and 30mg vitamin E (total vitamin E concentration 50mg) resulted in similar BW like the basal diet, but the increase of

supplemental vitamin E to 150mg or to 100mg significantly decreased BW and increased mortality.

2.6 Vitamin E and Immune Response

Lin and Chang (2006) studied the effect of dietary vitamin E supplementation on immune responses in breeder chickens during the maturing period. 17-week old female birds were fed corn-soybean meal based diets supplemented with either 0, 40, 80,120, or 160 mg vitamin E (all-rac-α-tocopherol acetate)/kg diet for 19 weeks. The results showed that supplemental vitamin E improved body weight gain of laying pullets during peak-laying period but had no significant effect on growth performance of cockerels. For cockerels, addition of 20 mg vitamin E/kg diet significantly enhanced ($p<0.05$) immune response to SRBC compared to those added with 0, 80 and 160 mg vitamin E/kg diet; addition of 20 mg vitamin E/kg diet had higher ($p<0.01$) antibody titer to IBDV than those added with 40-160 mg vitamin E/kg diet. No significant effects on immune response were observed in laying pullets fed supplemental vitamin E. The findings of the experiment suggested that moderate supplementation of vitamin E may enhance immune responses to selective antigens in cockerels but excessive vitamin E may depress specific immune response.

Niu *et al* (2009) evaluated the effect of dietary vitamin E on growth performance and immune response of broilers under heat stress (HS). Birds raised in either a thermos-neutral (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. 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 ($P < 0.05$). Numbers of AEC, percentage of macrophages in AEC, phagocytic macrophages, and internalized opsonized and opsonized 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.

Broiler chicks fed 80 IU vitamin E per kg (36.4 IU per lb) had increased innate and humoral immune response against coccidiosis vaccine and an Eimeria (protozoan parasites) challenge (Perez-Carbajoal *et al.* 2010).

Bhatti *et al* (2016) studied the effects of supplementations of vitamin C and E on humoral immune response against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) and on lymphoid organs in broiler birds. On the day 5th and 11th, the chicks were vaccinated against ND and Disease IBD. Booster doses of both vaccines were given on day 28. The chicks were offered Vitamin E (600 mg l-1), Vitamin C (600 mg l-1) and Vitamin E+C (300 mg l-1 each), for 5 consecutive days in drinking water on day 5 and 28. Weekly serum Hemagglutination Inhibition (HI) antibody titers against ND virus, total body weight, feed conversion ratio (FCR) and weight of lymphoid organs were recorded until the day 49. Geometric mean HI antibody titers against ND remained maximum in group C. At day 49, the total weight gain was maximum in group C (2196.0 gm) followed by group A (2155.0 g), group B (2146 g) and group D (2094 gm). The feed conversion ratio was the best in the group B (1.66) followed by group C (1.69) with a non significant difference. From the study, it was concluded that the combined effect of Vitamin E+C was better as compared to separate supplementation of Vitamin E and Vitamin C.

Dalia *et al* (2018) conducted an experiment and results showed that, Se and Vit E synergistic effect was clear in plasma IgM level at day 42 and in splenic cytokines expression (TNF-α, IFN-γ, IL-2, IL-10). The combination of 0.3 mg/kg ADS18-Se with 100 mg/kg VitE showed the highest IgM level. The combination of either SS or ADS18-Se with Vit E had no significant effect on IFN- γ and IL-10 compared to Vit E alone.

2.7. **Vitamin E and Antioxidant status:**

Vitamin E functions as a chain-breaking antioxidant, thereby neutralizing free radicals and preventing oxidation of lipids within membranes. Free radicals may damage not only their cell of origin but migrate and damage adjacent cells in which more free radicals are produced in a chain reaction leading to tissue destruction (Nockels, 1991). Vitamin E helps to interrupt production of free radicals at the initial stage.

Ognik and Wertelecki (2012) conducted an experiment on turkey hens and found that the supplemental natural form of RRR-d-α-tocopherol at the 2-fold lower dietary inclusion level or comparable with the dl-α-tocopherol acetate stimulated the antioxidant status of the body significantly better than the synthetic tocopherol acetate and, consequently, played a more efficient anti-oxidative role in the organism.

Vossen *et al*. (2011) studied the effect of dietary antioxidants on the plasma oxidative status of growing birds fed a diet containing 4% linseed oil and supplemented with single plant extracts rich in antioxidants (natural tocopherols, rosemary, grape seed, green tea, tomato) A diet with synthetic antioxidants with and without a-tocopheryl acetate (200 mg/kg feed) were also included. No significant effect of the dietary treatments was observed for FRAP as well as for TBARS. However, diet affected GSH-Px activity ($p = 0.002$) and a trend for an effect on SOD activity was observed ($p = 0.084$). A higher GSH-Px activity was found for 200 mg/kg tomato extract and natural a-tocopherol in relation to the corresponding 100 mg/kg treatment, and the lowest GSH-Px activity was measured for the synthetic antioxidants treatment. The lowest and highest SOD activity was found for the 200. and 100 mg/kg treatment with tomato extract, respectively. In conclusion, the oxidative status and lipid oxidation of plasma in broilers was not affected by feeding natural antioxidant extracts, but some changes in antioxidant enzyme activities were observed.

Saleh *et al.* (2017) studied the effects of supplementation with a-tocopherol acetate (a-Toc), pomegranate pomace extract (PPE) and pomegranate pomace (PP) into chicken feed on antioxidant status, oxidation susceptibility and quality of the thigh meat. Total phenolic content, lipid peroxidation level and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity in the thigh meat were significantly improved when chickens were fed diets containing a-Toc and PPE ($p < .05$). Supplementation of different antioxidant preparations into diets had no influence on plasma superoxide dismutase and glutathione peroxidase activities, in contrast, serum lipid peroxidation level was reduced in chickens fed diets supplemented with PPE (except 0.1 g kg1) and a-Toc. In conclusion, the broiler thigh meat may be successfully enriched with LC PUFA n-3 and its antioxidant potential and quality characteristics may be improved by supplementing diets with 0.2 and 0.3 g kg1 PPE. Moreover, the antioxidant potential of PPE supplementation was equal to that of a-tocopherol acetate in refrigerated meat.

2.8 Vitamin E and carcass quality:

Vitamin E improves carcass quality of broilers. In order to improve the oxidative stability, and thus increase the shelf life of meat, antioxidants have been successfully added to animal feeds. In the last few years, different compounds such as vitamin E, carotenoids, vitamin C, selenium and plant extracts have been tested in different experiments in order to verify their potential antioxidant effect on poultry meat (Bou *et al.* 2001; Bou *et al.* 2005; Surai, 2002; Surai *et al*. 2002; Açikgöz *et al.* 2011; Halici *et al*., 2012;among others). Of all of them, alpha tocopherol has demonstrated the highest biological efficiency in preventing the lipid oxidation in vivo (Barroeta, 2007). Lipid membranes have an abundance of unsaturated fatty acids and thus are very susceptible to peroxide formation. The peroxidation of membrane lipids can in turn cause oxidation of membrane proteins (Leeson and Summers, 2001). These reactions are enhanced by the presence of metal ions, especially iron. Broilers fed additional alpha-tocopherol of between 30 and 50 mg per kg (13.6 and 22.7 mg per lb) had reduction in lipid peroxidation as evidenced by the decrease in malondialdehyde (Zhang *et al*., 2009).

Cheng *et al.* (2016) conducted a study to compare the supplementation of natural (D-α-tocopherol) and synthetic (DL-α-tocopherol acetate) vitamin E on the growth performance, meat quality, muscular antioxidant capacity and genes expression related to oxidative status of broilers. Birds were given a basal diet (control group), and basal diet supplemented with either 20 IU D-α-tocopherol or DL-α-tocopherol acetate for 42 days, respectively. Compared with the control group, concentration of α-tocopherol in the breast muscle was increased by the supplementation of vitamin E. In the thigh, α-tocopherol content was also enhanced by vitamin E inclusion, and this effect was more pronounced in the natural vitamin E group ($p<0.05$). Vitamin E supplementation increased the redness of breast ($p<0.05$). In the contrast, the inclusion of synthetic vitamin E decreased lightness of thigh ($p<0.05$). Dietary vitamin E inclusion reduced drip loss at 24 h of thigh muscle ($p<0.05$), and this effect was maintained for drip loss at 48 h in the natural vitamin E group ($p<0.05$). Broilers given diet supplemented with vitamin E showed decreased malondialdehyde (MDA) content in the breast ($p<0.05$). Additionally, natural rather

than synthetic vitamin E reduced MDA accumulation in the thigh ($p<0.05$).They concluded that vitamin E supplementation, especially the natural vitamin E, can enhance the retention of muscular α-tocopherol, improve meat quality and muscular antioxidant capacity of broilers.

Kumbhar *et al*. (2018) carried out a study to investigate the effect of dietary selenium (Se) and vitamin E (VE) supplementation on mRNA level of heat shock proteins, selenoproteins, and antioxidant enzyme activities in the breast meat of broilers under summer heat stress conditions. Dietary Se, VE and Se + VE significantly enhanced the activities of superoxide dismutase (SOD). In addition, Se and Se + VE significantly enhanced the glutathione peroxidase (GPx) activity and the expression of *GPx1* and *GPx4* in breast muscle tissues. It is noteworthy that all the treatments significantly decreased malondialdehyde (MDA) level in the breast meat. Overall results showed that Se in combination with VE has maximal effects to mitigate heat stress. They recommended that Se + VE was a suitable dietary supplement for broilers to ameliorate the negative effects of summer heat stress.

Sasiadek *et al*. (2016) concluded from his findings that Vitamin E supplementation had a significant impact on meat quality by changing its pH value, reducing juice drip during heat treatment and increasing its WHC. Vitamin E applied in chicken feeding decreased lipid oxidation, improved sensory scores of the meat and changed the histological parameters in the muscles.

Daniel *et al*. (2016) conducted a study to evaluate the effect of vitamin E and selenium on performance, viability, productive efficiency, and yields of carcass, major cuts, and organs of broilers from 22 to 42 days submitted to cyclic-heat stress. The yields of carcass, major cuts, intestine, and heart were not influenced by the levels of selenium and vitamin E, whereas abdominal fat for the selenium level 0.1 mg/kg decreased linearly with the inclusion in vitamin E.

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3 MATERIALS AND METHODS

An experiment on broiler chicken was carried out to discern the comparative effect of synthetic and herbal vitamin E on production performance, nutrient utilization, haemato-biochemical, antioxidant status, immunity and carcass characteristics in broiler chicken. The experiment was conducted at Instructional Livestock Farm Complex, Bihar Veterinary College, Patna, Bihar.

In this chapter, a brief description of experimental design, selection and management of experimental birds and analytical techniques followed during the study have been presented here under:

3.1.1. Experimental feeds

Feed ingredients, supplements and feed additives in required quantities for formulation of experimental diets were procured from the local market nearby Patna, Bihar, India. Vitamin E samples were provided by Ayurvet Limited, Katha, Baddi, Solan, India. Standard basal diets for pre-starter (0-7 days) starter (8-21 days) and finisher (21-35 days) phases of growth of broiler chickens were prepared by mixing the different ingredients as shown in Table 3.1 to meet the nutrient requirements of broiler chicken as per recommendation of (BIS 2007). Proximate composition, phosphorus (AOAC 2003) and calcium (Talapatra *et al.* 1940) contents of the feed ingredients used in the experiments were determined following standard techniques. Calculated value of metabolisable energy, lysine, methionine were used to balance the ration to meet the nutrients by the broiler chickens. Feed costs were calculated on the basis of local price of individual feed ingredients used in the ration.

There were 4 dietary treatment groups as follows:

Treatment T_1: Basal diet without Herbal or synthetic Vitamin E (control)

Treatment T_2 : Basal diet with synthetic vitamin E @) 100 g/quintal feed

Treatment T_3 : Basal diet with herbal vitamin E @) 100 g/quintal feed

Treatment T_4 : Basal diet with Synthetic vitamin E @) 50 g/quintal feed

Herbal vitamin E @) 50g /quintal feed

Table 1: Ingredient and chemical composition (% DM basis) of basal diets of broiler chickens used during experiment

Ingredients	Pre-starter	Starter	Finisher
Maize	57.90	56.00	59.20
Soybean meal	26.50	29.00	25.50
Groundnut cake	6.00	4.00	3.00
Meat cum bone meal	5.00	4.00	3.70
Vegetable oil	1.82	3.89	5.32
Limestone powder	0.80	0.90	0.90
Dicalcium phosphate	0.70	0.85	1.00
Common salt	0.30	0.30	0.30
Trace mineral premix [1]	0.15	0.15	0.15
Vitamin Premix[2]	0.10	0.10	0.10
L-lysine	0.25	0.15	0.050
DL-Methionine	0.12	0.10	0.130
Choline chloride	0.06	0.06	0.150
Toxin binder	0.10	0.10	0.10
Coccidiostat	0.05	0.05	0.05
Sodium bicarbonate	0.150	0.150	0.150
Total	100	100	100
Chemical composition (% DM basis)			
Crude protein (%)	23.09	22.13	20.21
Ether extract (%)	8.11	8.23	8.23
Crude fibre (%)	3.81	3.90	3.90
Total ash (%)	6.26	6.34	6.34
Calcium (%)	1.00	1.00	1.00
Total phosphorus (%)	0.71	0.73	0.73
Available phosphorus (%)*	0.41	0.44	0.44
ME (kcal/kg)*	3012.96	3125.36	3219.87
Lysine (%)*	1.01	1.00	1.00
Methionine (%)*	0.45	0.44	0.44

*Calculated values

[1]Trace mineral premixsupplied (per kg diet): Magnesium- 300 mg, Manganese- 55 mg, Iodine-0.4 mg, Iron- 56 mg; Zinc- 30 mg and Copper 4 mg.

[2] Vitamin premix supplied (per kg diet): vitamin A-8250 IU, vitamin D_3- 1200 ICU; vitamin K- 1 mg; vitamin B_1- 2 mg, vitamin B_2- 4 mg; niacin- 60 mg, pantothenic acid-10 mg, cyanocobalamin-10 µg and choline-500 mg.

3.1.3. Experimental birds

For the experimental feeding trial one hundred and eighty, day- old Cobb 400Y strain broiler chicks were procured locally. All the chicks were individually weighed and wing banded. Thereafter, chicks were randomly allotted to four treatment groups each with three replicates of 15 chicks in such a way that average body weight was approximately similar for all the treatment groups.

3.1.4. Housing and feeding management

All the broiler chicks were housed in a deep litter system and provided *ad libitum* feed and water throughout the feeding trial. All the chicks were provided proper lighting throughout the experimental period. A layer of 4-5 cm rice husk was spread as litter material. The standard vaccination schedule was followed during the feeding trial. Standard manegmental practices were adapted identically to all treatments during the entire experimental period of 35 days. Proper ventilation free from dust was maintained throughout the experimental feeding trial. Weighed amount of the experimental feed was offered daily at 8.00 A.M to ensure *ad libitum* feeding at all the time, but taking care to avoid spillage and wastage of feed. Fresh and clean water was always made available in suitable water troughs to all the birds during the study period.

3.1.5. Production performance

The following parameters were recorded and calculated during the experimental period for growth studies.

3.1.5.1. Feed intake

Daily record of feed given to various groups was maintained. Left over of feed was weighed weekly. The feed intake in different groups was calculated by subtracting the weight of left over feed from the weight of total feed offered in a week.

3.1.5.2. Body weight gain

The body weight of individual birds was recorded at the start of the experiment and also at every week till the end of experiment. On the basis of these

Plate 1: Day Old experimental birds

Plate 2: Experimental birds at 35 days

Plate 3: Weighing of Day Old chick

Plate 4: Weighing of birds at 35 days

Plate 5: Weighing of feed additive

Plate 6: Birds during Metabolic trial

weights, weekly and overall body weight gains in different groups of broiler chicks were calculated.

3.1.5.3. Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was calculated by dividing the feed consumed from the body weight gain as per the formula given below:

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

3.1.5.4. Performance index (PI)

The performance index of broilers during different periods of growth was calculated by using the formula given by Bird (1995).

$$\text{Performance index} = \text{Body weight gain (g)} \div \text{FCR}$$

3.1.5.5. Cost economics

The cost of different rations used in the present study was calculated based on the actual market price of feed ingredients which prevailed at the time of experiment. The cost was calculated for a period of 5 weeks as follows:

$$\text{Feed cost (Rs./kg body weight gain)} = \frac{\text{Feed consumption (kg)} \times \text{Feed cost (Rs./kg)}}{\text{Body weight gain (kg)}}$$

3.1.6. Metabolism trial

A metabolism trial of three days was conducted to investigate the apparent total tract nutrient retention at the end of 5th week of feeding trial. Six broiler chicks (2 birds per replicates) representing the average body weight of the group were randomly selected and kept in metabolic cages. The experimental feed and fresh drinking water were provided *ad lib*. Initially, 3 days adaptation period was observed followed by 3 days excreta collection period. During the collection period, weighed amount of feed was offered to all broilers in the morning at 9 A.M. and the residue left was weighed next morning at the same time. Simultaneously, faecal trays covered with polythene sheets were placed for the collection of excreta. Care was taken to collect the excreta free from feed, feathers and scales. Excreta was pooled and dried in hot air oven at 70ºC for 48 hrs for dry matter estimation and thereafter stored for

further analysis. Representative samples of feed and excreta were drawn for chemical analysis. For nitrogen estimation, fresh samples of excreta were preserved in 5% sulphuric acid (v/v). The samples of the experimental diets and the dropping were analysed for proximate principles, phosphorus (AOAC, 2003),calcium (Talapatra *et al.*, 1940) and gross energy (Adiabatic bomb calorimeter, Sanyo Gallenkemp).

