EFFECT OF DIETARY SUPPLEMENTATION OF OREGANO AND THYME OIL ON THE PERFORMANCE OF TURKEY POULTS

THESIS SUBMITTED FOR PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE

OF

MASTER OF VETERINARY SCIENCE

IN

POULTRY SCIENCE

BY

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Enrollment No. V-2126/19

COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY

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(2021)

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This is to certify that the thesis entitled **"Effect of dietary supplementation of oregano and thyme oil on the performance of turkey poults"** submitted by **Abhilasha Rai**, Enrollment No. V-2126/19 in partial fulfilment of requirement for award of the **Master of Veterinary Science in Poultry Science** of the U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura- 281001 (UP), India, is a bonafide research work carried out by her under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

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ABBREVIATIONS

%	:	Percentage
@	:	At the rate
<	:	Less than
>	:	Greater than
°C	:	Degree Celsius
A/G	:	Albumin per Globulin
AB	:	Avilamycin
ADFI	:	Average daily feed intake
ADG	:	Average daily gain
AGP	:	Antibiotic growth promoters
AIA	:	Acid insoluble ash
ALP	:	Alkaline phosphate
ALT	:	Alanine aminotransferase
ANOVA	:	Analysis of variance
ANTs	:	Antibiotics
AOAC	:	Association of Official Agricultural Chemists
AST	:	Aspartate aminotransferase
BF	:	Basal Feed
BW	:	Body weight
Ca	:	Calcium
CBP	:	Commercial blend of phytogenics
CI	:	Chlorine
CK	:	Creatine kinase
CK-MB	:	Creatine kinase myocardial band
CMI	:	Cell mediated immune response
CO2	:	Carbon dioxide
COR	:	Chicken cortisol
COX-2	:	Cyclooxygenase-2
CP	:	Crude protein
Cu	:	Copper

DM	:	Dry matter
DMB	:	Dry matter basis
DMSO	:	Di-methyl-sulphoxide
Dpi	:	Day post immunization
DTH	:	Delayed type hypersensitivity
EE	:	Ether extract
ELISA	:	Enzyme-linked immunosorbent assay
EO	:	Essential oil
EOB	:	Blend of essential oils
EOE	:	Essential oil extract
EOM	:	Essential oil mixture
Eos	:	Essential oils
et al.	:	Et alli/alia
FCR	:	Feed conversion ratio
Fe	:	Iron
Fig	:	Figure
FWI	:	Foot web index
g	:	Grams
g/kg	:	Grams per kilogram
g/L	:	Grams per litre
GIT	:	Gastrointestinal tract
GOT	:	Glutamate oxaloacetate transferase
GPT	:	Glutamate pyruvate transferase
GRBC	:	Goat Red Blood Cells
H/L	:	heterophils per lymphocytes
HA	:	Haemagglutination
Hb	:	Haemoglobin
HDL	:	High Density Lipoprotein
HI	:	Haemagglutination inhibition
HMG-CoA	:	Hydroxymethylglutaryl- CoA
Hr	:	Hour

HSP60	:	Heat shock protein 60
HSP70	:	Heat shock protein 70
IAEC	:	Institutional Animal Ethics Committee
IBDV	:	Infectious bursal disease virus
ID	:	Identification
IgG	:	Immunoglobulin G
IgM	:	Immunoglobulin M
IU	:	International unit
K	:	Potassium
Kcal	:	Kilo calorie
Kg	:	Kilogram
LD	:	Lactate Dehydrogenase
LDL	:	Low density lipoprotein
LE	:	Labiatae extract
LPO	:	Lipid peroxidation
M	:	Mole
MDA	:	Malondialdehyde
MDH	:	Malate dehydrogenase
ME	:	Matrix-encapsulated form
ME	:	Mercaptoethanol
ME	:	Metabolizable energy
MER	:	Mercaptoethanol resistance
MES	:	Mercaptoethanol sensitive
mg	:	Milligram
Mg	:	Magnesium
Mg/Kg	:	Milligram per kilogram
MHC	:	Major histocompatibility complex
Min	:	Minute
mL	:	Millilitre
ml/L	:	Millilitre per litre
mM	:	Millimole

MOS	:	Mannanoligosaccharides
MTT	:	3-(4,5-di methyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUFA	:	Monounsaturated fatty acid
n	:	Number
Na	:	Sodium
NDV	:	New Castle disease virus
nM	:	Nanomole
NRC	:	National Research Council
NS	:	Non significant
OD	:	Optical density
OEO	:	Oregano essential oil
OS	:	*Origanum syriacum*
PF	:	Powdered form
P	:	Phosphorus
PBS	:	Phosphate buffer saline
PC	:	Positive control group
PEG	:	Polyethylene glycol
PHA-P	:	Phytohaemagglutinin- *Phaseolus vulgaris*
pNPP	:	p- Nitrophenyl Phosphate
POD	:	Peroxidase
PPAR-α	:	Peroxisome proliferator –activated receptor- alpha
ppm	:	Parts per million
PUFA	:	Polyunsaturated fatty acid
RBC	:	Red blood cell
Se	:	Selenium
SE	:	Standard error
SEM	:	Standard error mean
SFA	:	Saturated fatty acid
SOD	:	Superoxide dismutase
SRBC	:	Sheep red blood cells
T	:	Tonne

T	:	Temperature
T1	:	Treatment 1
T2	:	Treatment 2
T3	:	Treatment 3
T4	:	Treatment 4
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic Acid
TGs	:	Triglycerides
V/V	:	Volume per volume
VLDL	:	Very low density lipoprotein
Wk	:	Week
Zn	:	Zinc
µL	:	Microlitre
µM	:	Micromole

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(Abhilasha Rai)

Place: Mathura (U.P.)

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CONTENTS

ABSTRACT

The present study was conducted to assess the effect of dietary supplementation of oregano and thyme oil on the performance of turkey poults. Day old turkey poults (n=144), were divided into four treatment groups, having three replicates of 12 birds each. The study was conducted in turkey poults during 0-8 weeks of age. Poults were fed T1- basal diet, T2- basal diet supplemented with oregano oil @ 1% of diet, T3- basal diet supplemented with thyme oil @ 1g/kg of diet, T4- basal diet supplemented with oregano oil @ 1% and thyme oil @ 1g/kg of diet. Weekly body weight was significantly higher in poults of T1 and T3 than poults of T4 during 1st ($P<0.05$), 2nd ($P<0.01$), 3rd week ($P<0.01$), 4th ($P<0.05$), 5th ($P<0.01$), 6th ($P<0.01$), 7th ($P<0.01$) and 8th ($P<0.01$) week of age. Further, weekly body weight was apparently higher in T1 poults as compared to poults of other treatment groups from 1st to 3rd week of age. Thereafter, the weekly body weight was apparently higher in T3 as compared to other treatment groups. Weekly body weight gain was significantly higher in T1 and T3 than T4 during 2nd ($P<0.01$) and 7th ($P<0.01$) week of age. Phase wise body weight gain was significantly higher in T1 and T3 than T4 during 0 to 4 weeks ($P<0.05$), 4 to 8 weeks ($P<0.05$) and 0 to 8 weeks ($P<0.01$) of age. Further, phase wise body weight gain was apparently higher in T3 as compared to other treatment groups during 0-4 weeks, 4-8 weeks and 0-8 weeks of age. Weekly feed intake was significantly higher ($P<0.05$) in poults of T1, T2 and T3 than T4 poults at 2nd and 8th week of age. Phase wise feed intake was significantly higher in the poults of T1 and T3 than T4 during 0-4 weeks ($P<0.01$) and 0 to 8 weeks ($P<0.05$) of age. Weekly FCR was significantly better ($P<0.05$) in T1, T3 and T4 than T2 at 2nd week of age. At 3rd week of age, FCR of poults of T2, T3 and T4 were significantly better ($P<0.01$) than T1. At 7thweek of age, FCR of poults of T1, T2 and T3 were significantly better ($P<0.05$) than T4. There was no significant difference observed in the humoral immune response (response to 1% GRBC HA titre) among the different treatment groups. T2, T3 and T4 poults had significantly higher ($P<0.01$) foot web index compared to T1 poults. T2 group poults had significantly higher ($P<0.05$) concentration of serum IgG as compared to other treatment groups. Further, the concentration of serum IgG and IgM of all the oil supplemented groups were apparently higher than the control group. No significant difference was observed in plasma uric acid, AST, ALP, SOD and LPO values.T4 poults had significantly higher ($P<0.05$) total plasma protein than T1, T2 and T3.T2 and T3 poults had significantly higher ($P<0.05$) plasma ALT as compared to T4 poults. T3 and T4 poults had significantly higher ($P<0.05$) plasma cholesterol as compared to T1 poults. Further, T2 and T3 poults had significantly higher ($P<0.01$) plasma HDL values than T1 and T4.There was no significant difference in development of digestive organs, lymphoid organs, yield of giblet and cut up parts and carcass quality traits among the different treatment groups. T2 poults had significantly higher ($P<0.05$) ready to cook yield% than T1. No significant difference was observed in the chemical composition of breast and thigh meat. T2 and T3 poults had significantly higher ($P<0.05$) deposition of Mg in breast muscle as compared to T1. Further all the oil supplemented groups had apparently higher deposition of Mg in breast meat. Monounsaturated fatty acid was significantly higher ($P<0.01$) in T1 as compared to other treatment groups. Polyunsaturated fatty acid was significantly higher ($P<0.01$) in all the oil fed groups as compared to the control group. Thus, it may be concluded that thyme oil @ 1g/kg of diet may be supplemented to elicit growth performance, immunity, percent ready to cook yield, Mg deposition in breast meat cut and PUFA in meat of turkey poults.