The retention of a nutrient was calculated by applying the following formula:

$$\text{Retention of nutrient (\%)} = \frac{\text{(Nutrient intake – Nutrient outgo)}}{\text{Nutrient intake}} \text{X100}$$

3.1.7. Analysis of feed, excreta and meat samples

The representative samples of broiler starter feed, broiler finisher feed, laying hen feed, excreta obtained during metabolism trial and representative meat samples from breast and thigh were collected and analyzed for for proximate principles, phosphorus (AOAC, 2003),calcium (Talapatra *et al.*, 1940)

3.1.7.1. Determination of dry matter (DM)

A known quantity of ground sample (about 10-50 g) was taken in a pre-weighed moisture cup. The cup was placed in hot air oven at 100 ± 2^0C for 24 h. The loss in moisture content after drying was estimated and DM was calculated as follows:

$$\text{Dry matter (\%)} = \frac{\text{(Wt. of moisture cup + sample after drying – Wt. of moisture cup)}}{\text{Wt. of fresh sample}} \text{X 100}$$

Plate 7: Digestion of feed samples

Plate 8: Nitrogen Analysis of samples

Plate 9: Blood collection of birds

Plate 10: Processing of blood samples

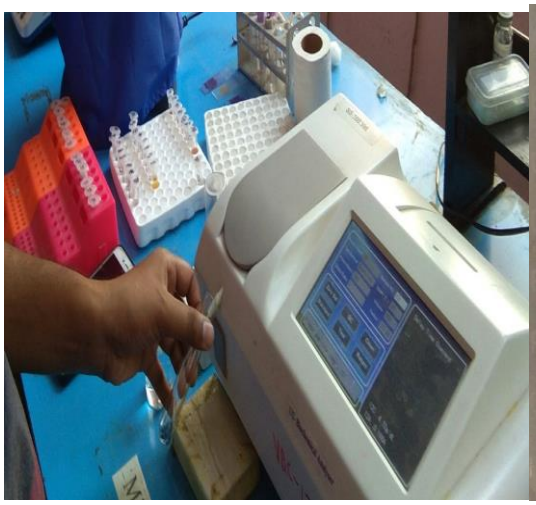

Plate 11: Biochemical analysis of

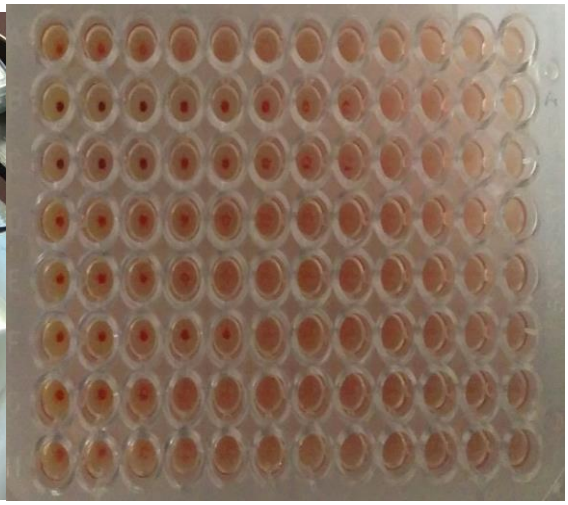

Plate 12: HI Assay of samples

3.1.7.2. Determination of nitrogen and crude protein

The crude protein content was determined by micro–Kjeldahl method. For this purpose 2 g of sample was taken in a digestion flask followed by addition of 5g of digestion mixture (K_2SO_4:$CuSO_4$ in 9:1) and 25 ml of concentrated sulphuric acid. The contents were digested till blue/ green transparent liquid was obtained. The volume of digested mixture was made up to 100 ml with distilled water. A 25 ml aliquot of digested mixture was distilled in Kjeldahl distillation apparatus (KELPLUS Nitrogen Analyzer) with excess of 40% NaOH solution and liberation of ammonia was collected in 20 ml of 2% boric acid solution containing 2 to 3 drops of mixed indicator (10 ml of 0.1% bromocresol green + 0.1% methyl red). A reagent blank was similarly digested and distilled which was titrated against N/10 H_2SO_4. Nitrogen content (%) in sample was calculated as follows:

Nitrogen (%) =

$$\frac{(\text{Sample Titre} - \text{Blank Titre}) \times \text{Normality of } H_2SO_4 \times 0.014 \times \text{Volume made up}}{\text{Aliquot} \times \text{Weight of sample taken}}$$

Nitrogen per cent was converted to crude protein per cent by multiplying with factor 6.25. This was based on the principle that all the protein contains 16% nitrogen.

3.1.7.3. Determination of ether extract

For estimation of ether extract Soxhlet extraction method was used. A known quantity of ground sample (about 2-3 g) was taken in a cellulose thimble and extracted for 6-8 hours with petroleum ether (Boiling point 40-60^{0}C) in Soxhlet extraction apparatus attached to a preweighed oil flask. The oil flask was removed and after evaporating the excess of ether, it was dried overnight in a hot air oven (60±2°C). The flask was cooled in a desiccator and weighed to a constant weight. The difference in weights gave the amount of ether extract in the sample as follows:

$$\text{Ether extract (\%)} = \frac{[A-B]}{C} \times 100$$

Where, A= Weight of oil flask with ether extract (g)

B= Weight of empty oil flask (g),

C= Weight of sample (g)

3.1.7.4. Determination of crude fibre

Moisture and fat free samples were transferred from thimbles to spoutless tall beaker of one litre capacity and in each beaker, 200 ml 1.25% H_2SO_4 was poured. It was refluxed for 30 minutes on hot plate after the boiling started and thereafter filtered through muslin cloth. The residue was washed 5-6 times with hot water until it became free from acid. The residual material on muslin cloth was again transferred to the respective spoutless tall beakers and in each beaker 200 ml of 1.25% sodium hydroxide (NaOH) solution was added. It was again refluxed for 30 minutes after the boiling started and thereafter filtered through muslin cloth and washed with hot water for 5-6 times until it became free from alkali. Thereafter, total residue was transferred in a clean, dry silica crucible and dried in hot air oven at 100^0C for 24 hours. Then, it was cooled in dessicators and weighed. The residue was then ignited in Muffle furnace at 600^0C for 2 hours. After 12 hrs silica crucibles containing ash were removed from the furnace and transferred into desiccators, cooled and weighed again.

Weight loss during ignition was recorded as the weight of crude fibre:

$$\text{Crude fibre (\%)} = \frac{(B-A)}{A} \times 100$$

Where, A = weight of samples on DM basis (g)

B= weight of silica crucible plus residue before ignition (g)

C = weight of silica crucible containing ash after ignition (g)

3.1.7.5. Determination of total ash

A known quantity of sample (5 g) was taken in pre-weighed silica crucible. After charring the sample on heater (till the smoke disappeared), the crucible was kept in Muffle furnace for ignition at 600°C for 2 h. The crucible was removed on cooling and kept in a desiccator and weighed again to find out weight of ash. The ash content was calculated as given below:

$$\text{Total ash (\%)} = \frac{\text{(Wt. of crucible + ash - Wt. of crucible)}}{\text{Wt. of sample (g)}} \times 100$$

3.1.7.6. Estimation of acid insoluble ash (AIA)

About 50 ml of 50 per cent hydrochloric acid was added to total ash in the above mentioned crucible and contents were boiled in the water bath for 10 minutes, filtered through Whatman's filter paper no. 42 after giving washings with the distilled water to washout the crucible completely. Filter paper along with the residue was transferred to the same crucible again and it was burnt on the heater and ignited in the Muffle furnace at 600°C for 2 hours. After cooling of Muffle furnace, the crucible containing acid insoluble ash were removed and kept in desiccators, cooled and weighed. The acid insoluble ash content was calculated by the formula given below:

$$\text{Acid insoluble ash (\%)} = \frac{(W_2 - W_1) \text{ X } 100}{W}$$

Where, W_1 = Weight of crucible (g)

W_2 = Weight of crucible with acid insoluble ash (g)

W = Weight of original sample (g)

3.1.7.7. Calcium estimation

Calcium was estimated in the acid mineral extract prepared from ashing of sample and then dissolving in dilute hydrochloric acid extraction (Talapatra *et al.* 1940). An aliquot of 10 ml mineral extract was transferred into a 250 ml beaker and to it 10 ml saturated ammonium oxalate solution was added with constant stirring. Then, two drops of methyl red indicator were added. Dilute ammonia solution was added drop wise to develop a faint yellow colour which was again adjusted by adding dilute HCl drop wise with constant stirring so as to get faint pink colour. The contents were heated on a hot plate for about 5 minutes and kept overnight for proper precipitation of calcium oxalate. On the next day, contents were filtered through Whatman filter paper no. 40 with minimum five washings with hot distilled water. After that, filter papers containing whitish calcium oxalate precipitate was quantitatively transferred into a beaker and dissolved by adding dilute sulphuric acid, followed by gentle heating and titrated against standard N/10 or N/100 potassium permanganate solution ($KMnO_4$) solution.

Calcium (%) in the sample was calculated as follows:

$$\text{Ca (\%)} = \frac{\text{N/10 } KMnO_4 \text{ used (ml) x 0.002 x dilution factor}}{\text{Wt of sample}} \times 100$$

3.1.7.8. Estimation of phosphorus

The phosphorus content of samples was estimated as per method of (AOAC 2003) using UV visible spectrophotometer as described below. In a large beaker 20 g ammonium molybdatetetrahydrate was taken and dissolved in about 300 ml hot distilled water and cooled by keeping in cold water trough. In a separate beaker two grams of ammonium metavandate was dissolved in 250 ml hot distilled water, cooled and 125 ml of 70% perchloric acid ($HClO_4$) was added to it slowly. Molybdate solution was added to vandate solution with constant stirring and transferred to a volumetric flask. The volume was made up to one liter mark with distilled water.

Different volumes of working standard solution were taken in the test tubes so as to have 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg P and volume was made to 6 ml by adding distilled water. Then, 4 ml of molybdo-vandate reagent was added to all the test tubes, mixed and OD was taken at 400 nm after 10 minutes of incubation in UV visible spectrophotometer. The amount of phosphorus present in the samples was determined by using regression equation obtained from the standard curve.

Collection of blood and separation of serum

Blood samples were collected from six experimental birds of each group i.e. two broiler chicks from each replicate on 35^{th} days of experimental feeding. Blood samples (about 3.0 ml) were collected aseptically from their wing vein, using sterilized syringes and needles. Collected blood samples were divided into two parts. One part (1.5ml) was transferred to the vials containing anticoagulant (EDTA) for analysis of haematological parameters. The second part (1.5 ml) of blood was used for separation of serum. For separation of serum, remaining 1.5 ml blood sample was allowed to stand at room temperature in slanting position for clot formation for three

to four hours. After clotting of blood, plunger was removed from the syringes and serum was stored at -20^{0}C with date and sample number for further analysis.

3.1.8. Haematological parameters

3.1.8.1. Haemoglobin (Hb)

Haemoglobin concentration was estimatedfollowing the method described by (Sharma and Singh 2000) using Sahli's haemoglobinometer with acid haematin method. The brown colour was matched with glass standard and haemoglobin concentration (g/ dl) was recorded.

3.1.8.2. Packed cell volume (PCV)

Packed cell volume was estimated by using micro haematocrit method as described by (Sharma and Singh 2000). Fresh anticoagulant added blood was drawn into micro capillaries and sealed with wax at one end. Capillaries were centrifuged at 10,000 rpm for 30 minutes. Packed cell volume was directly measured by using Citro Cap Microhaematocrit tube reader and expressed in per cent.

3.1.8.3. Total Erythrocyte Count (TEC)

The blood specimen is diluted with 1:200 with the RBC diluting fluid and cells are counted under high [40s objective] by using neubauers chamber. The number of cell in undiluted blood are calculated and reported as the total number of cell per cubic mm of whole blood. Total count is equal to total number of RBC in all the five squares (upper left, upper right,, lower right, lower left and central) multiplied by 10,000.

3.1.8.4. Mean Corpuscular Volume (MCV)

Mean Corpuscular Volume (MCV) is measure of the average volume of the red blood corpuscules. The measure is attained by multiplying a volume of blood by the proportion of blood i.e cellular and dividing that product by the number of erythrocyte in that volume. It is expressed in femto litre (fL). It was calculated by using the formula:

$$MCV = (PCV \times 10) / TEC$$

3.1.8.5. Mean Corpuscular Haemoglobin (MCH)

Mean Corpuscular Haemoglobin (MCH) is the average mass of haemoglobin per blood cell (RBC) in a sample of blood It is expressed in picograms per cells(pg). It was calculated by using the formula:

MCH=(Hb × 10) / TEC

3.1.9. Serum biochemical parameters

3.1.9.1. Serum glucose

Estimation of serum glucose was done by enzymatic GOD-POD method with the help of Span Diagnostic Kit at 505 nm wavelength against blank reagent (Sacks, 1998). In this procedure, glucose oxidase (GOD) oxidises glucose to gluconic acid and hydrogen peroxide. In 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 measured at 505 nm was directly proportional to glucose concentration in the sample. Concentration of serum glucose was expressed in mg/dl.

3.1.9.2. Serum protein profile

(a). Serum total protein

Total protein concentration in serum was estimated by biuret method with the help of Erba diagnostic kit at 540 nm wavelength (Johnson *et al.* 1999). The peptide bonds of proteins react with cupric ion in alkaline solution to form a colored chelate (Protein + Cu^{2+} → Cu-protein complex), which is measured at 578 nm. Concentration of plasma total protein was expressed in g/dl.

(b). Serum albumin

Albumin concentration in the serum was estimated by bromocresol green end point assay method (Albumin + bromocresol green → Green coloured complex) with the help of Autopak diagnostic kit at 630 nm wavelength (Johnson *et al.* 1999). Concentration of serum albumin was expressed in g/dl.

(c). Serum globulin

The serum albumin content was subtracted from serum total protein content to calculate globulin content and expressed in g/dl.

Globulin (g/dl) = Total protein (g/dl) – Albumin (g/dl)

(d). Albumin: Globulin Ratio (A: G ratio)

The albumin-globulin ratio was calculated by using following formula:

$$\text{Albumin- globulin ratio} = \frac{\text{Serum albumin (g/dl)}}{\text{Serum globulin (g/dl)}}$$

3.1.9.3. Serum enzyme profile

Following serum enzymes were estimated using the serum obtained from the blood samples.

a). Serum alkaline phosphatase (ALP)

The alkaline phosphatase activity in serum was assayed using Span diagnostic Kit at 510 nm wavelength (Bergmeyer *et al.* 1986). Concentration of serum ALP was expressed in IU/L.

b). Estimation of alanine aminotransferase (ALT)

Alanine amiontransferase (ALT) is also called as serum glutamate pyruvate transaminase (SGPT). For the estimation of ALT, 4 - DNPH method of (Reitman and Frankel 1957) was used. The principle in this reaction is that ALT catalyses the following reaction

α- Ketoglutarate + L- Alanine ↔ L- Glutamate + Pyruvate

Pyruvate so formed is coupled with 2, 4-Dinitrophenyl hydrazine (2, 4 - DNPH) to give the corresponding hydrazone which gives brown color in alkaline medium and this can be measured calorimetrically at 505 nm. ALT activity was expressed as IU/L

c). Serum aspartate aminotransferase (AST)

The activity of serum glutamate oxaloacetate transaminase (SGOT) or aspartate aminotransferase (AST) was measured following 2, 4 - DNPH method of Reitman and Frankel (1957) using a diagnostic kit (Span Diagnostic Ltd.). The principle in this reaction is that aspartate aminotransferase (AST) catalyses the transamination of L- aspartate and α- ketoglutarate to form oxaloacetate and L-glutamate.

α- Ketoglutarate + L- Aspartate ↔ L- Glutamate + Oxaloacetate

Oxaloacetate so formed is coupled with 2, 4 – Dinitrophenyl Hydrazine (2, 4 - DNPH) to give the corresponding hydrazone, a brown color complex in alkaline medium and this can be measured colourimetrically at 505 nm. AST activity was expressed as IU/L.

3.1.10. Serum Antioxidant status

3.1.10.1. Catalase activity

Serum catalase activity was estimated spectrophotometrically as per the method described by (Cohen *et al.* 1970). The reaction commences with the addition of 50µL of serum to 2.95ml of phosphate buffer- H_2O_2 solution. In the blank, sample was substituted by same amount of PBS. Phosphate buffer- H_2O_2 gives an absorbance of 0.5-0.6 at 240 nm. The decrease in absorbance was measured for every 20 seconds up to 1 minute. Since a decrease in absorbance of 0.05 at 240 nm corresponds to the disappearance of 3.45 µmoles of H_2O_2.

The units of catalase activity per ml serum was calculated as below.

0.05 change in absorbance = 3.45 µmoles of H_2O_2 disappeared

'A' change in absorbance in 50µl sample = 3.45 X A/ 0.05

So, 'A' change in absorbance in 1ml sample = 3.45 X A/ (0.05 X 0.05)

= 1380 A

3.1.10. 2. Lipid peroxidation

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) as per method given by (Buege and Aust 1978) modified by (Suleiman *et al*. 1996). First of all 0.2 ml serum sample was diluted to 1 ml with normal saline solution. Lipid peroxide levels were measured in the serum after addition of 2 ml of TBA-TCA reagent to 1ml of diluted serum. The mixture was heated in a boiling water bath for 15 minutes. After cooling, the suspension was centrifuged at 3000rpm for 10 minutes. The supernatant was separated and absorbance was measured at 535 nm. The MDA concentration was determined by specific molar extinction coefficient of $1.56 X10^5 mol^{-1} cm^{-3}$.

MDA value (µmoles/ml) = Absorbance X dilution factor/ $(1.56 X 10^5)$.

3.1.10. 3. Reduced glutathione

Reduced glutathione (GSH) level in serum was estimated using the method described by (Lin *et al.* 1988) with some modifications. An aliquot of serum (400µL) was mixed with 400µl of tris-EDTA buffer followed by addition of 40µl of 10mM 5,5'-dithiobis (2-nitro benzoic acid) [DTNB] and 3.16ml of absolute methanol. Colour was developed after incubation at 37^0C for 30 minutes. Now, the suspension was centrifuged at 3500 rpm for 10 minutes. The absorbance of the supernatant was measured at 412nm (A) and subtracted from a DTNB blank (B) and a blank containing the sample without DTNB. In agreement with (Sedlak and Lindsay 1968), a value of 0.03 at 412nm for the sample blank was consistently obtained. Consequently, individual sample blanks were not critical and were taken as 0.03. GSH levels were conveniently calculated using an absorptivity of $13600 cm^{-1} M^{-1}$ as follows: (A-B-0.03) X (4.0/0.4)/ 13.6 = (A-B-0.03) X 0.735 mM.

3.1.10. 4 Superoxide dismutase activity

Serum superoxide dismutase (SOD) activity was measured using the method as given by (Madesh and Balasubramanian 1998) with some modifications. In the micro-titre plate method, the assay mixture in a total volume of 300µl per well consisted of 120µl PBS, 10µl serum sample, 5µl of 1.25 mM MTT and 15µl of freshly prepared 1mM pyragallol solution to be added at the end. Sample was

replaced with PBS in the blank. After an incubation period of 15 minutes, 150µl DMSO was added and absorbance was taken in ELISA reader at 570 nm.

The percent inhibition by the presence of SOD was calculated from the reduction of the MTT colour formation as compared to the MTT formazan formed in the absence of SOD which was taken as 100%. One unit of SOD was defined as the amount of protein required to inhibit the MTT reduction by 50%.

SOD activity (units/ml) = 2 X 100 X A_T/ A_B

Where, A_T= Absorbance for test.

And A_B= Absorbance for blank.

3.1.11 Haemaglutination inhibition (HI) assay

Haemaglutination inhibition HI) assay was performed as per the procedure of (OIE 2002). Briefly, two-fold serial dilution of 50µL serum was made with PBS in round bottom plates. Then, 50 µl of Newcastle disease virus or antigen was added up to 11th well. The plates were kept at room temperature for more than 30 minutes to facilitate antigen-antibody reaction. Then, 50µL of 0.5% (v/v) chicken RBC suspension was added to each well. However, 11th well contains antigen and RBCs as the positive control and the 12th well contains only RBCs as the negative control. After gentle mixing, the RBCs were allowed to settle at room temperature for 40 minutes and agglutination was assessed by tilting the plates. Samples showing peculiar central button shaped settling of RBCs were recorded as positive and maximum dilution of each sample causing haemagglutination inhibition was considered as the end point, which was used to estimate the HI titer. The HI titer of each serum sample was expressed as reciprocal of the serum dilution (Hossain *et. al.* 2010).

3.1.12. Carcass quality traits

At the end of 42 days of experimental period, two representative broiler chicks from each replicate (six birds per treatments) of all treatment groups were sacrificed

for evaluation of carcass characteristics, organ weight, sensory evaluation, and proximate composition.

3.1.12. 1. Dressing yield and organs weight

The broiler chicks were off fed for 12 hours before slaughter. However, water was provided *ad libitum.* Before slaughter, each broiler chicks was weighed. The chicks were sacrificed by cervical dislocation and allowed to bleed completely by cutting the jugular vein. After complete bleeding, weight of the bled carcass was recorded. The weight was again recorded after defeathering manually. Head and shank were removed by giving cuts at atlanto-occipital and hock joints, respectively and their respective weights were taken. Thereafter, a horizontal cut was applied posterior to keel bone. Breast was pushed forward to expose the viscera, which was then pulled out. Weight of the carcass was then recorded as dressed yield by the following formula:

Dressing yield = live weigh – (weight loss as blood, feathers, head, shank

and viscera)

The weight of different cut up parts viz., thigh, breast, drumstick, back, neck and wings were recorded by separating them from carcass and expressed as a percentage of live body weight. Internal organs (heart, liver, gizzard and spleen) and small intestine were detached from rest of the viscera, weighed individually and expressed as percentage of live weight. Gall bladder was removed from liver. Gizzard was opened, its contents were removed and epithelial linings were detached. Around 100 g of meat sample from both breast and thigh muscles were collected for the nutritional analysis of meat (AOAC 2003).

3.1.12.2. Sensory evaluation of meat

The sensory quality of cooked meat samples was evaluated by the standard sensory evaluation method (Keeton and Feedings 1984). The broiler meat sample of breast muscle of broiler chickens from each group collected and was boiled by addition of 1.5% salt solution and served warm to panelist for sensory evaluation. A sensory panel (semi trained) of eight judges drawn from post graduate students and

teaching staff were requested to evaluate the meat sample for different sensory attributes *viz*., appearance, flavor, texture, juiciness and overall acceptability. All the food products were presented in small plates labelled with three digit random codes. Panelists were provided with drinking water to rinse their mouth between samples. The samples were coded and presented in random order and panelists were asked to rate their unbiased assessment of colour, taste, texture and overall acceptability on a 9-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely). A score of 5 or below was considered a limit of acceptability for all sensory attributes tested.

3.1.12.3. Meat cholesterol

Total cholesterol of meat was determined as per (Zaltkis *et al.* 1953) with little modification as described by (Rajkumar *et al.* 2004) using cholesterol test kit (Span Diagnostics Ltd., India) except that instead of blood serum, lipid extract was used. Lipid extract was prepared by taking one gram of minced meat sample and adding 10 ml of freshly prepared 2:1 chloroform: methanol solution and homogenized. Homogenate was filtered using Whatman No. 42 filter paper. To 5 ml of filtrate equal quantity of distilled water was added, mixed and centrifuged at 3,000 rpm for 7 min. Top layer (methanol) was removed by suction. Volume of the bottom (chloroform) layer having cholesterol was recorded. From this, 25 µl of the sample was pipetted in a test tube and kept in water bath for 2-3 minutes till it dried. To this 5 ml of cholesterol reagent was added, mixed and kept in a boiling water bath for 90 seconds for colour development. The O.D. of standard and test against blank was taken at 530 nm. Total cholesterol (mg %) was calculated as follows:

Total cholesterol (mg %) =

$$\frac{\text{O.D of sample} \times \text{volume of chloroform (ml)} \times \text{Concentration of standard}}{\text{O.D of standard} \times \text{Weight of sample taken}}$$

3.1.13. Statistical analysis

The experimental data obtained were analysed statistically (Snedecor and Cochran 1994) as a completely randomized design by analysis of variance (ANOVA) by using general linear model (GLM) procedure of SPSS. Difference between treatments means were compared using Duncan's Multiple Range Test (Kramer 1957). Statistical significance was declared at $P \leq 0.05$.

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4 RESULTS AND DISCUSSION

The present investigation was conducted to study the comparative efficacy of synthetic and herbal vitamin E supplementation on growth performance, haemato-biochemical constituents, oxidative status, immunity, nutrient utilization, and carcass characteristics in Cobb 400Y broiler chicks. The results obtained have been presented as follows:

4.1 Production performance

The data on growth performance of broilers of different groups supplemented with synthetic vitamin E, herbal vitamin E and their combination during 5 weeks experimental period are presented in Table 2 to 4.

4.1.1 Starter phase (0-21 days)

The cumulative growth performance of broilers in terms of average weight gain, feed intake, FCR and performance index as influenced by supplementation of synthetic vitamin E, herbal vitamin E and their combination (Synthetic and Herbal vitamin E) during starter phase (0-21 days) is presented in Table 2 and depicted in Figure 1 and 2.

During the starter phase (0-21 days) feed consumption, body weight gain, FCR and performance index in broilers of various treatment groups differed significantly ($P<0.05$). There was significantly ($P<0.05$) highest body weight gain, (653.05g) in the group supplemented with herbal vitamin E (T_3), that was followed by the groups supplemented with the combination of synthetic and herbal vitamin E (T_4) (640.34g), synthetic vitamin E (T_2) (635.80g) and the control group (T_1) (617.51g). Maximum feed intake ($P<0.05$) was recorded in T_3 group (1106.40g) which was followed by the groups T4 , T2 and T1 that is 1079.88g, 1068.57g and 1049.40g, respectively. Also, the performance index increased significantly ($P<0.05$) in the herbal vitamin E supplemented group (T3) (385.47) followed by T4, T2 and T1 that is 379.71, 378.30 and 363.37, respectively. There was significantly ($P<0.05$) better FCR (1.69) obtained in T3 group than T1 group (1.70) but it was comparable to T4 group (1.69). The best FCR (1.68) was recorded in herbal vitamin E supplemented (T2) group among the four groups.

Table-2 Effect of supplemental vitamin E on growth performance of broiler chicks during starter phase (0-21days)

Treat-ments	BWG(g)	FI(g)	FCR	PI
T_1	$617.51^{a}\pm11.52$	$1049.40^{a}\pm16.58$	$1.70^{c}\pm0.05$	$363.37^{a}\pm7.51$
T_2	$635.80^{b}\pm14.96$	$1068.57^{b}\pm18.74$	$1.68^{a}\pm0.05$	$378.30^{b}\pm4.37$
T_3	$653.05^{c}\pm10.39$	$1106.40^{d}\pm12.18$	$1.69^{bc}\pm0.03$	$385.47^{c}\pm3.09$
T_4	$640.34^{b}\pm12.83$	$1079.88^{c}\pm15.98$	$1.69^{ab}\pm0.06$	$379.71^{b}\pm8.91$
SEm	3.90	6.33	0.00	2.52
P value	0.001	0.001	0.007	0.001

^{a,b,c} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Table-3 Effect of supplemental vitamin E on growth performance of broiler chicks during starter phase (22-35 days)

Treat-ments	BWG(g)	FI(g)	FCR	PI
T_1	$905.49^{a}\pm10.92$	$1650.48^{a}\pm20.21$	$1.82^{c}\pm0.03$	$496.77^{a}\pm5.19$
T_2	$922.30^{b}\pm16.93$	$1672.71^{b}\pm18.16$	$1.81^{bc}\pm0.05$	$508.54^{b}\pm8.48$
T_3	$955.40^{d}\pm18.32$	$1704.36^{c}\pm14.70$	$1.78^{a}\pm0.02$	$535.57^{d}\pm6.02$
T_4	$934.67^{c}\pm13.06$	$1684.84^{b}\pm19.12$	$1.80^{b}\pm0.05$	$518.52^{c}\pm8.01$
SEm	5.57	6.32	0.00	4.33
P value	0.001	0.001	0.001	0.001

^{a,b,c,d} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Figure-1. Effect of supplemental vitamin E on growth performance of broiler chicks during starter phase (0-21days)

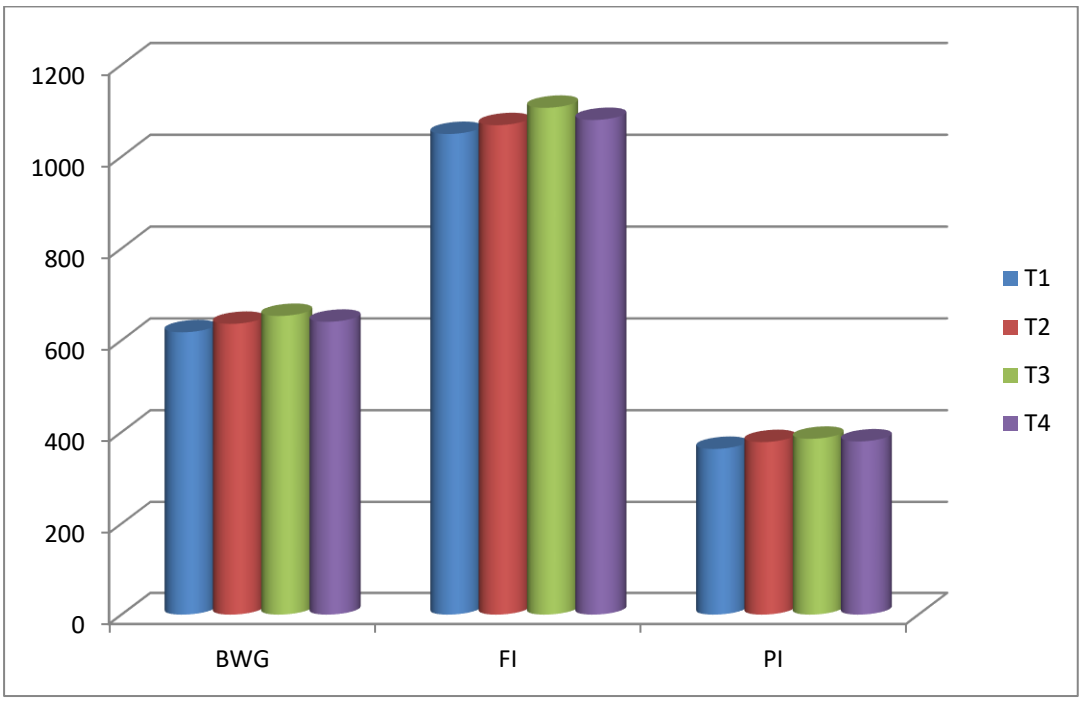
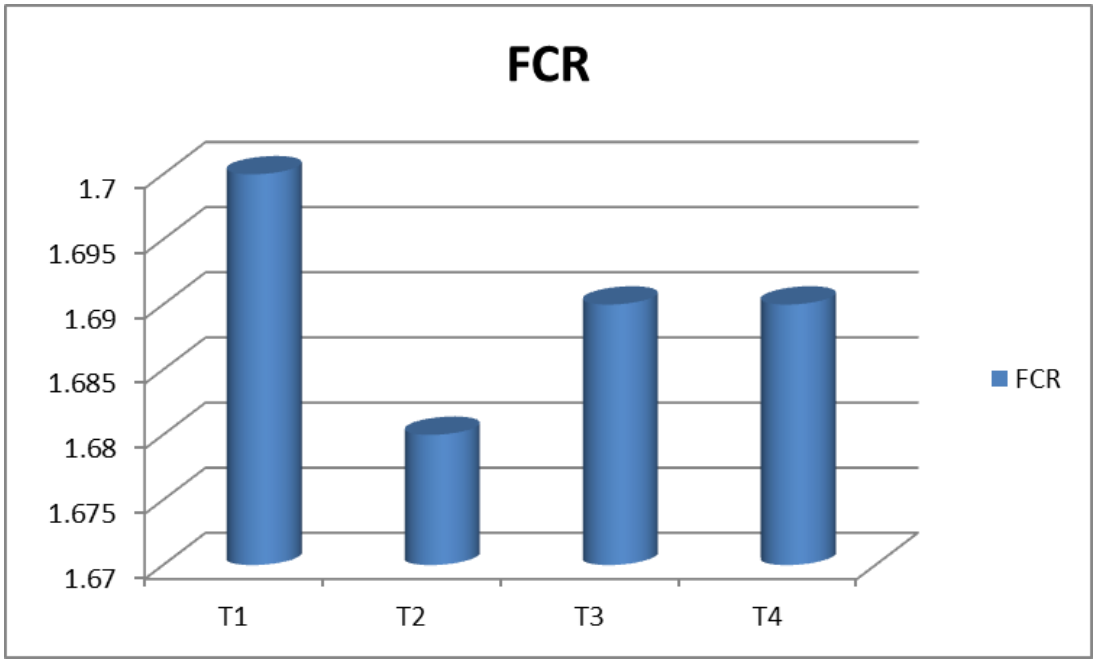


Figure-2. Effect of supplemental vitamin E on FCR of broiler chicks during starter phase (0-21days)



4.1.2 Finisher phase (22-35 days)

The cumulative growth performance of broilers in terms of average weight gain, feed intake, FCR and performance index and as influenced by synthetic vitamin E, herbal vitamin E and their combination (synthetic and herbal vitamin E) supplementation during finisher phase (22-35 days) is presented in Table 3 and depicted in Fig. 3 and 4.

During the finisher phase, the average weight gain of broilers was significantly ($P<0.05$) influenced by the dietary treatments. The body weight gain in broiler chickens fed diet supplemented with herbal vitamin E (T_3) group was significantly ($P<0.05$) higher (955.40g) than control (T_1) (905.49g) and those supplemented with synthetic (T_2) (922.30g) and their combination group (T_4) (934.67g). Feed intake follows the same pattern as in the body weight gain. Feed intake was significantly ($P<0.05$) higher in T_3 group (1704.36g) which was followed by T_4 (1684.84g), T_2 (1672.71g) and T_1 (1650.48 g) groups. Also, the performance index was significantly ($P<0.05$) higher in T_3 (535.57) group and it was followed by T_4 (518.52), T_2 (508.54) and T_1 (496.77) groups, respectively. FCR result was significantly lowest in the T_3 (1.78) group that was succeeded by T_4 (1.80) group. T_2 (1.81) and T1 (1.82) groups have higher FCR values than rest of the two groups but comparable with each other. There wassignificant ($P<0.05$) effect of dietary treatments on performance index of broiler chickens. Performance index was highest (535.57) in broilers fed diet containing herbal vitamin E (T_3) group, whereas birds fed diets containing synthetic vitamin E (T_2) and combination of herbal and synthetic vitamin E (T_4) had comparable performance index.

4.1.3 Overall period (0-35 days)

The cumulative growth performance of broilers in terms of average weight gain, feed intake, FCR, and performance index as influenced by synthetic vitamin E, herbal vitamin E and their combination (synthetic and herbal vitamin E) supplementation during overall experimental period (0-35 days) is presented in Table 4 and depicted in Fig. 5.

There was significant difference ($P<0.05$) observed in body weight gain among different groups. Maximum weight gain (1608.46 g) was recorded in broilersfed diet containing herbal vitamin E (T_3) followed by T_4 (1575.01g) , T_2 (1558.10g)

Figure- 3. Effect of supplemental vitamin E on growth performance of broiler chicks during finisher phase (22-35 days).

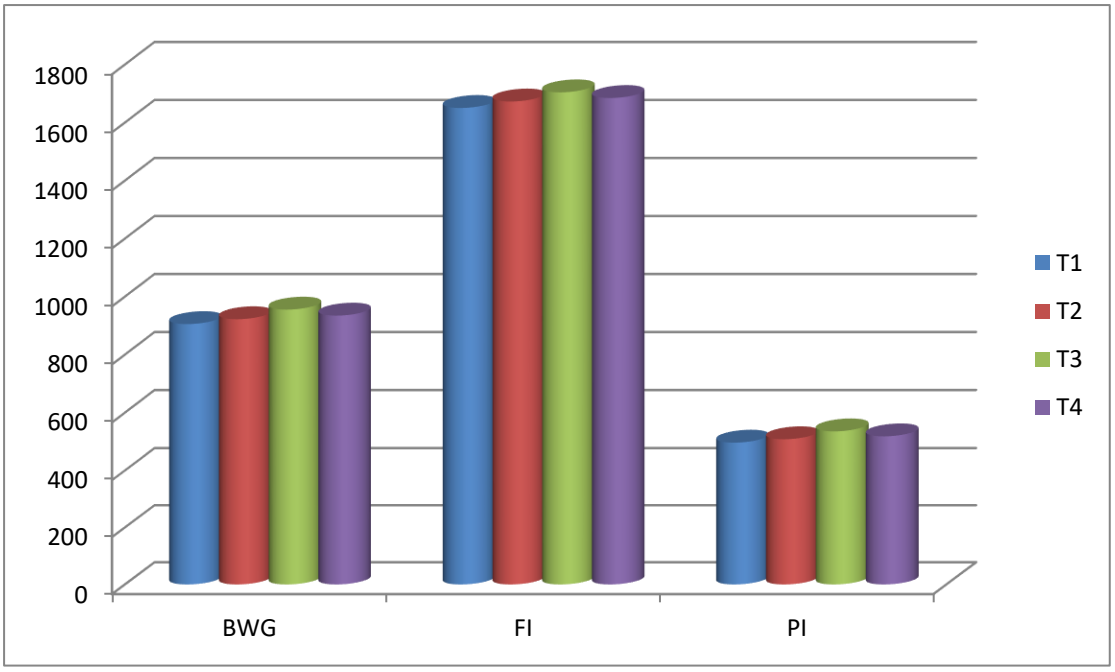
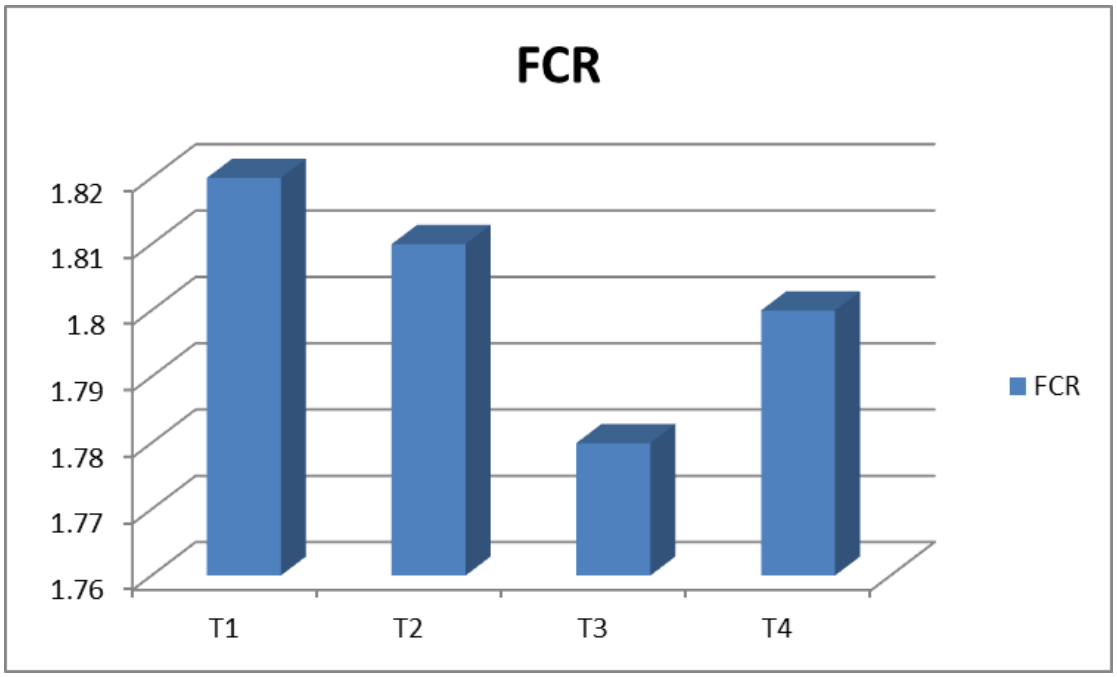


Figure- 4. Effect of supplemental vitamin E on FCR of broiler chicks during finisher phase (22-35 days)



and T_1 (1523.00g) groups. Feed intake and performance index also followed the same trend. Significant increase ($P<0.05$) in feed intake was recorded in different groups of which T_3 (2810.75g) has the highest feed intake followed by T_4 (2764.72g), T_2 (2741.28g) and T_1 (2699.89g) groups. Performance index was significantly ($P<0.05$) higher in T_3 (920.45) followed by T_4 (897.27), T_2 (885.60) and T_1 (859.13) groups. Also, there was significant difference ($P<0.05$) observed in FCR results among different groups. Lowest FCR (1.75) recorded in herbal vitamin E supplemented group (T_3) which was significantly higher as compared to synthetic vitamin E supplemented group (1.76) and the control group (1.77), but it was comparable with T_4 (1.76) group. Overall best FCR was recorded in herbal vitamin E supplemented group as compared with control group because herbal vitamin E helped in efficient utilization of feed.