Introduction

CHAPTER- 1

INTRODUCTION

Poultry is one of the important components of the farmer's economy in India and provides additional income and job opportunities for the rural population. India is the third-largest producer of eggs and ranks fifth in poultry meat production in the world (Prabhakaran et al., 2019). Poultry plays an important role in the livestock economy. Poultry sector in India can be broadly classified into three categories- Commercial chicken farming, backyard poultry farming and diversified poultry farming. The total poultry population in the country is 851.81 million (BAHS, 2019) and chickens account for more than 90% of the poultry production of India followed by ducks. Turkeys, Japanese quails, guinea fowls etc. have a very small share in Indian poultry production. Eggs and chicken meat are the cheapest source of animal protein affordable by the masses and account for more than 75% of the non-vegetarian items consumed in India.

Turkeys *(Meleagris gallopavo*) were introduced in India as a part of diversified poultry farming. Turkeys are native of North America and were first domesticated in Europe. In India, turkey is reared as a 'Winter bird'. Turkey is a rapidly growing bird and requires more energy, protein, vitamins and minerals compared to chicken. The turkey meat has good commercial value because the meat has low cholesterol and fat content. Under proper feeding and artificial lighting management, turkey hens lay as much as 60-100 eggs annually.

The sub therapeutic use of antibiotics in poultry nutrition for improvements in growth, feed utilization and FCR, is well documented. The opportunistic pathogens that are normally inhabitant of the intestinal tract may reduce the growth rate and has been related to the microbial load of the chicken's environment (Thomke and Elwinger, 1998). Thus, the need for the use of antibiotics to decrease the spread of disease (Waldroup et al., 2003) and as a growth enhancer is increasing day by day to sustain the growth of poultry production (Roura et al., 1992). However, the growing concern over the transmission and the proliferation of resistant bacteria via the food chain has led to a ban on the use of antibiotic growth promoters (AGP) in livestock

within the European Union since 2006. There has been a search for antibiotic alternatives to control the enteric diseases (Fritts and Waldroup, 2003; Ayed et al., 2004) and is also being encouraged by the World Health Organization (Humphrey et al., 2002). Thus, studies on various alternatives to antibiotics *viz.* prebiotics, probiotics, organic acid and essential oils etc. have been undertaken.

Phytogenic feed additives are plant-based feed additives that are used in natural substances in poultry nutrition. These substances are derived from herbs, spices, other plants and their extracts like essential oils. They are natural, less toxic, residue free and ideal feed additives for poultry when compared to synthetic antibiotics. The benefits of using phytogenic feed additives in poultry nutrition are increased feed intake, stimulation of digestion, increased growth performance, reduced incidence of disease, improved reproductive parameters, feed efficiency, profitability and reduced poultry house emissions. Further, these feed additives positively influence gut morphology in broilers and significantly increase nutrient digestibility. Moreover, it stimulates the production of antioxidant enzymes (Roofchaee et al., 2011). So phytogenic feed additives have been widely used to increase the performance of animals and are now used in poultry feeding practices extensively (Collington et al., 1990; Khan et al., 2007) not only to stimulate the growth and feed efficiency but to improve the health and performance of birds (Fadlalla et al., 2010; Abouelfetouh et al., 2012). As a result, new commercial additives derived from plants including aromatic plant extracts and their purified constituents have been examined as part of alternative feed strategies for the future. Such products have several advantages over commonly used commercial antibiotics since they are residue-free and they are also generally recognized as safe (Varel, 2002). Most common herbs and spices used as phytogenic feed additives in poultry production are oregano, thyme, garlic, horseradish, chili, cayenne, pepper, peppermint, cinnamon, anise, clove, rosemary and sage. The beneficial properties of these phytogenic compounds are due to their bioactive molecules *viz.* carvacrol, thymol, cineole, linalool, anethole, eugenol, allicin, capsaicin, allyl isothiocyanate and piperine. Many of these bioactive molecules are essential oils.

Essentials oils also called volatile or ethereal oils- are complex aromatic oily liquid typically obtained from plant materials by the process of distillation and have a characteristic fragrance of the source plant material such as flowers, buds, seeds,

leaves, twigs, bark, herbs, wood, fruits and roots (Burt, 2004). The essential oils are a complex mixture of secondary plant metabolites (Cowan, 1999) consisting of low-boiling-phenylpropenes and terpenes. Mostly, EOs are mixtures of hydrocarbons, oxygenated compounds and a small percentage of non-volatile residues (paraffin, wax, etc.). The term 'essential' was proposed by Paracelsus in his theory of 'Quinta essential', defined as "herbs" and spices" and described that this quintessence could be an effective element for medical use (Oyen and Dung, 1999). In medieval pharmacy, the term 'volatile oil' had been used. Essential oils can be obtained by various methods like expression, fermentation or extraction but they are typically extracted by distillation methods, in particular steam distillation (Greathead, 2003). Their active compounds can also be produced in 'nature-identical' form with identical chemical structure to the naturally occurring raw materials and their extracts. To achieve a recognised 'nature-identical' level under food legislation, these products must be at least 99.5% identical to the natural materials (Williams and Losa, 2001). Many aromatic plants, especially those from the plant family Labiatae (e.g. rosemary, thyme, oregano and sage), have been extensively studied for their antioxidant activity. This activity is not only related to the phenolic compounds but also non-phenolic compounds, which may show considerable antioxidant activity by stimulating the antioxidant enzyme production. The broilers fed diets supplemented with rosemary or its essential oils resulted in increased antioxidant status in meat, improved meat quality and its shelf life (Zhai et al., 2018). In general, essential oils (EOs) enhance the production of digestive secretions and nutrient absorption, reduce pathogenic stress in the gut, exert antioxidant properties and reinforce the animals immune status, which help to explain the enhanced performance observed in swine and poultry. However, the mechanisms involved are being explored, since data on the complex gut ecosystem, gut function, *in vivo* oxidative status and immune system are still lacking. Further, scanty information is available regarding the interaction between EOs and feed ingredients or other feed additives (Zeng et al., 2015).

Essential oil (EO) constituents are quickly absorbed after oral, pulmonary or dermal administration and mostly metabolized and either eliminated by kidneys in the form of glucuronides or exhaled as CO_2. Their accumulation in the body is unlikely due to rapid clearance and short half-life (Lee et al., 2004). The efficacy of EO mixture also depends upon the compatibility with the other ingredients of the mixture

in the feed. Around 3000 EOs are known till date, of which about 300 are commercially important and are used mainly in the flavours and fragrance market. Factors that may affect the quality of EOs in plants include soil type, climate, use of chemicals (fertilizers), variety of the plant, harvesting time, age of the plant, method of extraction etc.

There are two broad classes of essential oils: Terpenes and Phenylpropeness (Lee et al., 2004).

Essential oils may exhibit a range of potentially beneficial properties including antimicrobial (Hammer et al., 1999), antioxidant (Vichi et al., 2001), antiviral (Bishop, 1995), antiparasitic (Hafez and Hauck, 2006), digestive stimulant (Platel and Srinivasan, 2004), antioxygenic (Juglal et al., 2002), insecticidal (Karpouhtsis et al., 1998), immunomodulator (Hanieh et al., 2010), growth promoter (Elagib et al., 2013), hypolipidemic (Srinivasan, 2004) as well as inhibition of odour and ammonia control (Varel, 2002). EOs also stimulate blood circulation (Gopi *et al*., 2014).

Many studies have already depicted that dietary essential oil supplementation has a positive effect on the growth performance (Elagib et al., 2013) and immune system in poultry (Hanieh et al., 2010; Rahimi et al., 2011). Many herbal oils are rich in essential oils. Oregano and thyme are rich in essential oil.

Oregano (*Origanum vulgare*) is a flowering plant in the mint family (Lamiaceae). Oregano is derived from the Spanish *oregano,* Latin *origanum* and Greek *origanum* and has been used since the middle of 18^{th} century. This is a compound Greek term meaning "brightness of the mountain". Oregano is a perennial herb and it is sometimes called wild marjoram and it's close relative *O. majorana* is known as sweet marjoram. Oregano is used for the flavour of it's leaves, which can be more intense when dried than fresh. It has an earthy, warm and slightly bitter taste, which can vary in intensity.