In this study, birds fed the basal diet supplemented with vitamin E had significantly ($P < 0.05$) positive influence on average weight gain, feed intake, FCR and performance index during the period of 0-21days. There was higher BWG observed in herbal vitamin E supplemented group during 0-21 days. Similar results were obtained by Cheng et al. (2016). Whereas, Sheehy et al. (1991) reported that an increased vitamin E level from 5 to 180 mg/ kg diet did not affect the growth performance of broilers. Similar reports were found by Guo et al. (2001), who concluded that the growth performance of broilers was not significantly affected by the vitamin E treatments. It has been reported that the performance parameters were not modified either by the source or the dosage of the vitamin E supplementation to the turkeys (Rey et al. 2015). Likewise, Zhang et al. (2009) also suggested that supplemental vitamin E at 10, 30, and 50 mg/ kg did not affect the growth performance of broilers at 21 d of age due to the fact that basal level of α-tocopherol at 7.85 mg/ kg was adequate. FCR was significantly different ($P<0.05$) among different groups having best FCR values in group supplemented with synthetic vitamin E whereas herbal and combination groups had comparable values. Findings of Cheng et al. (2016) and Jhirwal et al. (2018) suggested that there was better FCR obtained in vitamin E supplemented groups and among supplemented groups best result was in synthetic vitamin E supplemented group.

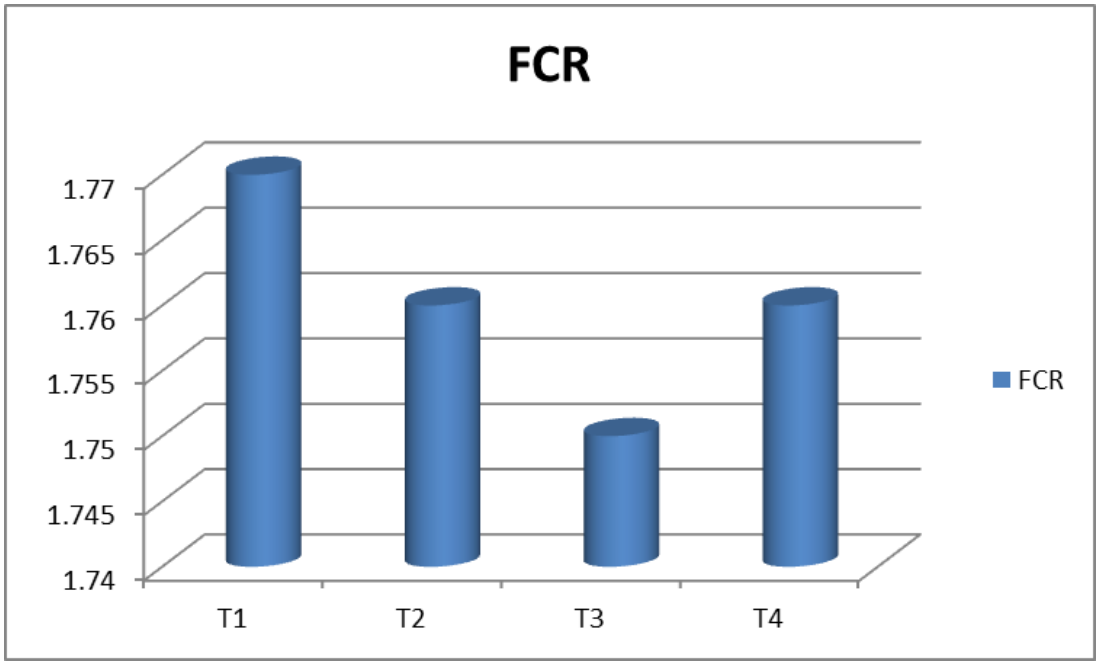
The feed conversion efficiency at this stage revealed that herbal vitamin E helped in efficient utilization of feed, which resulted in significantly lower feed

Table-4 Effect of supplemental vitamin E on growth performance of broiler chicks during starter phase (0-35 days)

Treat-ments	BWG(g)	FI(g)	FCR	PI
T_1	$1523.00^{a}\pm21.68$	$2699.89^{a}\pm39.87$	$1.77^{c}\pm0.02$	$859.13^{a}\pm12.47$
T_2	$1558.10^{b}\pm23.61$	$2741.28^{b}\pm34.52$	$1.76^{b}\pm0.05$	$885.60^{b}\pm12.66$
T_3	$1608.46^{d}\pm27.62$	$2810.75^{c}\pm44.25$	$1.75^{a}\pm0.03$	$920.45^{d}\pm16.89$
T_4	$1575.01^{c}\pm24.81$	$2764.72^{b}\pm32.91$	$1.76^{ab}\pm0.02$	$897.27^{c}\pm18.91$
SEm	9.40	12.47	0.00	6.74
P-value	0.001	0.001	0.002	0.001

^{a,b,c,d} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Figure-5: Effect of supplemental vitamin E on FCR of broiler chicks during 0-35 days



conversion ratio over control. During the period of 22-35 days better body weightgain, higher feed intake and better FCR obtained in vitamin E supplemented groups than control group. Similar findings were observed by Cheng et al. (2016). At fourth and fifth week of age, better results were obtained in herbal vitamin E supplemented group. These results were also in agreement with Silva et al. (2011).

During the entire period of 0-35 days there was significantly highest mean body weight gain was found in T_3 group followed by T_4, T_2 and T_1 group. Same trend was repeated in feed intake and performance index. Best FCR results were obtained herbal vitamin E supplemented group but it was comparable with combination group. Significantly highest overall body weight gain (week I-V) was observed in herbal vitamin E supplemented group followed by synthetic group supported by the findings of Jhirwal et al. (2018). Results the present study are in the agreement with the reports of Singh et al. (2005), Chae et al. (2006) and Shaiks et al. (2005). Feed intake during the entire rearing period (0-35 days) was found to be maximum in the herbal vitamin E group. These findings were akin to the findings of Chatterjee and Agarwal (2005), Shaik et al.(2005), Kumar and Singh (2005), Oliveros (2006) and Liu et al. (2009), Jhirwal et al. (2018), who observed significant improvement in feed consumption.

Significantly lower FCR values were recorded in herbal vitamin E supplemented group. The overall feed conversion efficiency (week I-V) best feed conversion efficiency was observed in group herbal vitamin E supplemented group but it was comparable with combination group. The best FCR of herbal vitamin E might be due to moderately higher weight gain, as compare to other groups. The results of significantly better feed conversion ratio due to incorporation of herbal vitamin E are similar to those of Panda et al. (2004), Shaik et al. (2005) and Liu et al. (2009), all of whom observed significant effect on feed conversion ratio by supplementing control diet with vitamin E.

Feed cost per kg body weight gain (Table. 7) was highest (Rs. 55.00) in control group and lowest (Rs. 53.84) in herbal vitamin E treatment group. Lowest feed cost in herbal vitamin E group might be due to better FCR, better nutrients utilization and lower price of herbal vitamin E as compared to synthetic vitamin E.

Table-5 Economics of broiler production in terms of feed cost per kg body weight gain of broiler chickens

Treatments	Feed Intake (gram)			Feed cost (Rs./ quintal)			Feed cost for body weight gain			Total feed cost for 0-35 days	Body weight gain (0-35 days)	Feed cost (Rs./Kg weight gain)
	Pre-starter (0-7 day)	Starter (8-21 days)	Finisher (22-35days)	Pre-starter feed (0-7 day)	Starter feed (8-21 days)	Finisher feed (22-35days)	Pre-starter (0-7 day)	Starter (8-21 days)	Finisher (22-35days)			
T_1	123.88	925.52	1750.48	3200	3000	2900	3.96	27.77	50.76	82.49	1523.00	55.00
T_2	128.85	940.07	1672.71	3360	3160	3060	4.33	29.71	51.18	85.22	1558.10	54.70
T_3	128.79	970.91	1704.36	3340	3140	3040	4.30	30.49	51.81	86.60	1608.46	53.84
T_4	127.38	954.96	1684.84	3350	3150	3050	4.27	30.08	51.39	85.74	1575.01	54.44

Cost of synthetic vitamin E: Rs. 320 per 200 gram

Cost of herbal vitamin E: 280 per 200 gram

4.2. Nutrient utilization

The data representing to the average total tract nutrient utilization in broilers during finisher phase are summarized in Table 6

Data pertaining to the average total tract nutrient utilization revealed that dry matter retention was significantly higher ($P<0.05$) in T_3 (75.11%) group from T_2 (72.59%) and T_1 (70.78%) but it was comparable with T_4 (73.71%) group. Crude protein retention was significantly higher in T_3 (67.09%) group was as compared to rest of the groups. Nitrogen retention was comparable in T_4 (65.30%) and T_2 (64.57%) groups whereas it was found to be lowest in the control (T_1) (62.43%) group. Ether extract retention was found to be significantly ($P<0.05$) higher in herbal vitamin E (77.32%) supplemented group that was comparable with the combination group (76.75%) but it was higher than synthetic vitamin E supplemented group (75.12%) followed by control group (73.78%).

There was no significant ($P>0.05$) change in the calcium retention although numerically higher retention observed in T_3 (46.9) group followed by T_4 (46.72), T2 (45.52) and T1 (45.02). There was no significant ($P>0.05$) change in the phosphorous retention among the groups supplemented with vitamin E or control group although numerically higher retention observed in T_3 (76.11) group followed by T4 (76.03), T2 (74.21) and T1 (72.62).

4.3. Haematological parameters

The data representing the haematological parameters (haemoglobin, packed cell volume, total erythrocyte count, mean corpuscular volume and mean corpuscular haemoglobin) in broilers fed diet supplemented with herbal vitamin E, synthetic vitamin E and their combination on 35^{th} day of feeding trial are summarized in Table 7 and depicted in Fig. 7

The average haemoglobin content in broiler chicks differed significantly ($P<0.05$) among different groups. Broiler chicks fed herbal vitamin E supplemented ration had numerically higher value (9.53) followed by combination group (9.11), synthetic vitamin E supplemented group (8.75) and control group (8.46). This result was in agreement with findings of El-Sheikh et al. (2006) and Shashikant et al., (2014) whereas Swain et al. (2000) didn't observed change in haemoglobin content in broiler chickens fed vitamin E supplemented diet.

Table-6 Effect of supplemental vitamin E on total tract nutrient retention (%) of broiler chickens

Treatments	DM retention (%)	Nitrogen retention (%)	EE retention (%)	Calcium retention (%)	Phosphorous retention (%)
T_1	70.78^{a}±0.70	62.43^{a}±0.55	73.78^{a}±0.43	45.02±0.88	72.62±1.41
T_2	72.59^{b}±0.32	64.57^{b}±0.42	75.12^{b}±0.20	45.52±0.27	74.21±1.19
T_3	75.11^{c}±0.33	67.09^{c}±0.47	77.32^{c}±0.63	46.91±1.20	76.11±1.07
T_4	73.71^{bc}±0.29	65.30^{b}±0.41	76.75^{c}±0.39	46.72±0.68	76.03±0.62
SEM	0.515	0.542	0.460	0.422	0.599
P value	0.001	0.001	0.002	0.329	0.114

^{a,b,c,d} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Table-7 Effect of supplemental vitamin E on haematological parameters of broiler chickens

Treatments	Hb	PCV	TEC	MCV	MCH
T_1	8.46^{a}±0.098	26.95^{a}±0.31	2.27^{a}±0.02	115±1.07	37.30±0.37
T_2	8.75^{ab}±0.084	28.30^{b}±0.43	2.35^{b}±0.03	120.57±2.73	37.27±0.68
T_3	9.53^{c}±0.213	30.11^{c}±0.50	2.59^{c}±0.02	116.25±1.25	36.79±0.66
T_4	9.11^{bc}±0.205	28.93^{bc}±0.39	2.42^{b}±0.01	119.57±1.63	37.67±0.81
SEM	0.112	0.360	0.027	0.961	0.313
P value	0.001	0.001	0.001	0.127	0.826

^{a,b,c,d} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Figure-6: Effect of supplemental vitamin E on total tract nutrient retention in broilers

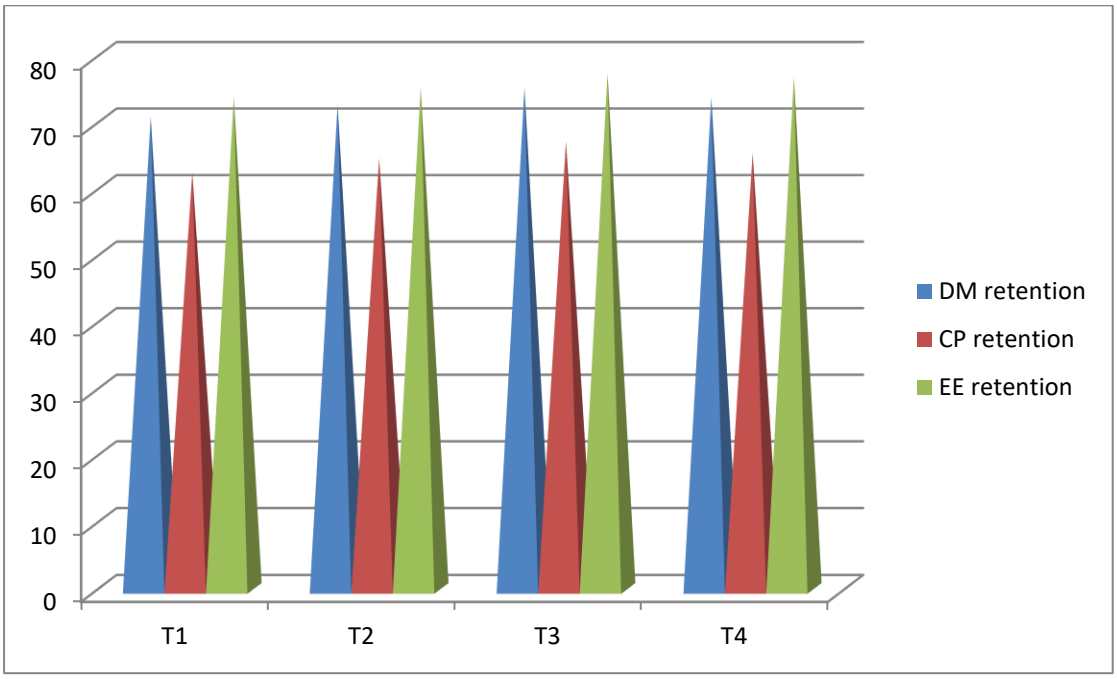
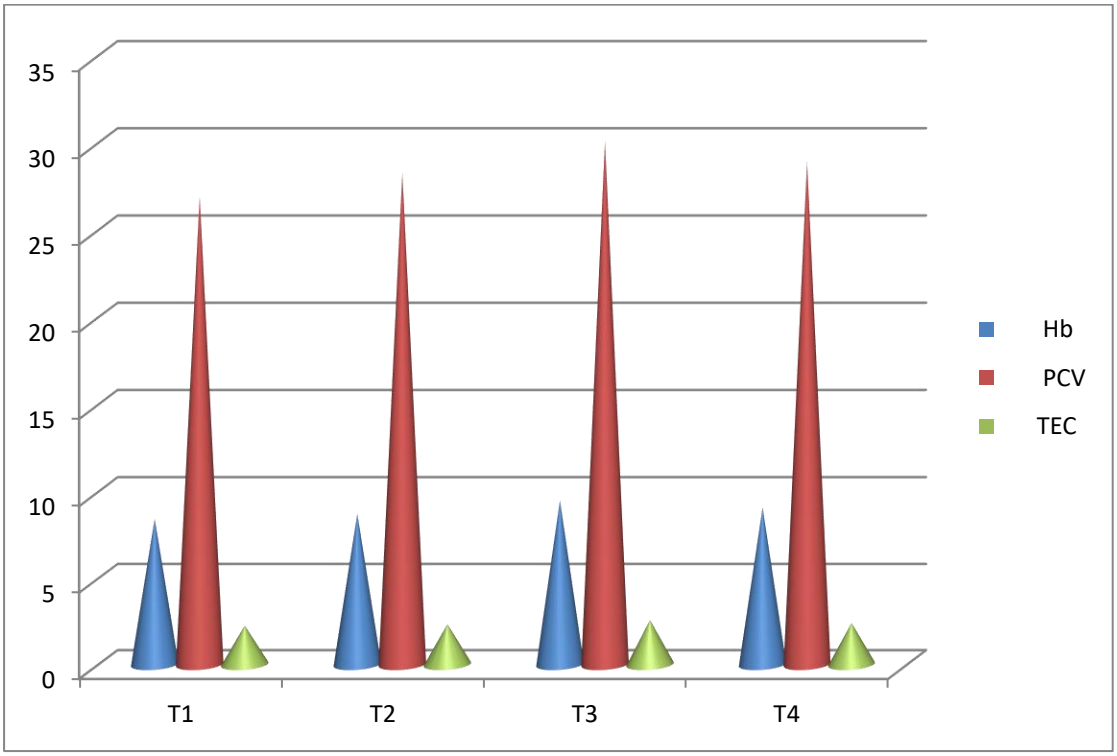


Figure-7: Effect of supplemental vitamin E Haematological parameters in broilers



The PCV values vary significantly (P<0.05) among supplemented and control groups. Highest PCV values recorded in T_3 (30.11) group which was comparable with T_4 (28.93). Birds of group T_2 (28.30) had comparable value with T_4 but the overall PCV of supplemented group was significantly higher than control group (26.11). This may be due to the effect of vitamin E on physiological parameters which increase immune system, thus, increase PCV concentration. El-Sheikh et al. (2006) reported that vitamin E has an essential role in improved performance or greater safety. This result of blood picture (PCV) could be attributed to the role of vitamin E as fat soluble antioxidant which protects the biological membranes from oxidative damage and decrease osmotic fragility of erythrocytes (Dlouha et al., 2008). This result was in agreement with the findings of (El-Sheikh et al., 2006); Shling, 2009). Similar result was found by Tayeb and Qader (2012).

Total erythrocyte count (TEC) was found to be significantly (P<0.05) higher in herbal vitamin E supplemented group (2.59 million /mm^3). It was followed by combination group (2.42 million/mm^3) and synthetic vitamin E supplemented group (2.35million/mm^3). These two have comparable values and there is least TEC found in the control group (2.27million/mm^3). Also Shashikant et al. (2014) got the same finding. In the present study there was no significant (P>0.05) change observed in MCV and MCH values among different groups. In contrast Shashikant et al. (2014) reported significantly (P<0.05) lower MCV and MCH values.

4.4. Serum biochemical parameters

The data on serum biochemical parameters in broiler chickens fed diet supplemented with different additives on 35^{th} day of feeding trial are summarized in Table 8.

4.4.1. Serum glucose

The values of mean serum glucose concentration in broiler chickens did not differ significantly (P>0.05) among different treatments (Table 4.1.7). Although there is numerically lower serum glucose concentrations found in the supplemented groups as compared to control group the change is insignificant. All values are within the normal range (130 to 270 mg/dl) as mentioned by Reece (2004). Also no significant difference in glucose was reported by the findings of Behera et al., (2019).

4.4.2 Serum total protein, albumin and globulin

In the present study no significant difference (P>0.05) was recorded in the total protein, albumin and globulin among all the treatment groups. Also Behera et al., (2019) have the similar findings. However there was significantly (P<0.05) higher serum total protein and albumin level than the control was suggested in the findings of Shashikant et al. (2014).

4.4.3 Total serum cholesterol

There is significant change (P<0.05) in the serum cholesterol level after supplementing vitamin E. There is clear difference of cholesterol level between vitamin E supplemented groups and unsupplemented group. Among the supplemented groups minimum cholesterol level was recorded in the group supplemented with herbal vitamin E (128.26) that was succeded by group supplemented with combination of two (133.73) and then synthetic vitamin E supplemented group (136.50). Control group have the maximum cholesterol level (158.02). From the above findings higher antoxidant property of the herbal vitamin E is very much clear. Result of the present study is similar to those of the previous findings. The serum cholesterol concentrations of all treatment groups were within the normal range for chickens (125 to 200 mg/dl) as reported by Reece (2004). Also behera have the similar findings. Shashikant et al. (2014) showed that total cholesterol levels were lower in treatment groups than the control group and the difference were significant (P<0.05). Habibian et al. (2013) found that chickens fed on a diet supplemented with vitamin E at both levels (125 and 250 mg/kg feed) produced significantly (P<0.05) lower LDL-cholesterol and significantly higher (P<0.05) HDL-cholesterol. Dieber Rotheneder et al. (1991) suggested vitamin E, most importantly α-tocopherol, is the major antioxidant transported in the LDL particle.

Kim et al., (2014) supplemented poultry diets with red ginseng marc and αtocopherol had a significant (P < 0.05) effect on serum cholesterol levels after 35 days, which was inconsistent with the results of prior studies. Bong et al (2011), Ao et al. (2011), Jang et al., (2007). In their study, increasing red ginseng marc level to 3% (T3) reduced the total cholesterol, low density lipoprotein cholesterol (LDL) and

Table-8 Effect of supplemental vitamin E on serum bio-chemical parameters of broiler chickens

Treatments	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total cholesterol (mg/dl)
T_1	258.43 ± 1.36	4.73 ± 0.11	3.12± 0.15	1.61 ± 0.21	158.02^{b} ± 3.58
T_2	250.43 ± 3.18	4.82 ± 0.18	3.01± 0.04	1.80 ± 0.18	136.50^{a} ± 3.51
T_3	251.46 ± 3.40	5.08 ± 0.15	3.13 ± 0.05	1.95 ± 0.17	128.26^{a}± 6.20
T_4	249.10 ± 3.95	5.00 ± 0.10	3.18 ± 0.04	1.82 ± 0.09	133.73^{a} ±4.15
SEm	1.642	0.073	0.052	0.079	3.155
P- value	0.294	0.324	0.599	0.547	0.001

^{a,b,c,d} Mean values with different superscripts within a column differ significantly (P<0.05).

triglyceride levels, and increased the high-density lipoprotein (HDL) cholesterol levels. Also Choi et al. (2010) reported that a combination of garlic powder and α-tocopherol significantly decreased serum cholesterol and increased HDL cholesterol in broiler blood ($P < 0.05$).

4.5. Serum enzymes

The data representing the activity of enzymes *viz.*, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in broilers fed diet supplemented with herbal vitamin E, synthetic vitamin E and the combination of two are shown in Table 9. In the present study there was no any significant ($P>0.05$) change observed in the AST, ALT and ALP. However, findings of Shashikant et al. (2014) suggested that there was decline in the ALT and AST values.

4.6. Immune response

4.6.1 Effect on lymphoid organ weight

There was no significant change ($P>0.05$) on lymphoid organs in broiler chicks fed diets supplemented with synthetic vitamin E, herbal vitamin E and their combination. This is presented in the Table 10 and depicted in Fig. 8.

Similar findings were observed in previous studies of Dalia et al. (2018). Vitamin E supplementation had no significant effect ($P>0.05$) on the lymphoid organ weights (thymus, bursa, and spleen) in broiler chickens. These findings are consistent with previous results of Niu et al. (2009) who revealed that vitamin E supplementation could not affect the lymphoid organ. Moreover, Habibian et al. (2014) indicated that dietary Se had no positive effect on the lymphoid organ weights under heat stress, however, the dietary inclusion of vitamin E showed improvement of the relative weights of lymphoid organs.

Cheng et al. (2017) also had the similar findings. The bursa of Fabricius and thymus are two primary lymphoid organs in the chicken. The bursa is unique to birds, and B-cell differentiation takes place in the bursa (Sharma 2000). The thymus gland is the primary lymphoid organ for T-cell differentiation. The spleen is another main development status and function of these immune organs determine the immunity

Table-9: Effect of supplemental vitamin E on serum enzymes of broiler chickens.

Treatments	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
T_1	27.63±0.85	170.31±2.41	214.66±3.15
T_2	28.05±0.75	168.01±2.73	208.97±3.10
T_3	29.52±0.64	167.87±1.16	216.38±5.73
T_4	26.85±0.87	171.89±2.77	218.12±5.84
SEM	0.419	1.156	2.285
P value	0.144	0.581	0.547

Table-10: Effect of supplemental vitamin E on lymphoid organ weight (% of body weight) and Antibody titre against response to Newcastle Disease vaccine in broiler chickens

Treatments	Spleen	Thymus	Bursa of Fabricius	ND Ab Titre in Log_2
T_1	0.11±0.005	0.35±0.001	0.07±0.005	4.52^{a}±0.12
T_2	0.11±0.007	0.36±0.017	0.08±0.005	5.40^{b}±0.14
T_3	0.11±0.007	0.35±0.004	0.07±0.005	6.12^{c}±0.13
T_4	0.11±0.005	0.36±0.002	0.08±0.003	5.68^{b}±0.10
SEm	0.003	0.002	0.002	0.135
P value	0.637	0.096	0.264	0.001

^{a,b,c} Mean values with different superscripts within a column differ significantly ($P<0.05$).

level of broilers. The relative weights of immune organs are commonly used to reflect the growth and development of organs (Chen et al. 2016). In the current study, inclusion of vitamin E in the diet had no effect on the relative weights of immune peripheral lymphoid organ of systemic immunity in birds and plays an important role in disease resistance (John 1994). Theorgans. These results are consistent with the finding of Niu et al. (2009), who reported that none of these organs (thymus, bursa, or spleen) of broilers were significantly affected by the level of vitamin E in the diet. In contrast, Zhang et al. (2009) indicated that broilers given a diet supplemented with 50 mg kg/1 RRRα-tocopherol succinate had higher relative weights of spleen and bursa than the control group. The differences in the results might be due to the types, usage, and levels of vitamin E in broilers. This is a little information available in literature on how these parameters are affected by the source of vitamin E supplementation or the dosage given to the birds. In the present study, our results showed that inclusion of natural vitamin E in the diet, as an alternative of the synthetic vitamin E, had no influence on the development of immune organs

4.6.2 Antibody titre against Newcastle Disease vaccine

The result of immune response to NewcastleDisease vaccine in chicken after supplementation of synthetic vitamin E, herbal vitamin E and their combination given in Table 10 and depicted in Fig. 9.

The data reveals that there was significant change ($P<0.05$) in the antibody titre value among the different groups. Further highest antibody titre was recorded in the group supplemented with herbal vitamin E (6.12) followed by combination group (5.68) and synthetic vitamin E supplemented group (5.40). But T_4 and T_2 groups have comparable titre values. Least antibody titre was observed in control group (4.52). The results of this study are also in correlation with earlier findings of Weber et al., (2008). Also Dalia et al. (2018) had similar findings. Dietary vitamin E and Se combination improved the humoral immunity and provided better immunity response through their role on free radical elimination and oxidative stress stability of the immune cells (Singh et al. 2006)

Figure-8: Effect of supplemental vitamin E on Lymphoid Organs weight (% of BW) in broilers

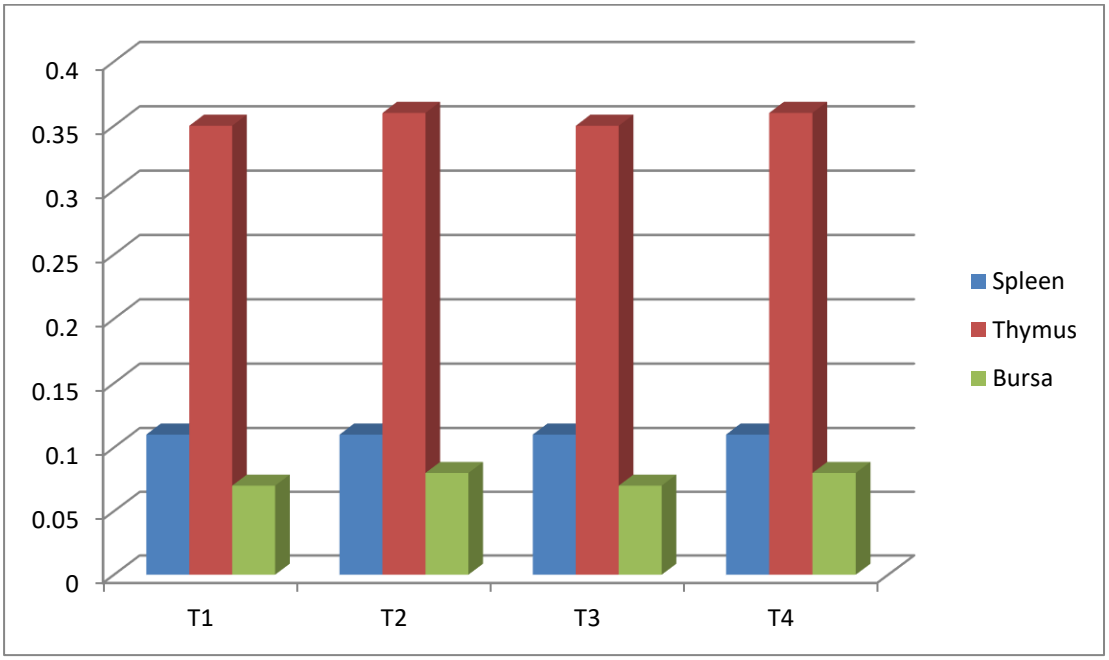
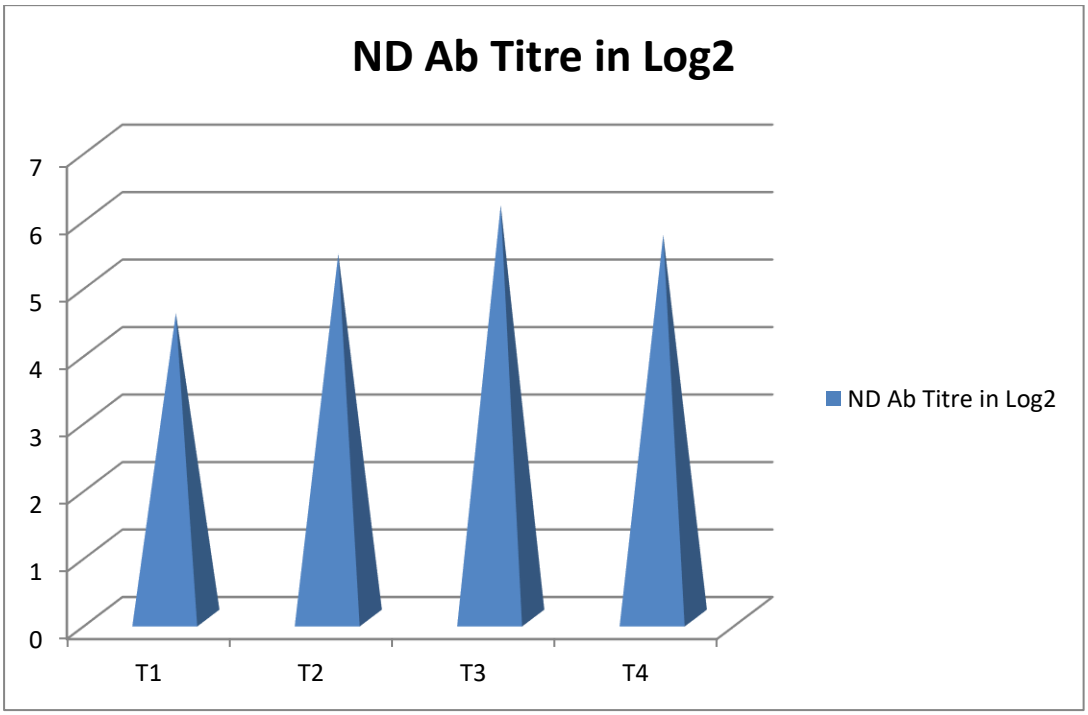


Figure-9: Effects of supplemental vitamin E on Antibody titre against Newcastle Disease Vaccine in broilers.



The present study is in agreement with Sasiadek et al. (2016) Vitamin E may also affect a decrease in mortality rate of chickens, which has been confirmed by results obtained in this study. A slightly lower mortality rate in the group administered vitamin E may be explained by the positive effect on the immune system. Vitamin E has a direct impact on the immunological system by inhibiting protein kinase C in cells of monocytes and lymphocytes, as well as by reducing the secretion of immunosuppressive factors, including hydrogen peroxide (ERF et al. 1998). Choct and Naylor (2004) also demonstrated the positive effect of vitamin E on the reduced mortality rate in chickens. This result was also consistent with the finding that vitamin E and Se combination improved the humoral immunity and provided better immunity response (Singh et al. 2006 , Habibian et al. 2014).

4.7 Antioxidant status of serum

4.7.1 Glutathione (GSH)

Effect of supplementation of synthetic vitamin E, herbal vitamin E and their combination on the activity of GSH is given in Table 11 and depicted in Fig. 10.

In the present study GSH activity (µmol/l) recorded is significantly higher ($P<0.05$) in the group supplemented with herbal vitamin E (9.96) that is followed by group containing both (9.21) and synthetic vitamin E supplemented group (8.89). The supplemented groups had significantly higher GSH activity than control group (6.31).

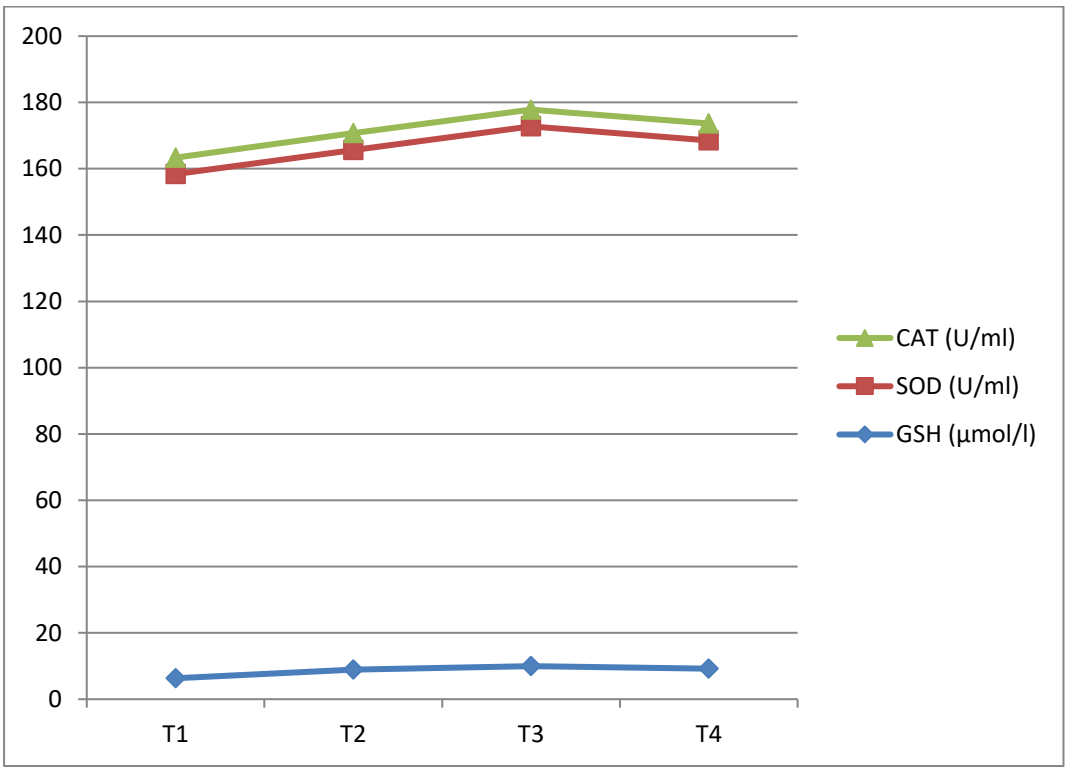
Behera et al. (2019) in their findings suggested significantly higher ($P<0.05$) GSH value in the serum of birds after supplementation of antioxidants. Cheng et al. (2017) also have the similar results. Ognik and Wertelecki. (2012) suggested that turkeys given with D-α-tocopherol had improved antioxidant status compared with those that received DL-α-tocopherol at the same dosage of 50 mg kg−1 , which was evidenced by enhanced glutathione level.

Regarding the glutathione concentration, in the groups supplemented with dietary natural tocopherol and monitored over the entire experimental period, glutathione concentration was significantly higher than in the control. Glutathione is the ubiquitous thiol found in cells, at high concentrations of 1 to 10 mmol/L. Its primary biological function is to act as a nonenzymatic reducing agent to keep the protein thiol groups in a reduced state, which, in numerous cases, is essential for the functional activity of proteins, particularly their antioxidant activity Bartosz. (2004); Weitzel et al. (1989).

Table-11: Effect of supplemental vitamin E on anti-oxidant parameters in blood of broiler chickens

Treatments	GSH (µmol/l)	SOD (U/ml)	CAT (U/ml)
T_1	6.31^{a}±0.20	152.08±2.28	4.96±0.06
T_2	8.89^{b}±0.08	156.76±2.94	5.12±0.05
T_3	9.96^{c}±0.14	162.81±3.99	5.05±0.16
T_4	9.21^{b}±0.07	159.31±2.16	5.16±0.04
SEM	0.293	1.594	0.046
P value	0.001	0.102	0.467

Figure-10: Effects of supplemental vitamin E on antioxidant status of serum in broilers



4.7.2 Superoxide Dismutase (SOD)

Effect of supplementation of synthetic vitamin E, herbal vitamin E and their combination on the activity of SOD is given Table 11 and depicted in Figure 10.

There is non significant (P>0.05) change in the SOD values in the supplemented groups was found as compared to the control group. Although. numercally highest SOD activity was observed in the group fed with herbal vitamin E (162.81) followed by mixture group (159.31) then synthetic vitamin E supplemented group (156.76). Control group have least (152.08) SOD activity. The result of the present study were found to be in agreement with the previous studies.