Oregano essential oil is extracted from the leaves of oregano plant. The essential oil of oregano is made by air drying the leaves and shoots of the plant. Once they are dried, the oil is extracted and concentrated by steam distillation. Oregano contains polyphenols including numerous flavones. Oregano oil is composed primarily of monoterpenoids and monoterpenes. Over 60 different compounds have been identified with the primary ones being carvacrol and thymol ranging more than

80%, while the lesser abundant compounds include p-cymene, γ- terpinene, caryophyllene, spathulenol, germacrene-D, β-fenchyl alcohol and δ-terpineol. Oregano contains rosmarinic acid, sterols, vitamin A and C as well as several minerals like Fe, Mg etc. Hippocrates, the father of medicine, used oregano as an antiseptic as well as for cure of stomach and respiratory ailments.

Thyme (*Thymus vulgaris*) is an aromatic perennial evergreen herb in the mint family Lamiaceae. Thymes are relatives of the oregano genus *Origanum.* It is commonly called as 'Ajwain' in India. They have culinary, medicinal and ornamental uses. They are used to purify rooms and to impart an aromatic flavour. Oil of thyme, the essential oil of common thyme, contains 20–54% thymol. Thyme essential oil also contains a range of additional compounds such as *p*-cymene, myrcene, borneol and linalool. The essential oil is obtained by steam distillation of the aerial parts of the plant, harvested during flowering. Thyme oil improves intestinal antioxidant status, reduces malondialdehyde content in the enterocytes and improves intestinal integrity (Attia *et al.*, 2017). They also exert anti-oxidant action to prolong the shelf life of the feed and the meat which is obtained from the animal fed with essential oils.

Few studies have been conducted on dietary supplementation of oregano and thyme oil in chicken (Fotea et al., 2004; Attia et al., 2017). However, limited work has been done in turkeys. Hence, the present study has been designed with the following objectives:

OBJECTIVES

1. To study the effect of supplementation of essential oil on growth performance of turkey poults.
2. To study the effect of dietary supplementation of essential oil on the immunocompetence traits and blood biochemical attributes of turkey poults.
3. To study the carcass quality of turkey poults fed on a diet supplemented with essential oil.

Review of Literature

CHAPTER-2

REVIEW OF LITERATURE

Essential oils (EOs) are complex mixtures of secondary plant metabolites (Cowan, 1999) consisting of low-boiling phenylpropenes and terpenes. In fact, essential oils are final terpenoid products and are formed by a huge group of enzymes known as terpene synthases (TPS). EOs constitute a distinctive aroma that is peculiar to that oil. EOs possess characteristic odour and are soluble in organic solvents like ether, benzene, acetone etc. They exist either in volatile or liquid form at the ambient temperature. Most of the essential oils have specific gravity between 0.8 and 1.17 and are lighter than water. These oils are sensitive to heat and light, therefore should be stored in dark bottles and cool places.

The shikimic acid pathway produces the aromatic amino acid phenylalanine, the products of which are cinnamic acid and p–coumaric acid, occurring in trans–configuration. The important phenylpropene compounds are eugenol, trans–cinnamaldehyde, safrole and also the pungent principles, capsaicin and piperine. These are classified as phenylpropenoids (Loza–Tavera, 1999). Biosynthesis of essential oils occurs through two complex natural biochemical pathways involving different enzymatic reactions. Mevalonic acid (six carbons) that is formed by condensation of three acetate units by HMG–CoA reductase, is converted to 5-carbon isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are the activated 5–carbon units of isoprene. In the particular plant cell part, prenyl diphosphate synthases condense isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) further to form prenyl diphosphates, which are used as substrates for geranyl diphosphate (GPP; C_{10}) or for fernesyl diphosphate (FPP; C_{15}).

Classification

There are two broad classes of essential oils: Terpenes and Phenylpropenes. Terpenes are subdivided based on the 5–carbon isoprene unit (building block) into mono ($C_{10}H_6$), sesqui ($C_{15}H_{24}$) and diterpenes ($C_{20}H_{32}$), while the phenylpropenes consist of 6-carbon aromatic ring having a 3-carbon side chain (C_6-C_3 compounds). More than 1000 monoterpenes and 3000 sesquiterpenes have been identified till today (Clegg et al., 1980; Cooke et al., 1998).

Poultry

Essential oils have wide range of activities *viz.* antibacterial, antioxidant, hyolipidemic, digestive stimulant, growth promoter, immunomodulator, antimycotic (Gopi et al., 2014). Among diverse categories of essential oils, carvacrol, thymol and linalool are of immense importance, enriched with specific health strengthening virtues.

Lovkova et al. (2001) studied the chemical composition related to the synthesis of physiologically active substances (alkaloids, terpenoids, glycosides, phenolic compounds, etc.) in medicinal plants. They suggested that chemical features of medicinal plants serve as an integral determinant of their species specificity and pharmacological properties and enable their wide use in medical practice.

Oregano

Oregano (*Origanum vulgare)* is a flowering plant in the mint family (Lamiaceae). Oregano is a perennial herb and it is sometimes called wild marjoram and its close relative *O. majorana* is known as sweet marjoram. Carvacrol being a phenolic monoterpenoid is a major component of oregano. It is one of the most copiously investigated essential oil components along with its closely related isomer thymol**.**

Antioxidant Activity

Rice-Evans et al. (1997) found that phenolics were found to be more potent antioxidants as compared to vitamins E and C and carotenoids.

Fernandez-Panchon et al. (2008) noted that antioxidant mechanisms of EOs were based on both their ability to donate a hydrogen or an electron to free radicals and their ability to delocalize the unpaired electron within the aromatic structure, thus protecting other biological molecules against oxidation.

Botsoglou et al. (2002) studied the effect of dietary oregano essential oil (50 and 100 mg/kg feed) on the performance of broilers and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. They observed that dietary oregano oil exerted an antioxidant effect on chicken tissues.

Antiparasitic activity

Carvacrol and thymol, the main ingredients of oregano oil, have anticoccidial action against *E. tenella* (Giannenas et al., 2003).

Giannenas et al. (2003) examined the effect of dietary supplementation of oregano essential oil @ 300 mg/kg and anticoccidial lasalocid at 75 mg/kg on performance of broiler chickens experimentally infected with *Eimeria tenella* at 14 days of age. Oregano essential oil exerted an anticoccidial action against *E. tenella* and mixed Eimeria species.

Growth performance

Many studies have shown positive effects on body weight gain after dietary supplementation of oregano oil. Broilers fed 1.0% oregano oil resulted in better average body weight and body weight gain and 4% improvement in feed efficiency (Fotea et al., 2004). Supplementation of a mixture of EOs (laurel, oregano, sage, citrus and anis) at a level of 24mg/kg diet in broilers significantly improved feed conversion (Cabuk et al., 2006a).

Oregano oil @ 300 mg/kg and oregano oil @ 600 mg/kg significantly increased the final body weight compared to the control group. On day 21, oregano oil @ 600 mg/kg increased average daily gain (ADG) and improved feed conversion ratio compared to control. On day 42, both Oregano oil @ 300 mg/kg and oregano oil @ 600 mg/kg significantly increased ADG and average daily feed intake (ADFI) compared to the control. Moreover, oregano oil @ 600 mg/kg increased ADG and ADFI compared with avilamycin. Avilamycin and oregano oil supplementation significantly improved the dressing percentage and eviscerated rate compared to control. Broilers fed with oregano oil @ 600 mg/kg had significantly higher breast muscle percentage and lowest abdominal fat percentage. All treatments had no effect on the villus height in the jejunum of broilers. However, oregano oil @ 300 mg/kg and oregano oil @ 600 mg/kg significantly decreased the crypt depth and significantly increased the villus height to crypt depth ratio compared with control and avilamycin resulting in improvement of growth performance (Peng et al., 2016).

Roofchaee et al. (2011) investigated the effects of dietary supplementation of oregano oil @ 300, 600 and 1200 mg/kg on broiler performance, cecal microflora and serum antioxidant activity. Inclusion of 600 mg/kg of oregano oil in grower diet

significantly increased body weight gain, Supplementation of 600 and 1200 mg/kg of oregano oil significantly improved feed conversion ratio. Populations of cecal *Escherichia coli* were significantly lower in 300 and 600 mg/kg. Although, serum antioxidant activity was not significantly affected by the treatments, antioxidant activity of serum was higher in OEO supplemented groups.

Abdel-Wareth et al. (2012) evaluated the effect of thyme or oregano @ 15 or 20 g/kg diet on growth performance and gastrointestinal microflora in broilers and found that improvements for body weight gain and feed conversion ratio ($P < 0.05$) were observed in both groups. However, feed intake was increased ($P < 0.001$) only by oregano addition. Colony forming units of Lactobacillus spp. were reduced ($P < 0.05$) for thyme and oregano supplemented groups, whereas no changes were observed for colony forming units of total plate counts. It was concluded that thyme and oregano supplemented @ 15 or 20 g/kg diet can be used as effective feed additives to improve performance of broilers.

Supplementation of oregano oil @50 mg/ kg diet in quail significantly increased body weight and body weight gain and resulted in numerically better FCR compared to control group (Badiri and Saber, 2016).

Tekce and Gul (2016) investigated the effects of *Origanum syriacum* (OS) essential oil and an antibiotic (Avilamycin) on performance parameters, intestinal microflora and histology under heat stress conditions of broilers. Birds were fed with 5 experimental diets: control (without feed additive), antibiotic (control + 100 mg/kg Avilamycin), OS 100 (control + 100 mg/kg OS), OS 300 (control + 300 mg/kg OS), OS 600 (control + 600 mg/kg OS). At the end of the study period, feed intake, feed-conversion ratio, body weight, average daily weight gain, ileum crypt depth, villus height and microflora decreased in groups under heat stress without additives.

Immunity

Asli and Rashti (2017) evaluated two commercial essential oils of oregano in broilers. Dietary treatments included: (1) control (without phytogenic), (2) commercial blend of phytogenics (CBP; 150 ppm), (3) oregano essential oil (OEO) (300 ppm), and (4) OEO (500 ppm). During the entire period, average daily feed intake ADFI as lower in CBP-fed birds than control, but was not different among CBP and either level of OEO. European production efficiency factor was greater for

broilers fed 300 ppm OEO than those fed control diet or CBP. Broilers fed 300 ppm OEO produced higher secondary total antibody titre against sheep red blood cell and their immunoglobulin G titre was higher than those fed control or CBP. Serum heterophil counts and heterophil to lymphocyte ratio were lower in birds fed 300 ppm OEO or CBP than the control. Supplementation of OEO at the rate of 300 ppm in diet led to beneficial effects on performance and immune response of broilers.

Blood biochemical indices

Tekce and Gul (2017) studied the effect of *Origanum Syriacum* (OS) essential oil on biochemical and haematological values of broilers fed under temperature stress. There were 8 different groups: 22 ºC Basal Feed (BF)-positive control group (PC), BF + 100 ppm OS; BF+OS-300 ppm OS; BF + 600 ppm OS; 36ºC Basal Feed (BF)-positive control group (PC), BF + 100 ppm OS, BF+300 ppm OS, BF + 600 ppm OS. Total bilirubin, cholesterol, HDL, TG, LDL-C, Na, Ca and Mg increased while glucose, ALT, AST, CK, CK-MB, urea, uric acid, CI and K decreased. However, there was no effect on haematological values.

Köksal and Kucukersan (2012) reported a decrease in serum total protein in response to 0.75 g/kg essential oil mix (*Origanum vulgare*, *Thymus vulgare*, garlic, anise seed and raziyane).

Thyme

Thyme (*Thymus vulgaris*) is an aromatic perennial evergreen herb in the mint family Lamiaceae. It is commonly called as ‘Ajwain’ in India. They have culinary, medicinal and ornamental uses. Thymol, also known as 2-isopropyl-5-methylphenol is a natural monoterpenoid phenol derivative and major constituent of thyme oil. It is obtained from *Thymus vulgaris* (common thyme) and various other kinds of plants as a white crystalline substance of a pleasant aromatic odour. Lately, thymol has received much consideration from the researchers. Thymol is structurally very identical to carvacrol, with the hydroxyl group at a different position on the phenolic ring.

Antimicrobial Activity

Essential oils like carvacrol and thymol cause disintegration of the membrane of bacteria and lead to release of membrane–associated material to the external medium.

Cowan (1999) observed that effectiveness of antimicrobial activities depends on pH, chemical structure, concentration of individual bioactive compound along with population and type of microorganisms affected by the chemical compound performing different activities, such as membrane disruption by terpenoids and phenolics, metal chelation by phenols and flavonoids, and effect on genetic material. Oregano and Thyme showed great antimicrobial activity.

Khaksar et al. (2012) reported that thyme essential oil @ 1g/kg fed to Japanese quail reduced the ileal *E. coli* and increased the Lactobacillus count after 35 days of feeding and significantly reduces total cholesterol and TGs.

Growth performance

Lee et al. (2003) conducted an experiment to describe the effect of thymol, cinnamaldehyde and a commercial preparation of essential oil components (CRINA Poultry), in female broilers. Feed intake, weight gain and feed: gain ratios were not different among the treatments. Water intake was significantly lowered by cinnamaldehyde. Relative liver weight (g/100g of body weight) was highest in birds given thymol, but this was seen only at the age of 21days and not at 40 days. Amylase activity in intestinal digesta was highest in chickens given CRINA Poultry for 21 days, but the effect had disappeared after 40 days. Plasma lipid concentrations were not changed by any dietary treatment. They found no effect of essential oil constituents on growth performance in female broiler chickens.

Denli et al. (2004) noted that addition of thyme essential oil @ 60 mg/kg diet and flavomycin 10 mg/kg diet in Japanese quails resulted in significantly higher body weight gains and better feed efficiency. Supplementation of diet with thyme essential oil decreased abdominal fat weight and abdominal fat percentage. It was found that intestinal weight and length, carcass weight, carcass yield and gizzard weight were not affected by any treatment. At the end of experiment, intestinal pH was lower in groups fed diet containing thyme essential oil.

Cross et al. (2007) studied the effect of dietary inclusion of 5 culinary herbs or their essential oils on the growth, digestibility and intestinal microflora status in female broiler chicks. A basal control diet without supplement was given to the one group of birds along with groups consisting of the basal diet with either 10g/kg herb (thyme, oregano, marjoram, rosemary or yarrow) or 1g/kg of essential oil. Dietary

thyme oil or yarrow herb inclusion had the most positive effect on chick performance, while oregano herb and yarrow oil were the poorest supplements. Only thyme and yarrow in these diets had a different effect when used as an herb or oil on weight gain. Dietary treatment had no effect on the intestinal microflora populations, apparent metabolizable energy (AME) or the calculated coefficients of digestibility. Sialic acid concentration was greatest in the birds given dietary thyme oil compared with all other treatments except those birds receiving marjoram oil, rosemary herb and control. However, less sialic acid was excreted in those birds given diets with oregano or rosemary oils, or oregano herb than in control. Thus, plant extracts in diets may affect chick performance, gut health and endogenous secretions, although chemical composition of the extract appears to be important in obtaining the optimal effects.

Al-Kassie et al. (2009) observed that chicks fed with 200 ppm essential oil derived from thyme and cinnamon had significantly higher feed intake, body weight gain, feed conversion ratio, reduced serum cholesterol and total increased protein.

Alali et al. (2013) determined the effect of an essential oil blend (carvacrol, thymol, eucalyptol, lemon) administered in drinking water on the performance, mortality, water consumption, pH of crop and ceca and colonization in broiler birds following Salmonella challenge and feed withdrawal. 0.05% EO administered in drinking water significantly reduced salmonella colonization in crops, improved FCR and increased weight gain. 0.025% and 0.015% essential oil in drinking water significantly increased weight gain compared with control, but did not significantly reduce salmonella colonization in crop. Thus, it was concluded that essential oil used in the study may control salmonella contamination in crop of broilers when administered in drinking water and therefore may reduce the potential for cross-contamination of the carcass when the birds are processed.

Wade et al. (2018a) reported that addition of thyme essential oil @100mg/kg resulted in significantly higher body weight gain, improved FCR, livability and profit in broiler production.

Attia et al. (2017) noted that dietary supplementation of thyme oil @ 1.0g/kg feed in broiler chicken reared under hot climate resulted in better FCR.

Egg quality

It has been observed that essential oil mixture and organic acid supplementation in commercial layer diets under heat stress have positive effect on egg weight and immune function. Essential oil mixture did not have a significant effect on egg productivity traits but the mixture did tend to increase egg weight. Essential oil mixture supplementation in diet significantly increased albumen height and haugh unit (Ozek et al., 2011).