The findings of Cheng et al. (2017) have similar result. The SOD catalyzes the dismutation of superoxide radical to hydrogen peroxide, which is detoxified into H2O and O2 by GSH-PX (Wang et al. 2015). In the study of Cheng et al., (2017) inclusion of vitamin E in the diet increased plasma T-SOD activity, GSH-PX activities. These results indicated that dietary supplementation with vitamin E enhanced antioxidant capacity of broilers, which is similar with the findings of Demerdash (2004) and Wang et al. (2015). Also Ognik and Wertelecki (2012) in turkey found that inclusion of both forms of vitamin E in the diets of birds did not produce any detectable changes in the activity dynamics of the antioxidative enzymes studied (i.e., superoxide dismutase and catalase). Superoxide dismutase and catalase are antioxidant enzymes that, by dismutation reaction, are capable of eliminating peroxides from the environment, thereby preventing the formation of other ROS and their derivatives. Their inactivation also relies on micro-molecular (non-enzymatic) substances present in a cell or extracellular space.

Saleh et al. (2018) in their findings suggested that a-tocopherol did not influence the activity of SOD. Vossen et al. (2011) after supplementing antioxidant suggested it is not significant for SOD activity. Superoxide dismutase is a major antioxidant enzyme that protects cells and organisms from the damaging effects of superoxide anion. SOD works in conjunction with catalase and peroxidases to diminish the harmful effects of ROS. In addition to extracellular SOD, two other known SOD forms exist: an intracellular form, Cu2+/Zn2+ SOD, and a mitochondrial form, Mn2+ SOD (Ottaviano et al. 2008).

4.7.3 Catalase (CAT)

Effect of supplementation of synthetic vitamin E, herbal vitamin E and their combination on the activity of CAT is given in Table 11 and depicted in Figure 10.

There was no significant change in the groups supplemented with vitamin E. The numerical values however shows rise in catalase activity (in U/ml). Maximum value was in the group T_4 (5.16) followed by T_2 (5.12) group and T_3 (5.05)group. Control group (4.96) shows least numerical value.

Findings of the present study corroborated with earlier studies of Ognik and Wertelecki (2012) in the turkey hens. Also Behera et al. (2019) have the similar findings in broilers after supplementing antioxidants in their diet.

4.8 Carcass traits

Data pertaining to the average dressing yield, processing losses, organ weight, cut- up parts and abdominal fat of broilers of different treatment groups due to supplementation of Synthetic vitamin E , Herbal vitamin E and their combination (Synthetic and Herbal vitamin E) are presented in Table 11 to 12 and expressed as per cent of pre-slaughter live weight.

4.8.1 Dressing yield, processing losses and cut-up parts

The results of dressing percentage, processing losses, relative weight of some internal organs and cut-up parts of broiler chickens due to addition of Synthetic vitamin E , Herbal vitamin E and their combination (Synthetic and Herbal vitamin E) in the diet of broiler chickens are presented in Table 11 and Table. 12.

The carcass characters *viz*., dressing yield, eviscerated yield, blood loss, feather loss, meat to bone ratio and relative weights of giblet, heart, liver and gizzard were found to be statistically similar ($P>0.05$) among the broilers of different treatment groups. Similarly different cut-up parts (% of live weight) *viz.* thigh, drumstick, breast, back, wing and neck did not differ among the different dietary treatments. These results are in agreement with the findings of Daniel et al. (2017) who reported that feeding vitamin E had no significant ($P>0.05$) effects on carcass cuts and visceral organs ofbroilers . The results of this study agree in part with those obtained by other workers.

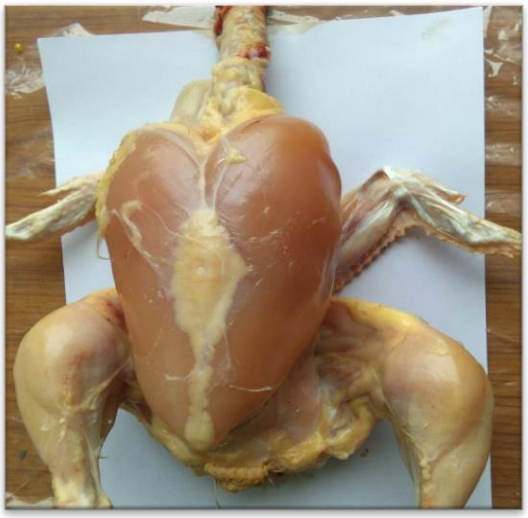


Plate 15: Dressed Carcass

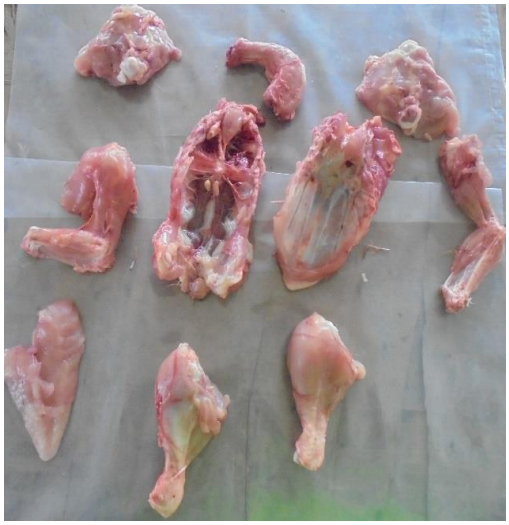


Plate 16: Cut-up parts

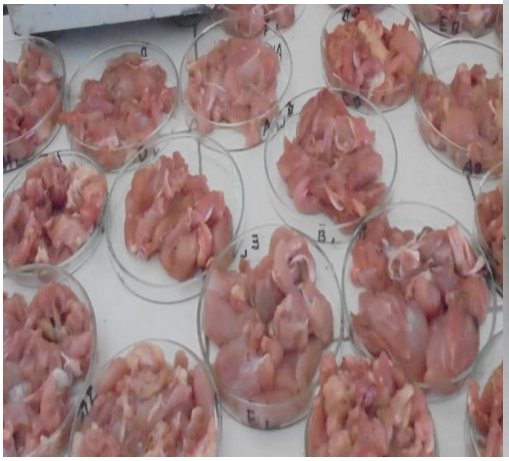


Plate 15: Meat samples

Plate 16: GIT of experimental birds

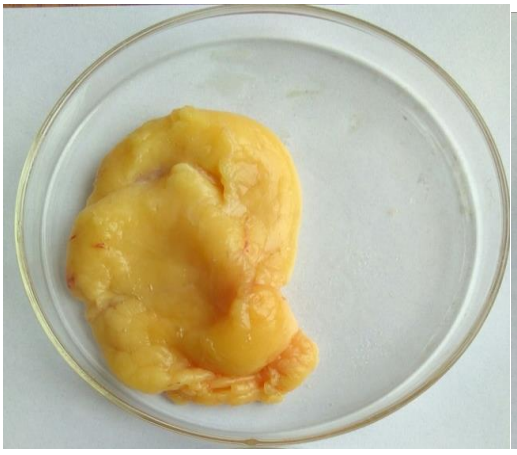


Plate 17: Abdominal fat of birds

Plate 18: Liver of experimental birds

Table-12 Effect of supplemental vitamin E on carcass characteristics of broiler chickens

Treatments	Blood loss	Feather loss	Dressing yield	Eviscerated yield	Giblet	Heart	Liver	Meat: Bone
T_1	3.80±0.34	5.01±0.10	68.39±0.55	63.68±0.49	5.25±0.07	0.62±0.03	2.34±0.08	2.12±0.04
T_2	4.21±0.17	5.13±0.23	69.12±0.93	63.96±0.39	5.27±0.13	0.64±0.03	2.36±0.04	2.25±0.08
T_3	4.05±0.31	5.06±0.07	71.48±0.89	65.34±0.52	5.35±0.12	0.71±0.02	2.51±0.13	2.31±0.16
T_4	3.67±0.35	5.18±0.17	70.44±0.38	65.01±0.32	5.28±0.05	0.66±0.02	2.39±0.08	2.28±0.11
SEm	0.15	0.07	0.42	0.25	0.05	0.01	0.04	0.05
P-value	0.553	0.881	1.420	0.734	1.151	1.473	0.663	0.851

Table-13 Effect of supplemental vitamin E on cut-up parts (% of body weight) of broiler chickens

Treatments	Thigh	Drumstick	Breast	Back	Wing	Neck	Abdominal fat
T_1	10.17±0.20	8.84±0.24	18.15±0.18	16.86±0.22	8.10±0.31	4.26±0.08	1.64^{c}±0.02
T_2	10.19±0.18	9.21±0.21	18.24±0.43	17.05±0.38	8.58±0.25	4.48±0.13	1.49^{b}±0.01
T_3	10.51±0.16	9.30±0.12	19.14±0.30	17.28±0.48	8.64±0.19	4.64±0.14	1.41^{a} ±0.02
T_4	10.36±0.22	9.26±0.04	18.29±0.71	17.06±0.40	8.63±0.16	4.61±0.17	1.45^{ab} ±0.01
SEm	0.09	0.09	0.23	0.18	0.12	0.07	0.196
P-value	0.408	0.161	0.263	0.252	0.482	0.546	0.001

^{a,b,c} Mean values with different superscripts within a column differ significantly (P<0.05).

Choct and Naylor (2004) who found no differences in the absolute weight of carcass, breast, or thigh+ drumstick in broilers slaughtered at 38 days testing different sources of selenium (0.1 mg organic or inorganic selenium) and vitamin E levels (50 and 100 mg/kg). Tayeb and Qader (2012) reported vitamin E had no significant effect each carcass parts percentage (breast, thigh, back, neck, wing and abdominal) % between treatment groups, also there were no significant differences between treatment groups compared with control group. There was significant decrease ($P<0.05$) in the abdominal fat percent in group supplemented with herbal vitamin E (1.41) followed by combination group (1.45) and synthetic group (1.49). Control group have the maximum abdominal fat percent (1.64). The results obtained in the present study corroborated with previous studies.

Ognik and wertelecki. (2012) reported significant influence of both forms of tocopherol on the slaughter traits in turkey. Dressing percentage ranged between 80 and 84%. The turkey hens receiving dietary RRR-d-α-tocopherol 5.2% higher dressing percentage as compared with the group fed supplementary dl-α-tocopherol acetate. The weight of edible giblets also differed significantly in the experimental groups, but no differences were observed for leg muscles. The highest breast muscle content was determined in carcasses of the turkey hens receiving dietary RRR-d-α-tocopherol supplementation. The groups of birds fed vitamin E in the natural form showed a significantly ($P \leq 0.05$) lower gizzard weight and lesser adiposity as compared with the groups of turkey receiving the synthetic form of vitamin E. Malczyk et al.,(2001) who studied vegetable oils and α-tocopherol added at various levels to chicken diets, found that they did not influence dressing percentage or breast and leg muscle contents. Sasiadek et al. (2016) however recorded higher carcass yield in chickens from the experimental group ($P< 0.01$). There was no significant difference in the percentage of breast and leg muscle depending on diet. The experimental group chickens had a higher percentage of heart and gizzard ($P<0.05$) of the body weight but a lower percentage of liver ($P<0.01$).

The addition of vitamin E affected a decrease of abdominal fat in birds from the experimental group. Skrivan et al. (2010) suggested a positive effect of vitamin E on the fat content of breast muscles in the carcass at appropriately high supplementation. Castellini et al. (2002) emphasised the significance of reduced adiposity in an extended rearing period. Daniel et al. (2017) reported relative weight

of abdominal fat with the increase in vitamin E, this demonstrates that vitamin E strongly influences the lipid metabolism.

4.8.2. Meat cholesterol level

Data related to cholesterol level in breast meat of broiler chicks is presented in Table. 14 and depicted if Figure. 11.

There is significant change ($P<0.05$) in the meat cholesterol level after supplementing vitamin E. There is clear difference of cholesterol level between vitamin E supplemented groups and un-supplemented group. Among the supplemented groups minimum cholesterol level was recorded in the group supplemented with herbal vitamin E (39.23) that was succeeded by group supplemented with combination of two (40.52) and then synthetic vitamin E supplemented group (42.66). Control group have the maximum cholesterol level. From the above findings higher antoxidant property of the herbal vitamin E is very much clear. Result of the present study is similar to those of the previous findings. Sasiadek et al. (2016) suggested meat of chickens fed with the higher dose of vitamin E was characterized by a lower cholesterol level. The addition of vitamin E as well as type of the muscles had a significant effect on the fatty acid profile. The recorded increase in the content of n-3 polyunsaturated fatty acids (PUFAs) and a decrease in the n-6/n-3 fatty acid ratio indicate a positive effect of vitamin E on the fatty acid profile. The supplementation of the higher dose of vitamin E caused a significantly lower content of cholesterol in tissue of breast muscle ($P \leq 0.01$) and leg muscle ($P \leq 0.05$). Sudarman et al. (2011) applied *Pluchea indica* in broilers feeding and observed over 8% decrease in cholesterol content in meat. Strong antioxidative properties of *P. indica* have been widely known and described by Sen et al. (2002). Souza and Silva (2006) found that the addition of 200 mg of vitamin E in pigs' feed cause as much as a 20% decrease in cholesterol content in meat. Their studies show a strong correlation between the level of cholesterol in the meat and the amount ofvitamin E in animal diet. They found a decrease of 10%, 20%, and 30% of cholesterol content in meat after the application of 100, 200, and 400 mg vitamin E in diet, respectively. The level of cholesterol is usually linked with adipose tissuecontent (Konjufca et al. 1997), which is also confirmed with the present result.

Rymer and Givens (2010) emphasize that poultry meat may be a fine source of polyunsaturated fatty acids. They suggest that desired traits of poultry meat may be achieved upon bird diet supplementation with vitamin E at the level of at least 100 mg/ kg.

4.8.3 Antioxidant status of breast meat:

4.8.3.1 Malondialdehyde (MDA)

Effect of supplementation of synthetic vitamin E, herbal vitamin E and the combination of two on the accumulation of MDA in the breast muscle is given in Table 14 and depicted in Figure 12.

There was significant change ($P<0.05$) in the MDA accumulation in the breast muscle among the different groups. The lowest MDA was accumulated in the group supplemented with herbal vitamin E (0.45). Maximum accumulation occurred in the control group (0.48). Groups supplemented with synthetic vitamin E (0.47) and combination of both (0.46) have intermediate values. MDA is the main end product of lipid peroxidation by radical oxygen species, is an important indication of lipid peroxidation. Vitamin E supplementation decreased muscular MDA accumulation. The reduced MDA accumulation was in agreement with the simultaneously enhanced α-tocopherol retention and improved meat quality, which in turn suggested that vitamin E may improve the meat quality by enhancing muscular αtocopherol content which could inhibit lipid peroxidation as evidenced by reduced generation of MDA. Thus, the present study shows that there is least lipid peroxidation in the herbal vitamin E supplemented group.

This study is corroborated with the findings of Cheng et al. (2016) in the breast and thigh muscle of broiler chicken after feeding herbal and synthetic vitamin E. Also Ognik and Wertelecki. (2012) have similar findings in the liver of turkey hens where they found significantly ($P<0.05$) lower MDA accumulation in the groups supplemented with natural RRR-D-a-tocopherol than the control group.

Table-14 Effect of supplemental vitamin E on cholesterol and antioxidant status of meat in broiler chickens

Treatments	Breast meat cholesterol (mg%)	MDA (nmol/mg protein)	GSH-Px (nmol/mg of protein)	SOD (U/mg of protein)
T_1	48.53^{b}±1.31	0.48^{d}±0.001	14.83±0.18	40.59±0.57
T_2	42.66^{a}±1.27	0.47^{c}±0.001	14.98±0.27	41.23±0.10
T_3	39.23^{a}±1.63	0.45^{a}±0.001	15.46±0.13	40.87±0.15
T_4	40.52^{a}±2.29	0.46^{b}±0.001	15.06±0.12	41.02±0.10
SEm	1.082	0.002	0.100	0.151
P-value	0.004	0.001	0.145	0.532

^{a,b,c} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Figure- 11: Effects of supplemental vitamin E on Breast meat cholesterol in broiler chickens

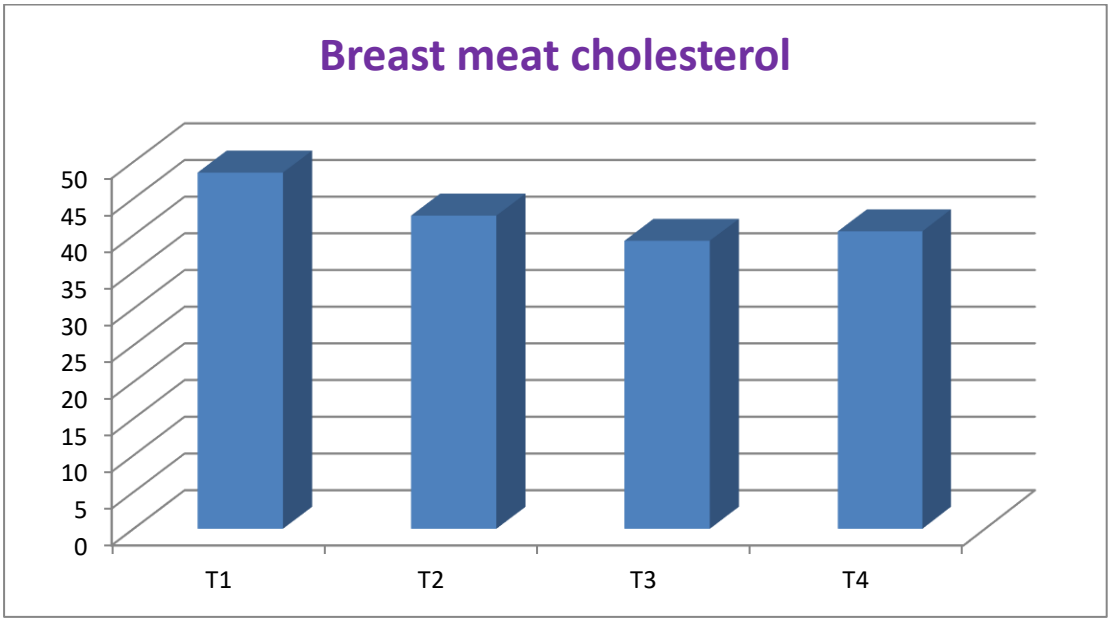
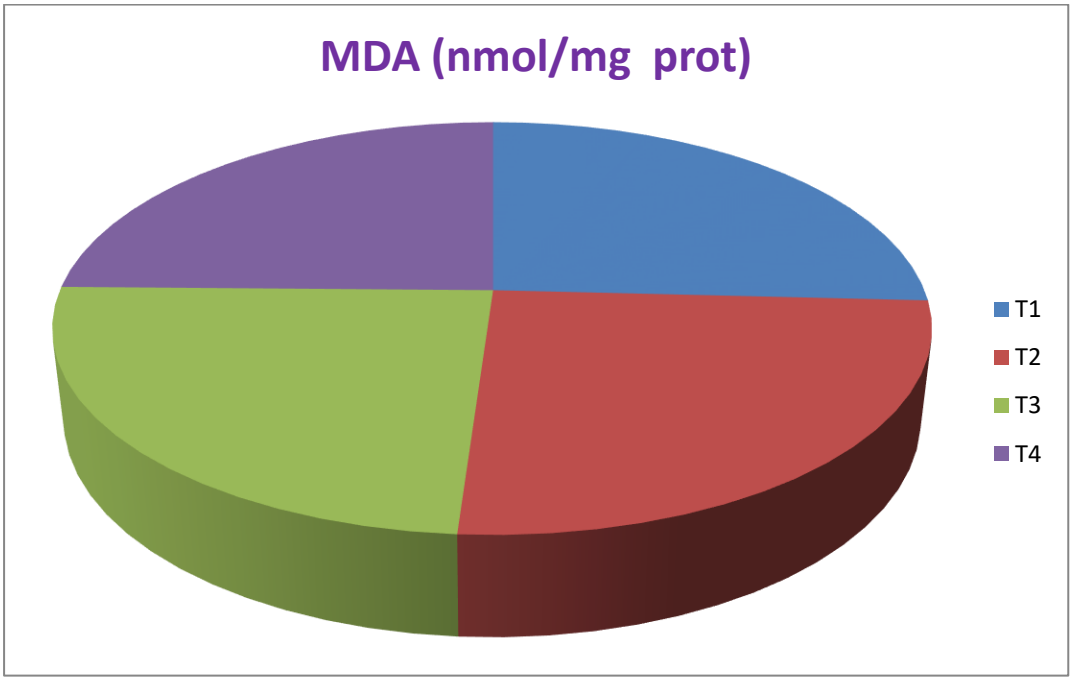


Figure- 12: Effects of supplemental vitamin E on antioxidant status of meat in broilers.