Olgun (2016) investigated the effect of seven different levels (0, 25, 50, 100, 200, 400, and 600 mg/kg) of a phytogenic feed additive containing a mixture of essential oils from thyme, black cumin, fennel, anise and rosemary on performance, eggshell quality, bone biomechanical properties and bone mineralization in laying hens. Egg weight and egg mass were positively linearly affected by essential oil mixture supplementation. Also, eggshell thickness was increased quadratically by essential oil mixture supplementation. The biomechanical properties and tibia mineral content were adversely affected by essential oil mixture supplementation at the level of 600 and 400 mg/ kg respectively. Dietary supplementation with a low or medium concentration of essential oils improved bone parameters, while high levels adversely affected laying hens

Immunomodulatory activity

The immunomodulatory potential of various EOs have been studied by several researchers. Placha et al. (2014) evaluated the duodenal wall integrity, antioxidant status as well as some immunological parameters of broiler chickens supplemented with 0.5g *Thymus vulgaris* essential oil /kg diet and 0.4 mg Se/kg DM (dry matter) derived from sodium selenite. Thyme oil decreased malondialdehyde (MDA) concentration in duodenal mucosa and kidney, increased immunoglobulin A (IgA) concentration in duodenal mucosa, stimulated phagocytic activity in blood, improved intestinal barrier integrity. Further, thyme oil with selenium increased thioredoxin reductase (TrxR) activity in duodenal mucosa.

Saleh et al. *(*2014) reported that dietary supplementation of thyme and ginger oils @200 and 100 mg per kg feed respectively in chicken resulted in stimulation of innate immunity and humoral immunity by increasing phagocytic capacity of heterophils and antibody production.

Doaa et al. (2017) reported that supplementation of thyme leaves powder in the broiler's diet improved the immune status and antioxidant activities in broilers and also resulted in production of broilers meat with low levels of lipid peroxidation products.

Antioxidant property

Thymol and carvacrol in thyme have been found to possess strong antioxidant property (Baratta et al., 1998).

Economou et al. (1991) conducted an experiment to determine the anti-oxidative properties of several extracts of plant oils such as oregano, thyme, marjoram, spearmint, lavender and basil and it was mixed to lard kept at 75 °C. The antioxidant action was determined by their impact in stabilizing the lard and it was found that extract containing oregano was the most potent followed by thyme, marjoram and lavender.

Bölükbaşı et al. (2006) conducted a study to investigate the effects of dietary vitamin E (E) and thyme oil (TO) supplementation on the growth performance, lipid oxidation, fatty acid concentration of tissues and the serum lipoprotein levels of male broilers. The addition of thyme oil @ 200 ppm to the broiler feed led to a significant reduction in the saturated (SFA) and polyunsaturated fatty acid (PUFA) concentrations of the leg and breast tissues. The monounsaturated fatty acid (MUFA) concentrations in these tissues increased. Thyme oil supplementation also led to increased plasma levels of triglycerides, LDL-cholesterol and HDL-cholesterol in broilers. Based on the results of this study, it was recommended that broilers may be supplemented with 200 mg/kg of thyme oil as an antioxidant.

Carcass quality

Wade (2018b) conducted an experiment to study the effect of thyme essential oil @ 100, 200, 300 mg/kg diet on the carcass quality of broiler chickens. Higher dressing yield was obtained n 100mg/kg diet thyme supplemented group. Breast %, thigh % and back % was significantly higher in thyme oil supplemented group than control. However, giblet yield and drumstick % were not affected in thyme oil supplemented group. In conclusion, the feed supplemented with thyme essential oil at 100mg/kg resulted in improved dressing yield and cut up parts of carcass traits.

Alcicek et al. (2003) reported that effects of blend of essential oils @ 48 mg /kg had no additional beneficial effect on the carcass yield of turkeys.

Biochemical indices

Attia et al. (2017) studied the effect of dietary supplementation of thyme oil at graded levels and mannanoligosaccharides (MOS) in broilers raised under hot climate from 1 to 28 days of age. The experiment comprised of five dietary treatments : (a) positive control group fed the basal diet supplemented with MOS at 1g/kg feed; (b) negative control group fed the basal diet without supplementation; (c) thyme oil supplementation @ 1.0 g/kg feed (d) thyme oil supplementation @ 1.5 g/kg feed and (e) thyme oil supplementation @ 2.0 g/kg feed. Thyme oil @ 1.0 g/ kg feed displayed a better feed conversion ratio than other treatments. The MOS and thyme oil supplemented groups had higher plasma total protein than control and also increased plasma globulin compared to the control, but decreased plasma albumin/globulin ratio. Moreover, the thyme oil supplement groups significantly decreased the plasma AST. MOS and thyme oil supplemented groups diets showed higher white blood cells than control group. In addition, MOS and thyme oil supplemented groups exhibited a greater antibody titre than the control group. In conclusion, thyme oil @ 1.0 g/kg diet may be used as a potential growth enhancer for broilers in hot climate during 1-28 days.

Behboudi et al. (2016) studied the effect of drinking water containing lemon juice and thyme supplemented diet on growth performance, serum concentration of cholesterol and triglycerides, heterophils, lymphocytes, H/L ratio and enzyme activity of glutathione peroxidase (GPX) in broilers under heat stress condition. The experiment was carried out as a 2 × 3 factorial arrangement with 2 levels of lemon juice (0 and 2 ml/L of drinking water) and 3 levels of thyme (0, 0.5 and 1% of diet). The birds were kept at 34 ˚C ± 2 (9.00 to 17.00 hours) from 25 to 42 days of age. Feed intake, body weight gain, feed conversion ratio, H/L ratio, GPX activity and serum cholesterol concentration were not affected by individual addition of lemon juice or thyme; whereas, combination effect of the two additives improved performance and reduced H/L ratio and serum triglycerides concentration. Concurrent usage of 0.5% thyme and 2 ml/L of lemon juice increased feed intake and body weight gain by 229 and 165 g, respectively. Addition of 0.5 and 1% thyme to the diet

and 2 ml/L lemon juice to drinking water reduced the serum triglycerides concentration by 9.8, 11.1 and 8.5 mg/dL respectively.

Hosseini et al. (2018) evaluated the effect of a blend of essential oils (cinnamaldehyde and thymol) (EOB) and stocking density on the performance, gut microflora, meat quality and physiological stress markers of broilers. The results indicated that overcrowding stress decreased growth performance parameters, blood immunoglobulin IgG and heterophil: lymphocyte (H:L) ratio but increased IgA and IgM levels. Essential oils supplementation elevated H: L ratio but decreased breast meat redness and pH. Significant interactions between blend of essential oils and stocking density were observed for corticosterone level and mRNA levels of heat shock protein 70 (HSP70) in brain and heart. Although high stocking density of 20 birds/m^2 increased corticosterone and HSP70 when compared to low stocking density of 10 birds/m^2, the effects of the former were inconsistent with EOB supplemented diets. Thus, dietary supplementation with blend of essential oils could improve some of the biomarkers associated with overcrowding stress in broiler chickens.

Absorption and utilization of nutrients

Hafeez et al. (2016) demonstrated that inclusion of phytogenic feed additives in feed may enhance performance of broilers. Essential oils in powdered form (characterized by menthol and anethole) at 150 mg/kg (P-150) and matrix-encapsulated form (characterized by carvacrol, thymol, and limonene) at 100 mg/kg (ME-100) were supplemented in the diet. Body weight of birds at 42 days and overall body weight gain was higher in treatment ME-100 than the birds in control treatment. Feed efficiency during finisher phase was improved in treatment ME-100 as compared to control and P-150 groups, the apparent ileal digestibility of crude protein was higher in ME-100 group as compared to control and P-150 groups. Apparent ileal absorption of phosphorus was higher in ME-100 treatment than control treatment. A powdered phytogenic additive characterized by menthol and anethole at 150 mg/kg had no effect but only a tendency towards improved growth performance and apparent ileal absorption (AIA) of phosphorus, whereas essential oils addition in encapsulated form characterized by caravacol, thymol, and limonene at 100 mg/kg improved growth performance as well as apparent ileal digestibility of nutrients in broilers, possibly due to improved secretion of digestive enzymes.

Jang et al. (2007) studied that a supplementation of blend of essential oils (EO) @ 50 mg /kg feed resulted in a significant increase in certain digestive enzyme activities of the pancreas (pancreatic trypsin and pancreatic α-amylase) and intestine. However, weights of the digestive organs including liver, pancreas, intestine and mucosal tissues were not affected by the dietary supplementation of EO in broiler chickens.

Thyme and oregano

Hernandez et al. (2004) conducted a trial to study the influence of 2 plant extracts on growth performance, digestibility and digestive organ weights in broilers. There were 4 treatment groups: control, 10 ppm avilamycin (AB), 200 ppm essential oil extract (EOE) from oregano, cinnamon, and pepper and 5,000 ppm Labiatae extract (LE) from sage, thyme, and rosemary. No differences in feed intake and feed conversion ratio were observed. From 14 to 21 days of age, broilers fed the LE diet grew faster than broilers fed the control and EOE feeds. Antibiotic and plant extract supplementation improved apparent whole-tract and ileal digestibility of the nutrients. For starter feed, LE supplementation improved apparent fecal digestibility of dry matter and all additives increased ether extract digestibility. However, no effect was detected for crude protein digestibility. At the ileal level, the AB, EOE and LE supplementation of the starter feed increased dry matter and starch digestibility but not crude protein digestibility. All additives improved apparent fecal digestibility of dry matter and crude protein of the finisher diet. No differences were observed for proventriculus, gizzard, liver, pancreas and intestine weight. Thus, it was concluded that both the plant extracts improved the digestibility of feed for broilers thereby improving the performance slightly, but this effect was not statistically significant.