4.8.3.2 Glutathione Peroxidase (GSH-Px)

Effect of supplementation of synthetic vitamin E, herbal vitamin E and their combination on the activity of GSH-Px in the breast muscle is given in Table. 14.

The perusal of the table suggest that there is insignificant (P>0.05) change in the activity of GSH-Px among different groups. Although numerically there was increase in the T_3 (15.46) group followed by T_4 (15.06) and T_2 (14.98) groups which is higher than control (14.83) group. The result of present study is in agreement with earlier findings. Cheng et al. (2016) also got similar findings in broiler chickens after supplementation of herbal and synthetic vitamin E.

4.8.3.3 Superoxide Dismutase (SOD)

Effect of supplementation of synthetic vitamin E, herbal vitamin E and the combination of two on the activity of SOD in the breast muscle is given in Table 14.

There was insignificant (P>0.05) change in SOD level in the breast muscle of different groups. The result of present study is in agreement with earlier findings. The result was in corroboration with the findings of Cheng et al. (2016) where he suggested that there is no effect of vitamin E supplementation on SOD activity in broilers. On contrary Ognik and Wertelecki (2012) got different result in turkey after supplementing natural RRR-D-a-tocopherol. Changes in superoxide dismutase activity manifesting with a significantly higher activity in groups where RRR-d-α-tocopherol were administered. Guo et al. (2003) observed no influence of increased dietary vitamin E in broiler chicken diets on superoxide dismutase.

4.8.4 Organoleptic test (Hedonic test) of meat

The results of the sensory characteristics of meat due to dietary addition of synthetic vitamin E, herbal vitamin E either alone or in combination are shown in Table 15.

Dietary treatments significantly (P<0.05) affected the tenderness, juciness and overall acceptability of meat of broiler chicken. The tenderness, juciness, and overall acceptability enhanced significantly(P<0.05). Meat appearance and flavor didn't differsignificantly (P>0.05) among dietary treatments.

Table-15 Effect of supplemental vitamin E on sensory evaluation of broiler chicken breast meat

Treatments	Appearance	Flavour	Juciness	Tenderness	Overall Acceptability
T_1	6.33±0.21	6.00±0.25	5.33^{a}±0.21	5.33^{a}±0.21	5.50^{a}±0.22
T_2	6.50±0.22	6.33±0.33	6.33^{b}±0.21	6.16^{ab}±0.40	6.16^{ab}±0.30
T_3	6.33±0.33	6.16±0.30	6.83^{b}±0.30	6.83^{b}±0.30	7.00^{b}±0.36
T_4	6.66±1.42	6.83±0.16	6.66^{b}±0.42	6.50^{b}±0.22	6.16^{ab}±0.30
SEm	0.147	0.143	0.185	0.180	0.180
P-value	0.849	0.193	0.010	0.012	0.021

^{a,b,c} Mean values with different superscripts within a column differ significantly ($P<0.05$).

However, numerically better appearance and flavor was observed in the chicken meat samples.

The above results of sensory evaluation (appearance, flavour, juiciness, tenderness and overall acceptability) are in agreement with the previous findingsof Cheng et al. (2016) and Sasiadek et al. (2016). Improved meat color through dietary vitamin E supplementation has been demonstrated in swine (Kim et al. 2015), beef (Sherbeck et al. 1995) and
poultry (Rey et al. 2015). Literature data (Niu et al. 2009; Adebiyi et al. 2011; Khattak et al. 2012) demonstrated no or limited effect effects of stress on meat quality in the case of animals administered vitamin E in diet. According to Buckley et al. (1995), the main factors responsible for water holding capacity decrease include low pH, too high temperature of meat after slaughter and a low level of antioxidants, vitamin E in particular.

Protection of membranal lipids against lipid oxidation by vitamin E has been suggested to be the mechanism responsible for the positive influence of dietary vitamin E on the water holding capacity since the integrity of the cell membrane is thought to be associated with drip loss (Asghar et al. 1991). MDA, the main product of lipid peroxidation by radical oxygen species, is an important indication of lipid peroxidation. Vitamin E supplementation decreased muscular MDA accumulation. Vitamin E may improve the meat quality by enhancing muscular αtocopherol content, which could inhibit lipid peroxidation as evidenced by reduced generation of MDA. In the present study there is considerably less oxidative changes were observed.

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5 SUMMARY AND CONCLUSIONS

Feed accounts for 65- 70% of broiler production cost, thus, feed cost deserves befitting attention. The key nutrients that need to be supplied by the dietary ingredients are amino acids contained in proteins, vitamins and minerals. Presently, feed supplements and feed additives have assumed prime importance in poultry production. Vitamins are a group of organic compounds that poultry require in small quantities, but they are essential to regulate the biological processes in the body. There are 13 essential vitamins categorised into fat-soluble (vitamin A, D, E, K) and water soluble vitamins (vitamin B-complex and vitamin C). Each vitamin has specific function in animal body. A deficiency of one or more vitamins can lead to a number of diseases or syndromes and ultimately, uneconomical poultry production.

Vitamin E is a fat-soluble vitamin, which is essential for the integrity of the reproductive, muscular, circulatory, nervous and immune system. Vitamin E is very essential for optimum poultry production. There is proven role of vitamin E dietary supplementation in broilers for proper metabolism, growth performance, immunity and quality of animal products. Vitamin E is known to be a major chain-breaking antioxidant and helpful to improve both cell-mediated and humoral immunity in broiler chicks. However, the effect of this vitamin depends upon dose, age and genetics of the broiler chicks (Khan *et al*., 2016). Part of the vitamin E that poultry need comes from the ingredients in their diet. Concentration of vitamin E greatly varies due to several factors, including the quality of the ingredients and their state of preservation. This is why poultry nutritionists choose to ensure suitable levels using specific sources of Vitamin E that are added to the feed.

Eight naturally occurring substances have been found to have vitamin E activity: α-, β-, γ-, and δ-tocopherols and α-,β-, γ-, and δ-tocotrienols. Of these, the α-tocopherol is the most biologically active and most widely distributed form. Vitamin E is found in high quantities in vegetable oils. Synthetic DL-α-tocopherol acetate consists of all eight possible stereoisomers. Vitamin E is usually added to poultry feeding is the synthetic form of DL-α-tocopherol acetate. Previous studies have reported that natural (D-α-tocopherol) vitamin E has a superior bioavailability than synthetic (DL-α-tocopherol acetate) vitamin E in turkeys, cows, and pigs. However,

there is not much information that shows the effects of replacing synthetic vitamin E with natural vitamin E (D-α-tocopherol) on growth performance, antioxidant capacity, immunity and carcass characteristics of broiler chickens.

The present study was therefore, codcucted to compare the effects of supplementation of DL-α-tocopherol acetate (Synthetic source of vitamin E) and D-α-tocopherol (Herbal source of vitamin E) in broiler chickens with the following objectives:

5. To study the effect of synthetic and herbal vitamin E supplementation on growth performance of broilers
6. To study the effect of synthetic and vitamin E supplementation on hemato-biochemical parameters and oxidative status of broilers
7. To study the effect of synthetic and herbal vitamin E supplementation on nutrients utilization of broilers
8. To study the effect of synthetic and vitamin E supplementation on carcass characteristics of broilers

A feeding trial of 35 days was conducted to to compare the effects of supplementation of DL-α-tocopherol acetate (Synthetic source of vitamin E) and D-α-tocopherol (Herbal source of vitamin E) on the growth performance, nutrients utilization, haemato-biochemical profile meat quality, antioxidant capacity, immunity and carcass quality of broiler chickens.

There were 4 dietary treatment groups as follows:

Treatment T_1:Basal diet without Herbal or synthetic Vitamin E (control)

Treatment T_2: Basal diet with synthetic vitamin E @) 100 g/quintal feed

Treatment T_3:Basal diet with herbal vitamin E @) 100 g/quintal feed

Treatment T_4:Basal diet with Synthetic vitamin E @) 50 g/quintal feed

Herbal vitamin E @) 50g /quintal feed

A total of one hundred and eighty, day- old Cobb 400Y strain broiler chicks were procured, weighed individually and randomly allotted to four treatment groups each with three replicates of 15 chicks in such a way that average body weight was approximately similar for all the treatment groups. Standard basal diets for pre-starter

(0-7 days) starter (8-21 days) and finisher (21-35 days) phases of growth of broiler chickens were prepared by mixing the different ingredients to meet the nutrient requirements of broiler chicken as per recommendation of BIS (2007).. The chicks were provided the experimental feeds and water *ad libitum.* Body weight of individual chick and feed consumption of each replicate in different group was recorded weekly upto 5 weeks of age, and feed conversion ratio (FCR) and performance index was calculated. Blood samples were collected from wing vein of two birds from each replicates on 35^{th} day for analyses of haemato-biochemical parameters, anti-oxidant status, immunity in terms of antibody titre against New Castle disease. A metabolism trial was also conducted on two birds from each replicate during the 5^{th} week of feeding trial to know the nutrient utilization. At the end of feeding trial, two chicks per replicate in each treatment group were sacrificed for the study of carcass yield, cut up parts, organ weights, processing losses and, cholesterol level and antioxidant status in breast meat.

The results of the present study are summarized as under:

I. Effect of supplementation of synthetic and herbal vitamin E on growth performance of broiler chicken:

Result on cumulative growth performance of broilers in terms of average weight gain, feed intake, FCR and performance index as influenced by supplementation of synthetic vitamin E, herbal vitamin E and their combination (Synthetic and Herbal vitamin E) are divided into during starter phase (0-21 days) and finisher phase (22-35 days) and overall period (0-35 days).

During the starter phase (0-21 days) feed consumption, body weight gain, FCR and performance index in broilers of various treatment groups differed significantly ($P<0.05$). There was significantly ($P<0.05$) highest body weight gain, (653.05g) in the group supplemented with herbal vitamin E (T_3), that was followed by the groups supplemented with the combination of synthetic and herbal vitamin E (T_4) (640.34g), synthetic vitamin E (T_2) (635.80g) and the control group (T_1) (617.51g). Maximum feed intake ($P<0.05$) was recorded in T_3 group (1106.40g) which was followed by the groups T4 , T2 and T1 that is 1079.88g, 1068.57g and 1049.40g, respectively. Also, the performance index increased significantly ($P<0.05$) in the herbal vitamin E supplemented group (T3) (385.47) followed by T4, T2 and T1 that is 379.71, 378.30 and 363.37, respectively. There was significantly ($P<0.05$) better FCR

(1.69) obtained in T3 group than T1 group (1.70) but it was comparable to T4 group (1.69). The best FCR (1.68) was recorded in herbal vitamin E supplemented (T2) group among the four groups.

During the finisher phase (22-35 days), the average weight gain of broilers was significantly ($P<0.05$) influenced by the dietary treatments. The body weight gain in broiler chickens fed diet supplemented with herbal vitamin E (T_3) group was significantly ($P<0.05$) higher (955.40g) than control (T_1) (905.49g) and those supplemented with synthetic (T_2) (922.30g) and their combination group (T_4) (934.67g). Feed intake follows the same pattern as in the body weight gain. Feed intake was significantly ($P<0.05$) higher in T_3 group (1704.36g) which was followed by T_4 (1684.84g), T_2 (1672.71g) and T_1 (1650.48 g) groups. Also, the performance index was significantly ($P<0.05$) higher in T_3 (535.57) group and it was followed by T_4 (518.52), T_2 (508.54) and T_1 (496.77) groups, respectively. FCR result was significantly lowest in the T_3 (1.78) group that was succeeded by T_4 (1.80) group. T_2 (1.81) and T1 (1.82) groups have higher FCR values than rest of the two groups but comparable with each other. There was significant ($P<0.05$) effect of dietary treatments on performance index of broiler chickens. Performance index was highest (535.57) in broilers fed diet containing herbal vitamin E (T_4) group, whereas birds fed diets containing synthetic vitamin E (T_3) and combination of herbal and synthetic vitamin E (T_4) had comparable performance index.

During overall experimental period (0-35 days) there was significant difference ($P<0.05$) observed in body weight gain among different groups. Maximum weight gain (1608.46 g) was recorded in broilers fed diet containing herbal vitamin E (T_3) followed by T_4 (1575.01g) , T_2 (1558.10g) and T_1 (1523.00g) groups. Feed intake and performance index also followed the same trend. Significant increase ($P<0.05$) in feed intake was recorded in different groups of which T_3 (2810.75g) has the highest feed intake followed by T_4 (2764.72g), T_2 (2741.28g) and T_1 (2699.89g) groups. Performance index was significantly ($P<0.05$) higher in T_3 (920.45) followed by T_4 (897.27), T_2 (885.60) and T_1 (859.13) groups. Also, there was significant difference ($P<0.05$) observed in FCR results among different groups. Lowest FCR (1.75) recorded in herbal vitamin E supplemented group (T_3) which was significantly higher as compared to synthetic vitamin E supplemented group (1.76) and the control group (1.77), but it was comparable with T_4 (1.76) group. Overall best FCR was recorded in herbal vitamin E supplemented group as compared with control group

because herbal vitamin E helped in efficient utilization of feed. Feed cost per kg body weight gain was highest (Rs. 55.00) in control group and lowest (Rs. 53.84) in herbal vitamin E treatment group.

II. Effect of supplementation of synthetic and herbal vitamin E on nutrients utilization in broiler chicken:

Total tract nutrient utilization in broilers during the metabolism trial revealed Data pertaining to the average total tract nutrient utilization in broilers during finisher phase of different treatment groups revealed that dry matter retention was significantly higher ($P<0.05$) in T_3 (75.11%) group from T_2 (72.59%) and T_1 (70.78%) but it was comparable with T_4 (73.71%) group. Crude protein retention was significantly higher in T_3 (67.09%) group was as compared to rest of the groups. Nitrogen retention was comparable in T_4 (65.30%) and T_2 (64.57%) groups whereas it was found to be lowest in the control (T_1) (62.43%) group. Ether extract retention was found to be significantly ($P<0.05$) higher in herbal vitamin E (77.32%) supplemented group that was comparable with the combination group (76.75%) but it was higher than synthetic vitamin E supplemented group (75.12%) followed by control group (73.78%). There was no significant ($P>0.05$) change in the calcium retention although numerically higher retention observed in T_3 (46.9) group followed by T_4 (46.72), T2 (45.52) and T1 (45.02). There was no significant ($P>0.05$) change in the phosphorous retention among the groups supplemented with vitamin E or control group although numerically higher retention observed in T_3 (76.11) group followed by T4 (76.03), T2 (74.21) and T1 (72.62).

III. Effect of supplementation of synthetic and herbal vitamin E on haematological parameters in broiler chicken:

Haematological parameters viz. haemoglobin, packed cell volume (PCV), Total erythrocyte count (TEC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) values in broiler chicks fed diets supplemented with synthetic vitamin E, herbal vitamin E and their combination (Synthetic and Herbal vitamin E) differed significantly ($P<0.05$)

Broiler chicks fed herbal vitamin E supplemented ration had numerically higher haemoglobin concentration (9.53) followed by combination group (9.11), synthetic

vitamin E supplemented group (8.75) and control group (8.46). The PCV values were significantly (P<0.05) different among supplemented and control groups. Highest PCV values recorded in T_3 (30.11) group which was comparable with T_4 (28.93). Birds of group T_2 (28.30) had comparable value with T_4 but the overall PCV of supplemented group was significantly higher than control group (26.11). Total erythrocyte count (TEC) was found to be significantly (P<0.05) higher in herbal vitamin E supplemented group (2.59 million /mm^3). It was followed by combination group (2.42 million/mm^3) and synthetic vitamin E supplemented group (2.35million/mm^3). These two have comparable values and there is least TEC found in the control group (2.27million/mm^3). There was no significant (P>0.05) change observed in MCV and MCH values among different groups.

IV. Effect of supplementation of synthetic and herbal vitamin E on serum biochemical parameters in broiler chicken:

Serum glucose, total protein, albumin and globulin levels and serum enzymes *viz.*, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in broiler chickens did not differ significantly (P>0.05) whereas there was significant change (P<0.05) in the serum cholesterol level in broiler chickens due to supplementation of synthetic and herbal vitamin E or their combination. Among the supplemented groups minimum cholesterol level was recorded in the group supplemented with herbal vitamin E (128.26) that was succeded by group supplemented with combination of two (133.73) and then synthetic vitamin E supplemented group (136.50). Control group have the maximum cholesterol level (158.02).

V. Effect of supplementation of synthetic and herbal vitamin E on immunity in broiler chicken:

There was no significant change (P>0.05) in lymphoid organs weights viz. spleen, thymus and bursa of fabricius among different groups however, there was significant change (P<0.05) in the antibody titre against Newcastle Disease vaccine due to supplementation of synthetic and herbal vitamin E. The highest antibody titre against Newcastle Disease vaccine was recorded in the group supplemented with herbal vitamin E (6.12) followed by combination group (5.68) and synthetic vitamin E

supplemented group (5.40). But T4 and T2 groups have comparable titre values. Least antibody titre was observed in control group (4.52).

VI.Effect of supplementation of synthetic and herbal vitamin E onanti-oxidant status in broiler chicken:

There was significant (P<0.05) effect of supplementation of synthetic and herbal vitamin E on Glutathione (GSH). GSH activity was significantly higher (P<0.05) in the group supplemented with herbal vitamin E (9.96) followed by group containing both (9.21) and synthetic vitamin E supplemented group (8.89). The supplemented groups had significantly higher GSH activity than control group (6.31).

There was non-significant (P>0.05) change in the superoxide dismutase (SOD). SOD values in the supplemented groups was found as compared to the control group. Although numerically highest SOD activity was observed in the group fed with herbal vitamin E (162.81) followed by mixture group (159.31) then synthetic vitamin E supplemented group (156.76). Control group had least (152.08) SOD activity. Similarly There was no significant change in catalase (CAT) activity in the groups supplemented with vitamin E. The numerical values however shows rise in catalase activity. Maximum value of SOD was in the group T_4 (5.16) followed by T_2 (5.12) group and T_3 (5.05) group. Control group (4.96) shows least numerical value.