Khattak et al. (2014) evaluated the effect of a commercial preparation of natural blend of essential oils from basil, caraway, laurel, lemon, oregano, sage, tea, and thyme (Tecnaroma Herbal Mix PL) on growth performance, blood biochemistry, cecal morphology, and carcass quality of broilers. Tecnaroma Herbal Mix PL was supplemented at 0, 100, 200, 300, 400, and 500 g/ tonne of feed. Birds fed diets supplemented with the herbal mix had significantly heavier body weight, higher weight gain and improved feed to gain ratio as compared to control group during grower phase and overall performance. The blood biochemistry results showed no differences between treatments. The carcass weight, breast weight and relative

percentage of breast meat increased when diets were supplemented with Tecnaroma Herbal Mix PL compared with that from birds fed the control diet. The inclusion level of 300 g of Tecnaroma Herbal Mix PL/t of feed was optimum for enhancing breast meat yield and nutrient utilization as indicated by increased cecal villus surface area.

TURKEY

Growth performance

Dietary oregano leaves (1·25, 2·5 and 3·75 g/kg) improved feed conversion efficiency (FCE) in female early maturing turkeys during 43-84 days of age with the lowest level of inclusion (1·25 g/kg) being most economical (Bampidis et al., 2005)

Antioxidative property

Florou-Paneri et al. (2005) reported that oregano essential oil (OEO) supplementation at 100 mg/kg was more effective in delaying lipid oxidation.

Materials and Methods

CHAPTER-3

MATERIALS AND METHODS

The experiment was carried out to study the effect of dietary supplementation of oregano and thyme oil on the performance of turkey poults. The experimental procedure and analytical techniques followed in the present study have been described in this chapter.

3.1 Oregano and Thyme oil

Oregano and Thyme oil were procured from India Aroma Oils and Company, Kanpur, India.

Feed was procured from Central Avian Research Institute, Izatnagar, India.

3.2 Birds

One hundred forty four straight run turkey poults were divided into four treatment groups comprising of three replicates and twelve turkey poults in each replicate. The poults were wing banded, weighed individually and distributed randomly on uniform body weight basis in the treatment groups. The poults were housed in deep litter system. Water was offered *ad lib*. The experiment was conducted at Poultry Farm of Department of Poultry Science, U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwa Vidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura after due approval of the IAEC. There were four dietary treatments.

3.3 Preparation of experimental diet

The dietary treatments offered were:

T1- Control, Basal diet (turkey starter ration; NRC, 1994)

T2- Basal diet+ supplementation of oregano oil @ 1% of diet

T3- Basal diet + supplementation of thyme oil @ 1g/kg of diet

T4- Basal diet + supplementation of oregano oil 1% and thyme oil @ 1g/kg of diet

Table 3.1: Composition of basal diet (Turkey Starter)

Feed Ingredients	Starter (0-8 wks)
Gross composition (kg/100kg)	
Maize	42.52
Soya bean meal	43.75
Fish Meal	8.0
Animal Fat	2.25
Dicalcium phosphate	2.0
Limestone powder	1.0
Mineral Mixture [1]	0.1
Vitamin Mixture [2]	0.025
Choline Chloride (60%)	0.16
Salt	0.1
Methionine	0.1
Chemical composition (%)	
Crude Protein	28.31
Metabolizable energy (K cal/kg)	2843.84
Calcium	1.65
Phosphorous (Total)	0.9

1. Each gram contains: Copper-15 mg, Iron-250 mg, Iodine-6 mg, Manganese-300 mg and Zinc-300 mg
2. Each gram contains: Vitamins A - 82,500 IU, B_2 -50 mg, D_3 – 12,000 IU, K – 10 mg. B_1– 8 mg, B_6 – 16mg, B_{12} – 80 mg, E – 80 mg, niacin – 120 mg, calcium pantothenate – 80 mg.

3.4 Growth performance parameters

Weekly body weight, group feed consumption and mortality were recorded. Feed conversion ratio (feed intake: body weight gain) of 0 to 8 weeks were calculated at the end of the experiment.

3.5 Immunocompetence traits

The general innate immune-competence status of turkey poults were assayed by measuring two important immunocompetence traits i.e. antibody response to 1% GRBC and cell mediated immune response to PHA-P after 8 weeks of age (Corrier and De Loach, 1990).

3.5.1 Antibody response to Goat Red Blood Cells (GRBC)

The micro-titre plate haemagglutination procedure as described by Siegel and Gross (1980) with slight modifications was followed to measure total HA antibody

titres in turkey poults on day zero and day 5 post injection. The procedure followed is described below.

3.5.1.1 Preparation of 1% Goat Red Blood Cells (GRBC) suspension

Blood from jugular vein was collected from healthy goat in Alsever's solution. The red blood cells were washed thrice in PBS (Phosphate Buffer Saline, pH 7.2). Finally 1% suspension of GRBC in PBS (V/V) was prepared.

3.5.1.2 Preparation of reagents

Alsever's solution and Phosphate Buffer Saline (PBS) was prepared as per the given composition:

A) Alsever's solution

Dextrose	2.05 g
Tri sodium citrate dehydrate	0.80 g
Sodium Chloride (NaCl)	0.42 g
Citric acid	0.055 g
Distilled water	100 ml

The pH of the solution was adjusted to 6.5 by addition of citric acid and stored in refrigerator at 4°C.

B) Preparation of Phosphate Buffered Saline (PBS)

Sodium Chloride (NaCl)	8.00 g
Potassium Chloride (KCl)	0.20 g
Potassium di- hydrogen phosphate (KH_2PO_4)	0.20 g
Di-sodium hydrogen phosphate (Na_2HPO_4, H_2O)	1.44 g
Distilled water	1000 ml
pH	7.20

3.5.1.3 Immunization and harvesting of immune serum

1 ml of 1% (V/ V) of GRBC suspension was injected to 10 poults of each treatment group. At 5 days post immunization (dpi), about 3ml of blood was collected from jugular vein. The blood was endorsed to clot in an incubator at 37^0C for 1 hr. The clot was endorsed to retract after detaching it from sides of its container and left at 4^0C. Centrifugation of blood was carried out at 2000 rpm for 5-10 minutes to

facilitate rapid collection of the serum. Required quantity of immune serum was harvested and stored at-70^{0}C for subsequent testing.

3.6.1 Haemagglutination test (HA test) for total immunoglobulin (total HA titre)

The antibody titre was determined by HA methods (Vander Zijpp, 1983 & Siegel and Gross, 1980). The micro-titre plates (U bottom) were cleaned, rinsed with PBS and dried. The HA test was done in triplicate for each sample.

Procedure

- 50 µl of PBS was distributed in each well of the micro titre plate.
- 50 µl of serum was added in the first well.
- Two fold serial dilutions were made up to column 11 and column 12 was kept as control.
- 50 µl of 1% GRBC was added in each well and mixed by gentle tapping.
- The plates were covered and then kept at temperature 37^{0}C for 1 hour incubation.
- The plates were read under bright light.
- The reciprocal of highest dilution showing clear agglutination was the end titre.

The titres were expressed as log 2 value.

The response titre was the result of the difference between HA titre before and after 1% GRBC immunization

3.6.2 Mercaptoethanol resistant antibodies (MER or IgG) against GRBC

Antibodies were determined by means of a mercaptoethanol (ME) HA test as per the method described by Martin et al. (1989) with slight modification. 0.2 M Mercaptoethanol (2ME) was prepared by adding 1.4 ml of 2-Mercaptoethanol to 98.6 ml of PBS. It can be stored at room temperature having shelf life for 5 days.

Procedure

- 50 µl of a 0.2 M solution of 2-ME in PBS was distributed in each well.
- 50 µl of test serum was added in the first well.
- After incubation for 30 minutes at room temperature, a serial dilution was made.
- 50 µl of a 1% GRBC in PBS was added to each well and mixed.

- The plates were covered with adhesive plastics.
- The micro-titre plates were incubated for 1 hour at 37^{o}C and read under bright light as above and the titre was recorded as 2-ME resistant antibody and expressed as log 2 values.

3.6.3 Mercaptoethanol sensitive antibodies (MES or IgM) against GRBC

The reduction of total titre due to 2-ME treatment was called 2-ME sensitive antibody and the titre was expressed as log 2 value (Total HA titre-MER=MES).