VII.Effect of supplementation of synthetic and herbal vitamin E onantioxidant status of breast meat of broiler chicken:

There was significant (P<0.05) effect of supplementation of synthetic and herbal vitamin E on anti-oxidant status of breast meat. There was significant change (P<0.05) in the Malondialdehyde (MDA) accumulation in the breast muscle among the different groups. The lowest MDA was accumulated in the group supplemented with herbal vitamin E (0.45). Maximum accumulation occurred in the control group (0.48). Groups supplemented with synthetic vitamin E (0.47) and combination of both (0.46) have intermediate values. There was insignificant (P>0.05) change in the activity of Glutathione Peroxidase (GSH-Px) enzyme activity among different treatment groups , however, Superoxide Dismutase (SOD) level in meat didn't differ (P>0.05) due to supplementation of synthetic and herbal vitamin E.

VIII. Effect of supplementation of synthetic and herbal vitamin E on carcass characteristics of broiler chicken:

The carcass characters *viz*., dressing yield, eviscerated yield, blood loss, feather loss, meat to bone ratio and relative weights of giblet, heart, liver and gizzard were found to be statistically similar (P>0.05) among the broilers of different treatment groups. Similarly different cut-up parts (% of live weight) *viz.* thigh, drumstick, breast, back, wing and neck did not differ among the different dietary treatments. There was significant decrease (P<0.05) in the abdominal fat percent in group supplemented with herbal vitamin E (1.41) followed by combination group (1.45) and synthetic group (1.49). Control group have the maximum abdominal fat percent (1.64). There is significant change (P<0.05) in the meat cholesterol level after supplementing vitamin E. There is clear difference of cholesterol level between vitamin E supplemented groups and un-supplemented group. Among the supplemented groups minimum cholesterol level was recorded in the group supplemented with herbal vitamin E (39.23) that was succeded by group supplemented with combination of two (40.52) and then synthetic vitamin E supplemented group (42.66). Control group have the maximum cholesterol level.

Dietary treatments significantly (P<0.05) affected the tenderness, juciness and overall acceptability of broiler chicken. The tenderness, juciness, and overall acceptability enhanced significantly. Meat appearance and flavor didn't differ significantly (P>0.05) among dietary treatments. However, numerically better appearance and flavor was observed in the chicken meat samples.

Conclusion:

I. Dietary supplementation of Synthetic or Herbal vitamin E and their combination @ 100 gm/quintal feed improved the growth performance, antioxidant status, carcass quality and immune status of broiler chickens.

II. Herbal Vitamin E was economical and more effective as compared to the synthetic vitamin E in improving the performance of broiler chickens.

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APPENDICES

Table-I: Effect of supplemental vitamin E on average growth performance of broiler chicks during first week (0-7days)

Treatments	BWG	FI	FCR	PI
T_1	80.23±1.56	123.88±2.47	1.56±0.03	51.96±1.04
T_2	82.34±2.09	128.85±2.51	1.54±0.10	52.66±2.78
T_3	83.87±2.49	128.79±3.13	1.53±0.08	54.77±2.16
T_4	83.20±2.62	127.38±2.53	1.53±0.05	54.49±3.04
SEM	0.690	1.822	0.019	0.859
P value	0.290	0.800	0.950	0.653

Table-II: Effect of supplemental vitamin E on average growth performance of broiler chicks during second week (8-14days)

Treatments	BWG	FI	FCR	PI
T_1	176.67^{a}±2.59	296.98^{a}±4.79	1.68^{b}±0.11	105.10^{a}±2.92
T_2	181.65^{b}±1.78	299.89^{ab}±5.47	1.65^{ab}±0.09	110.03^{b}±3.78
T_3	190.86^{d}±3.54	312.88^{c}±5.04	1.64^{a}±0.12	116.46^{c}±3.13
T_4	186.80^{c}±2.48	307.35^{bc}±4.85	1.65^{a}±0.08	113.54^{bc}±2.39
SEM	1.70	2.19	0.01	1.36
P value	0.001	0.011	0.036	0.001

^{a,b,c,d} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Table-III: Effect of supplemental vitamin E on growth performance of broiler chicks during third week (15-21days)

Treatments	BWG	FI	FCR	PI
T_1	$360.61^{a}\pm5.03$	$628.54^{a}\pm12.54$	$1.74^{b}\pm0.08$	$206.89^{a}\pm3.70$
T_2	$369.83^{b}\pm8.73$	$640.18^{b}\pm11.79$	$1.73^{a}\pm0.03$	$213.65^{b}\pm4.30$
T_3	$378.32^{d}\pm9.50$	$658.03^{d}\pm9.81$	$1.74^{ab}\pm0.05$	$217.51^{d}\pm2.37$
T_4	$373.69^{c}\pm11.63$	$647.61^{c}\pm11.86$	$1.73^{a}\pm0.07$	$215.63^{c}\pm4.11$
SEM	1.99	3.34	0.00	1.22
P value	0.001	0.001	0.035	0.001

^{a,b,c,d} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Table-IV Effect of supplemental vitamin E on growthperformance of broiler chicks during fourth week (22-28days)

Treatments	BWG	FI	FCR	PI
T_1	$441.68^{a}\pm5.45$	$791.05^{c}\pm11.94$	$1.79^{c}\pm0.05$	$246.61^{a}\pm3.17$
T_2	$451.76^{b}\pm9.08$	$805.03^{bc}\pm8.33$	$1.78^{bc}\pm0.02$	$253.52^{b}\pm3.92$
T_3	$469.17^{d}\pm10.38$	$821.37^{a}\pm9.54$	$1.75^{a}\pm0.04$	$268.00^{d}\pm4.83$
T_4	$458.34^{c}\pm8.89$	$811.72^{b}\pm6.41$	$1.77^{b}\pm0.05$	$258.81^{c}\pm2.58$
SEM	3.05	3.53	0.00	2.39
P value	0.001	0.001	0.002	0.001

^{a,b,c,d} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Table-V Effect of supplemental vitamin E on growth performance of broiler chicks during fifth week (29-35days)

Treatments	BWG	FI	FCR	PI
T_1	463.81^{a}±6.05	859.43^{a}±10.76	1.85^{c}±0.08	250.31^{a}±4.02
T_2	470.54^{b}±8.86	867.67^{ab}±11.15	1.84^{c}±0.05	255.17^{b}±3.64
T_3	486.23^{d}±11.98	882.99^{c}±13.67	1.82^{a}±0.06	267.75^{d}±5.24
T_4	476.33^{c}±7.01	873.13^{bc}±9.79	1.83^{b}±0.09	259.86^{c}±4.89
SEM	2.57	2.90	0.00	1.98
P value	0.001	0.004	0.001	0.001

^{a,b,c,d} Mean values with different superscripts within a column differ significantly ($P<0.05$).

Table. VI: Analysis of variance (ANOVA) of weekly growth performance

Particulars	Source of variance	d.f	S.S	M.S	F
1st week	Between Groups	3	22.539	7.513	1.485
BWG	Within Groups	8	40.474	5.059	
	Total	11	63.013		
FI	Between Groups	3	49.04	16.341	.336
	Within Groups	8	389.38	48.673	
	Total	11	438.405		
FCR	Between Groups	3	.002	.001	.113
	Within Groups	8	.047	.006	
	Total	11	.049		
PI	Between Groups	3	17.059	5.686	.566
	Within Groups	8	80.372	10.047	
	Total	11	97.431		
2nd week	Between Groups	3	342.509	114.170	24.605
BWG	Within Groups	8	37.121	4.640	

	Total	11	379.630		
FI	Between Groups	3	467.438	155.813	7.416
	Within Groups	8	168.081	21.010	
	Total	11	635.518		
FCR	Between Groups	3	.003	.001	3.155
	Within Groups	8	.003	.000	
	Total	11	.006		
PI	Between Groups	3	215.187	71.729	19.014
	Within Groups	8	30.180	3.773	
3rd week	Total	11	245.367		
BWG	Between Groups	3	508.771	169.590	100.93
	Within Groups	8	13.442	1.680	
	Total	11	522.213		
FI	Between Groups	3	1387.95	462.653	44.818
	Within Groups	8	82.583	10.323	
	Total	11	1470.54		
FCR	Between Groups	3	.000	.000	4.736
	Within Groups	8	.000	.000	
	Total	11	.000		
PI	Between Groups	3	192.874	64.291	117.59
	Within Groups	8	4.374	.547	
4th week	Total	11	197.248		
BWG	Between Groups	3	1199.195	399.732	104.771
	Within Groups	8	30.522	3.815	
	Total	11	1229.718		
FI	Between Groups	3	1459.609	486.536	20.658

	Within Groups	8	188.414	23.552	
	Total	11	1648.023		
FCR	Between Groups	3	.003	.001	13.162
	Within Groups	8	.001	.000	
	Total	11	.003		
PI	Between Groups	3	732.336	244.112	80.600
	Within Groups	8	24.230	3.029	
5th week	Total	11	756.565		
BWG	Between Groups	3	811.850	270.617	36.782
	Within Groups	8	58.858	7.357	
	Total	11	870.707		
FI	Between Groups	3	879.102	293.034	10.195
	Within Groups	8	229.953	28.744	
	Total	11	1109.055		
FCR	Between Groups	3	.002	.001	29.843
	Within Groups	8	.000	.000	
	Total	11	.002		
PI	Between Groups	3	496.254	165.418	58.395
	Within Groups	8	22.662	2.833	
	Total	11	518.916		

Table. VII: Analysis of variance (ANOVA) for the effects of treatments on weeklyy growth performance

Particulars	Source of variance	d.f	S.S	M.S	F-value
Growth Performance (0-21 days)					
BWG	Between Groups	3	1949.235	649.745	89.933
	Within Groups	8	57.798	7.225	
	Total	11	2007.033		
FI	Between Groups	3	5104.052	1701.351	74.109
	Within Groups	8	183.660	22.958	
	Total	11	5287.712		
FCR	Between Groups	3	.001	.000	8.650
	Within Groups	8	.000	.000	
	Total	11	.001		
PI	Between Groups	3	798.716	266.239	57.837
	Within Groups	8	36.826	4.603	
	Total	11	835.543		
Growth Performance (22-35 days)					
BWG	Between Groups	3	3978.185	1326.062	91.778
	Within Groups	8	115.588	14.449	
	Total	11	4093.773		
FI	Between Groups	3	4580.067	1526.689	17.729
	Within Groups	8	688.909	86.114	
	Total	11	5268.976		
FCR	Between Groups	3	.003	.001	24.130
	Within Groups	8	.000	.000	
	Total	11	.003		
PI	Between Groups	3	2428.202	809.401	124.182

	Within Groups	8	52.143	6.518	
	Total	11	2480.345		
Growth Performance (0-35 days)					
BWG	Between Groups	3	11385.412	3795.137	110.317
	Within Groups	8	275.217	34.402	
	Total	11	11660.629		
FI	Between Groups	3	19276.747	6425.582	41.048
	Within Groups	8	1252.298	156.537	
	Total	11	20529.045		
FCR	Between Groups	3	.001	.000	13.199
	Within Groups	8	.000	.000	
	Total	11	.001		
PI	Between Groups	3	5852.763	1950.921	103.203
	Within Groups	8	151.229	18.904	
	Total	11	6003.992		

Table.IX: **Analysis of variance (ANOVA) for the effects of treatments on total tract nutrients retention**

Particulars	**Source of variance**	**d.f**	**S.S**	**M.S**	**F**
DM Retention	Between Groups	3	30.199	10.066	16.727
	Within Groups	8	4.815	.602	
	Total	11	35.013		
CP retention	Between Groups	3	33.454	11.151	16.719
	Within Groups	8	5.336	.667	
	Total	11	38.789		
EE Retention	Between Groups	3	23.235	7.745	13.145
	Within Groups	8	4.714	.589	
	Total	11	27.949		
Ca Retention	Between Groups	3	15.180	5.060	1.217
	Within Groups	20	83.176	4.159	
	Total	23	98.356		
P Retention	Between Groups	3	50.006	16.669	2.245
	Within Groups	20	148.527	7.426	
	Total	23	198.533		

Table. X: Analysis of variance (ANOVA) for the effects of treatments on haematological parameters

Particulars	Source of variance	d.f	S.S	M.S	F
Hb	Between Groups	3	.371	.124	2.438
	Within Groups	20	1.015	.051	
	Total	23	1.386		
PCV	Between Groups	3	8.221	2.740	1.804
	Within Groups	20	30.388	1.519	
	Total	23	38.610		
TEC	Between Groups	3	.056	.019	1.235
	Within Groups	20	.303	.015	
	Total	23	.359		
MCV	Between Groups	3	213.800	71.267	.958
	Within Groups	20	1487.252	74.363	
	Total	23	1701.053		
MCH	Between Groups	3	2.984	.995	.266
	Within Groups	20	74.783	3.739	
	Total	23	77.768		

Table XI: Analysis of variance (ANOVA) for the effects of treatments on growth performance

Particulars	Source of variance	d.f	S.S	M.S	F
Glucose	Between Groups	3	312.138	104.046	1.768
	Within Groups	20	1177.300	58.865	
	Total	23	1489.438		
Protein	Between Groups	3	.463	.154	1.233
	Within Groups	20	2.506	.125	

	Total	23	2.969		
Albumin	Between Groups	3	.086	.029	.638
	Within Groups	20	.901	.045	
	Total	23	.987		
Globulin	Between Groups	3	.341	.114	.728
	Within Groups	20	3.119	.156	
	Total	23	3.460		
Serum Cholesterol	Between Groups	3	3066.822	1022.274	8.413
	Within Groups	20	2430.152	121.508	
	Total	23	5496.973		
ALT	Between Groups	3	22.593	7.531	2.015
	Within Groups	20	74.736	3.737	
	Total	23	97.329		
AST	Between Groups	3	67.335	22.445	.669
	Within Groups	20	671.223	33.561	
	Total	23	738.558		
ALP	Between Groups	3	283.701	94.567	.728
	Within Groups	20	2598.618	129.931	
	Total	23	2882.320		

Table-XII: Analysis of variance (ANOVA) for the effects of treatments on lymphoid organ weight(% of body weight) and Antibody titre against response to Newcastle Disease vaccine in broiler chickens

Particulars	**Source of variance**	**d.f**	**S.S**	**M.S**	**F**
Spleen	Between Groups	3	.000	.000	.577
	Within Groups	20	.005	.000	

	Total	23	.006		
Thymus	Between Groups	3	.001	.000	2.424
	Within Groups	20	.002	.000	
	Total	23	.002		
Bursa	Between Groups	3	.001	.000	1.429
	Within Groups	20	.003	.000	
	Total	23	.003		
log2	Between Groups	3	8.206	2.735	27.451
	Within Groups	20	1.993	.100	
	Total	23	10.198		

Table. XIII: Analysis of variance (ANOVA) for the effects of treatments \ on carcass characteristics of broiler chicken

Particulars	**Source of variance**	**d.f**	**S.S**	**M.S**	**F**
Dressing yield	Between Groups	3	34.022	11.341	3.601
	Within Groups	20	62.979	3.149	
	Total	23	97.001		
Eviscerated yield	Between Groups	3	11.609	3.870	3.326
	Within Groups	20	23.267	1.163	
	Total	23	34.876		
Giblet	Between Groups	3	.039	.013	.226
	Within Groups	20	1.159	.058	
	Total	23	1.199		
Heart	Between Groups	3	.031	.010	2.282
	Within Groups	20	.089	.004	
	Total	23	.120		
Liver	Between Groups	3	.112	.037	.809

	Within Groups	20	.924	.046	
	Total	23	1.036		
Gizzard	Between Groups	3	.131	.044	.624
	Within Groups	20	1.395	.070	
	Total	23	1.525		
Abdominal fat	Between Groups	3	.034	.011	.168
	Within Groups	20	1.368	.068	
	Total	23	1.403		
Blood loss	Between Groups	3	1.059	.353	.640
	Within Groups	20	11.033	.552	
	Total	23	12.091		
Feather loss	Between Groups	3	.095	.032	.220
	Within Groups	20	2.894	.145	
	Total	23	2.990		
Thigh	Between Groups	3	.461	.154	.704
	Within Groups	20	4.365	.218	
	Total	23	4.827		
Drumstick	Between Groups	3	.810	.270	1.499
	Within Groups	20	3.601	.180	
	Total	23	4.410		
Leg quarter	Between Groups	3	.869	.290	.841
	Within Groups	20	6.894	.345	
	Total	23	7.763		
Breast	Between Groups	3	3.798	1.266	1.040
	Within Groups	20	24.358	1.218	
	Total	23	28.156		

Back	Between Groups	3	.552	.184	.212
	Within Groups	20	17.347	.867	
	Total	23	17.899		
Wing	Between Groups	3	1.199	.400	1.220
	Within Groups	20	6.552	.328	
	Total	23	7.751		

Table.XIV : Analysis of variance (ANOVA) for the effects of treatments Onanti-oxidant enzymes

Particulars	**Source of variance**	**d.f**	**S.S**	**M.S**	**F**
MDA	Between Groups	3	.003	.001	62.299
	Within Groups	20	.000	.000	
	Total	23	.003		
GSH	Between Groups	3	43.761	14.587	159.663
	Within Groups	20	1.827	.091	
	Total	23	45.588		
SOD	Between Groups	3	29.654	9.885	61.186
	Within Groups	20	3.231	.162	
	Total	23	32.885		
GSH	Between Groups	3	45.298	15.099	132.893
	Within Groups	20	2.272	.114	
	Total	23	47.570		
SOD	Between Groups	3	10275.238	3425.079	63.256
	Within Groups	20	1082.923	54.146	
	Total	23	11358.161		
CAT	Between Groups	3	.137	.046	.883
	Within Groups	20	1.033	.052	

	Total	23	1.170		

Table. XV: Analysis of variance (ANOVA) for the effects of treatments on Sensory evaluation of meat of broiler chicken

Particulars	**Source of variance**	**d.f**	**S.S**	**M.S**	**F**
Appearance	Between Groups	3	.458	.153	.266
	Within Groups	20	11.500	.575	
	Total	23	11.958		
Flavour	Between Groups	3	2.333	.778	1.728
	Within Groups	20	9.000	.450	
	Total	23	11.333		
Juciness	Between Groups	3	8.125	2.708	5.000
	Within Groups	20	10.833	.542	
	Total	23	18.958		
Tenderness	Between Groups	3	7.458	2.486	4.735
	Within Groups	20	10.500	.525	
	Total	23	17.958		
Overall Acceptability	Between Groups	3	6.792	2.264	4.055
	Within Groups	20	11.167	.558	
	Total	23	17.958		

Brief Bio-data of the student

Name : Dr.Suman Kumar Prasad

Father's Name : Sri Daya Kishore

Mother's Name : Smt. Saraswati Devi

Date of Birth : 31st December 1986

Permanent Address : Village- Langhaura, P.O.-Powari, P.S.- Harnaut, District-Nalanda, State- Bihar.

Academic qualification :

Details of qualification	**Year of passing**	**Name of School/College**	**Name of Board/ University**	**Marks(%) / OGPA**	**Division**
10th class	2002	G & H High School, Ranchi	CBSE	60%	1st
12th Class(Science)	2004	Marwari College, Ranchi	J.A.C. Ranchi	59%	2nd
B.V.Sc. & A.H.	2017	Ranchi Veterinary College, Ranchi	Birsa Agricultural University, Ranchi	6.947	1st
M.V.Sc.	2019	Bihar Veterinary College, Patna	Bihar Animal Sciences University, Patna	8.682	1st

Thesis title in M.V.Sc. : Study on comparative efficacy of synthetic and herbal vitamin E supplementation on performance of Broiler chickens.

(**Suman Kumar Prasad)**