3.6.4 *In-vivo* cell mediated immune response

The cellular immune response was assessed by cutaneous basophilic hypersensitivity test *in vivo* by using PHA-P (Phytohaemagglutinin, lectin from *Phaseolus vulgaris*). Turkey poults were injected intradermally between 3rd and 4th toe of the right foot with 0.1 mg PHA-P in 0.1 ml of PBS (1 mg PHA-P/ml of PBS). The left foot received 0.1 ml of PBS and served as control. The thickness of inter-digital skin was measured using micrometer (AMES) at 0 and 24 hr after injection. The skin swelling was calculated by subtracting the skin thickness at 0 hr from that of after 24 hours of injection. The foot web index was determined as the difference between inter-digital swelling values of PHA-P injected and control foot.

Foot web index (in mm) = (thickness after 24 hrs of inj of PHA-P of right foot - thickness before inj of the same foot) - (thickness after 24 hrs of inj of PBS of left foot - thickness before 24 hrs of inj of PBS of the same foot).

3.7 ELISA for determination of concentration for serum Cortisol, IgG and IgM antibodies.

3.7.1 Serum Cortisol

Cortisol was determined in the serum of experimental birds by Chicken Cortisol ELISA Test kit, Bioassay Technology Laboratory, Shanghai, China.

Principle

The principle on which the ELISA kit works is as follows. This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with chicken cortisol (COR) antibody.COR present in the sample is added and binds to antibodies coated on the wells and then biotinylated Chicken COR antibody is added

and binds to COR in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated COR antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and colour developed in proportion to the amount of Chicken Cortisol. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagents required

Components	Quantity
Standard Solution(64ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 x 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (30x)	20ml x1
Biotin- Conjugate Anti Chicken COR Antibody	1ml x1

Reagents Preparation

Standard Preparation

120µl of the standard (64ng/ml) was reconstituted with 120µl of standard diluents to generate 4.8ng/ml standard stock solution. The standard was allowed to settle for 15 min with gentle agitation prior to making dilutions. Duplicate standard was prepared by 2.4ng/ml, 1.2ng/ml, 0.6ng/ml and 0.3ng/ml solutions. Standard diluents served as the zero standard (0 ng/ml).Dilution of standard solutions carried out were as follows:

4.8ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
2.4ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
1.2ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
0.6ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
0.3ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
9.6ng/ml	4.8ng/ml	2.4ng/ml	1.2ng/ml	0.6ng/ml	0.3ng/ml

Wash Buffer

20ml of Wash Buffer Concentrate 25X was diluted into deionized or distilled water to yield 500 ml of 1X Wash Buffer.

Assay Procedure

The reagents were brought to room temperature before use. The assay was performed at room temperature. 50µl standard was added to standard well and 40µl sample was added to sample wells and also 10µl anti-COR antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). It was mixed thoroughly and the plate was covered with a sealer and was incubated for 60 minutes at 37°C. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.The plate was blotted on paper towels.50µl substrate solution A was added to each well. After that 50µl substrate solution B was also added to each well. The plate was covered with a new sealer and was incubated for 10 minutes at 37°C in the dark. After that 50µl stop solution was added to each well, and then blue color was changed into yellow colour immediately. The optical density (OD value) of each well was determined immediately with the help of microplate reader at 450 nm wavelength.

Calculation of Results

A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit line curve was drawn through the points on the graph. And the best fit line was determined by regression analysis method.

3.7.2 IgG

IgG was determined in the serum of experimental birds by Chicken IgG ELISA Test kit, Bioassay Technology Laboratory, Shanghai, China.

Assay Principle

The principle on which the ELISA kit works is as follows. This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre- coated with chicken IgGantibody. IgG present in sample is added and binds to antibodies coated on the wells and then biotinylated chicken IgG Antibody is added and binds to IgG in sample. Then Streptavidin-HRP is added and binds to the Biotinylated IgG antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and colour developed in proportion to the amount of chickenIgG. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagents required

Components	Quantity
Standard Solution(64ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 x 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (30x)	20ml x1
Biotinylated Chicken IgG Antibody	1ml x1

Reagents Preparation

Standard Preparation

120µl of the standard (128µg/ml) was reconstituted with 120µl of standard diluents to generate 64µg/ml standard stock solution. The standard was allowed to settle for 15 min with gentle agitation prior to making dilutions. Duplicate standard was prepared by serially diluting the standard stock solution (64µg/ml) 1:2 with standard diluent to produce32µg/ml, 16µg/ml, 8µg/mland 4µg/ml solutions. Standard diluents served as the zero standard (0 µg/ml). Dilutions of standard solutions were prepared as follows:

64µg/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
32µg/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
16µg/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
8µg/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
4µg/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
128µg/ml	64µg/ml	32µg/ml	16µg/ml	8µg/ml	4µg/ml

Wash Buffer

20ml of Wash Buffer Concentrate 25X was diluted into deionized or distilled water to yield 500 ml of 1X Wash Buffer.

Assay Procedure

The reagents were brought to room temperature before use. The assay was performed at room temperature. 50µl standard was added to standard well and 40µl sample was added to sample wells and also 10µl anti-IgG antibody was added to sample wells. Then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). It was mixed thoroughly and the plate was covered with a sealer and was incubated for 60 minutes at 37°C. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted on paper towels.50µl substrate solution A was added to each well after that 50µl substrate solution B was also added to each well. The plate was covered with a new sealer and was incubated for 10 minutes at 37°C in the dark. After that 50µl stop solution was added to each well, then blue colour was changed into yellow immediately. The optical density (OD value) of each well was determined immediately with the help of micro plate reader at 450 nm wavelength.

Calculation of Results

A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the Horizontal (X) axis

and a best fit line curve was drawn through the points on the graph. Best fit line was determined by regression analysis method.

The concentration of serum IgM was determined by the same procedure described above by chicken IgM ELISA Test kit by Bioassay Technology Laboratory, Shanghai, China.

3.8 Biochemical parameters

Blood was collected from 10 turkey poults of each group at the end of the biological experiment from the wing vein with the help of heparinized syringe and poured into a sterile tube. The blood samples were centrifuged for 10-15 min at 2500 rpm. Plasma was separated and stored in refrigerator (-20^{0}C) until analyzed.

3.8.1 Plasma cholesterol

Plasma cholesterol was determined by standard diagnostic kit (Span Cogent Diagnostics product). The procedure of the assay is mentioned below: Cholesterol esters are hydrolyzed by Cholesterol Esterase (CE) to give free cholesterol and fatty acids. In subsequent reaction, cholesterol oxidase (CHOD) oxidizes the 3-OH group of free cholesterol to liberate cholest -4-en-3-one and hydrogen peroxide. In presence of peroxidase (POD), hydrogen peroxide couples with 4-Aminoantipyrine (4-AAP) and phenol to produce red quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of total cholesterol concentration in the sample.

S. No.	Reagents	Composition
1	Cholesterol Reagent	Good's Buffer (pH 6.7) Cholesterol Esterase, Cholesterol Oxidase Peroxidase, 4- Aminoantipyrine Stabilisers
2	Cholesterol Standard	Cholesterol Preservative Stabiliser

Procedure

Pipetted into tubes marked	Blank	Standard	Test
Plasma	-	-	10µL
Reagents 2	-	10µ	-
Reagents 1	1000µL	1000µL	1000µL

It was mixed well. Then, it was incubated at room temperature for 30 minutes. The analyzer was programmed as per assay parameters.

1) The analyser was blanked with Reagent Blank.
2) The absorbance of standard was measured followed by test.
3) Results were calculated as per given calculation formula.

Calculation

Cholesterol concentration (mg/100 ml) = Absorbance of Test/Absorbance of Standard × 200

3.8.2 HDL Cholesterol

Low density lipoprotein (LDL), very low density lipoprotein (VLDL), cholesterol and chylomicron fractions are precipitated by addition of polyethylene glycol 6000 (PEG). After centrifugation, high density lipoprotein (HDL) fraction remains in the supernatant and is determined with CHOD-PAP method.

Reagent No.	Reagent	Composition
1	Cholesterol Reagent	Goods Buffer (pH 6.7) Cholesterol Esterase, Cholesterol Oxidase Peroxidase 4-Aminoantipyrine Stabiliser
2	Cholesterol standard	Cholesterol Preservative Stabiliser
3	Precipitating Reagents	PEG 6000 Stabiliser Preservative
4	HDL Cholesterol Standard	Cholesterol Preservative Stabiliser

Step 1: HDL- Cholesterol separation.

Pipette into tube marked	Test
Plasma	200 μL
Reagent 3	200 μL

It was mixed well and kept at room temperature (15-30º C) for 10 minutes, centrifuged for 15 minutes at 200 rpm and the clear supernatant was separated. The supernatant was used for HDL-Cholesterol estimation.

Step 2: HDL- Cholesterol estimation

Procedure

Pipette into tube marked	Blank	Standard	Test
Supernatant from step 1	-	-	100 µL
Reagent 4	-	100 µL	-
Reagent 1	1000 µL	1000 µL	1000 µL

It was mixed well and incubated at room temperature for 30 minutes. The analyzer was programmed as per assay parameters.

1) The analyzer was blanked with Reagent Blank.
2) Absorbance of standard was measured followed by the test.
3) Results were calculated as per given formula.

Calculation

HDL-Cholesterol concentration (mg/dL) = Absorbance of test /absorbance of standard ×50 ×2

(2=Dilution factor, as Sample is diluted 1:1)

3.8.3 Plasma Protein

Plasma protein was estimated by standard diagnostic kit (Span Cogent Diagnostics product) as per modified Biuret method.

The peptide bonds of proteins react with cupric ions in alkaline solution to form a coloured chelate, the absorbance of which is measured at 578 nm. The Biuret reagent contains sodium-potassium tartrate, which helps in maintaining solubility of this complex at alkaline pH. The absorbance of final colour was proportional to the concentration of total protein in the sample.

$$\text{Protein} + Cu^{++} \text{ Alkaline pH} \longrightarrow \text{Cu- Protein complex}$$

Reagent No.	Reagent	Composition
1	Biuret Reagent	Copper Sulphate, Sodium Hydroxide, Sodium-Potassium Tartate Surfactant
2	Protein Standard	BSA Preservative

Procedure

Pipette into tube marked	Blank	Standard	Test
Plasma	-	-	10µL
Reagent 2	-	10µL	-
Reagent 1	1000µL	1000µL	1000µL

It was mixed well and incubated at 37º C for 5 minutes. The analyzer was programmed as per assay parameters.

1) The analyzer was blanked with Reagent Blank.
2) Absorbance of the standard was measured followed by the test.
3) Results were calculated as per the given calculation formula

Calculation

Total Protein concentration (g/dL) = Absorbance of test / Absorbance of Standard × 6.5

3.8.4 Plasma Uric Acid

Plasma uric acid was estimated by standard diagnostic kit (Span Cogent Diagnostics product). The procedure for assay has been mentioned below:

Uric acid is oxidized to allantoin& hydrogen peroxide by the enzyme uricase. In presence of peroxidase, released hydrogen peroxide is coupled with aniline derivative and 4- amino antipyrine (4-AAP) to form colouredchromogen complex. Absorbance of coloured dye is measured at 550 nm and it is proportional to uric acid concentration in the sample

$$\text{Uric acid} + 2H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{Aniline derivative} + \text{4- AAP} \xrightarrow{\text{POD}} \text{chromogen complex} + H_2O_2$$

Reagent No	Composition	Concentration
1	Uric acid Mono Reagent	Tris buffer (pH 8.25) Uricase, Aniline Derivative 4- Aminoantipyrine Peroxidase
2	Uric acid Standard	Uric acid Stabiliser Preservative

Procedure

Pipetted into tube marked	Blank	Standard	Test
Plasma	-	-	20µL
Reagent 2	-	20µL	-
Reagent 1	1000µL	1000µL	1000µL

It was mixed well and incubated at 37º C for 5 minutes. The analyzer was programmed as per assay parameters.

1) The analyzer was blanked with Reagent Blank.
2) Absorbance of the Standard was measured followed by the Test.
3) Results were calculated as per the given calculation formula.

Calculation

Plasma Uric acid (mg/dL) = Absorbance of test / Absorbance of Standard × 6

3.8.5 Plasma ALT/GPT

Plasma GPT was determined by standard diagnostic kit (Span Cogent Diagnostics product) as mentioned below:

Alanine aminotransferase (ALT) catalyses the transamination of L- Alanine and α- Ketoglutarate to form pyruvate and L- Glutamate. In subsequent reaction, lactate dehydrogenase (LD) reduces pyruvate to lactate with simultaneous oxidation of Nicotinamide Adenine Dinucleotide [reduced] (NADH) to Nicotinamide Adenine Dinucleotide (NAD). The rate of oxidation of NADH was measured kinetically by monitoring the decrease in absorbance at 340 nm. LD rapidly and completely reduces endogenous sample pyruvate during the initial incubation period, so that it does not interfere with assay.

Sl. No	Reagent	Composition
1	Buffer	Tris buffer (pH7.5), L-Alanine, LD
2	Substrate	α- Ketoglutrate NADH

Procedure

Sl. No	Pipetted into tube marked	Test
1	Plasma	100 µL
2	Working ALT reagent	1000 µL

It was mixed well and aspirated immediately for measurement. The analyzer was programmed as per assay parameters.

1) The analyzer was blanked with purified water.
2) Absorbance was examined after 60 seconds. Reading was repeated after every 30 seconds i.e. up to 120 seconds at 340 nm wavelength.
3) Mean absorbance was determined as change per minute (Δ A/minute).

Calculation

ALT activity (IU/L) = ΔA/ min × Kinetic factor

Where ΔA/ minute= change in absorbance per minute

Kinetic factor (K) = 1768

3.8.6 Plasma AST/GOT

Plasma GOT was determined by standard diagnostic kit (Span Cogent Diagnostics product) as mentioned below:

Aspartate aminotransferase (AST) catalyzes the transamination of L-Aspartate and α- Ketoglutarate to form L- glutamate and oxaloacetate. In subsequent reaction, malate dehydrogenase (MDH) reduces oxaloacetate to malate with simultaneous oxidation of nicotinamideadeninedi nucleotide [reduced] (NADH) to nicotinamideadenine dinucleotide (NAD). The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340 nm and is directly proportional to AST activity in the sample. Lactate dehydrogenase (LD) is added to enzyme system to prevent endogenous pyruvate interference, which is normally present in the serum.

Sl. No.	Reagents	Composition
1	Buffer	Tris buffer (pH7.8), L-Aspartate, MDH, LD
2	Substrate	α- Ketoglutarate NADH

Procedure

Sl. No.	Pipetted into tube marked	Test
1	Plasma	100 µL
2	Working AST reagent	1000 µL

It was mixed well and aspirated immediately for measurement. The analyzer was programmed as per assay parameters.

1) The analyzer was blanked with purified water.
2) Absorbance was examined after 60 seconds. Reading was repeated after every 30 seconds i.e. up to 120 seconds at 340 nm wavelength.
3) The mean absorbance change per minute (ΔA/minute) was determined.

Calculation

AST activity (IU/L) = ΔA/ minute × Kinetic factor

where ΔA/ minute = change in absorbance per minute

Kinetic factor (K) = 1768

3.8.7 Plasma Alkaline Phosphate Test

At pH 10.3, alkaline phosphate (ALP) catalyzes the hydrolysis of colourless p-Nitrophenyl Phosphate (pNPP) to yellow coloured p-Nitrophenol and Phosphate. Change in absorbance due to yellow colour formation was measured kinetically at 405 nm and was proportional to ALP activity in the sample

p-nitrophenyl Phosphate + H_2O $\xrightarrow{ALP}$ P-Nitrophenol + Phosphate

Sl. No.	Reagents	Composition
1	AMP (2-amino-2-methyl-1 propanol buffer)	AMP magnesium acetate zinc sulphatechelator
2	pNPP substrate	pNPPStabiliser

Procedure

Sl. No.	Pipetted into tube marked	Test
1	Plasma	20 µL
2	Working AST reagent	1000 µL

It was mixed well and aspirated immediately for measurement. The analyzer was programmed as per assay parameters.

1) The analyzer was blanked with purified water.
2) Absorbance was examined after 30 seconds. Reading was repeated after every 30 seconds i.e. up to 120 seconds at 405 nm wavelength.
3) The mean absorbance was determined as change per minute (Δ A/minute).

Calculations

ALP activity (IU/L) = ΔA/ minute × kinetic factor

where ΔA/ minute = Change in absorbance per minute

Kinetic factor (K) = 2712

3.8.8 Superoxide dismutase (SOD) activity in Serum

Reagents

Sl. No.	Chemicals
1	1.25 mM MTT
2	1mM pyragallol
3	Di-methyl-sulphoxide (DMSO)

Procedure

Superoxide dismutase (SOD) activity in serum was measured using the method as described by Madesh and Balasubramanian (1998) with some modifications. It involves generation of superoxide by pyrogallol antioxidation and the inhibition of superoxide-dependent reduction of the tetrazolidium dye MTT to its formazan was measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazan formed and the color evolved is stable for many hours. Pyrogallol used as source of superoxide based on the inhibition of MTT formazan formation from the reaction of MTT and superoxide. MTT is a tetrazolidium compound which can be reduced to its coloured formazan by superoxide.

Procedure

The total volume of mixture 3000 µl consisted of 1200 µl PBS, 100 µl serum, 50 µl of 1.25 mM MTT (1.035 mg/ml) and 150 µl of 1 mM pyrogallol solution (0.126 mg/ml) was added at the end. Sample was replaced with PBS in the blank. After incubation period of 15 minutes 1500 µl DMSO was added and absorbance was taken in double beam spectrophotometer at 570 nm. The percent inhibition by the presence of SOD was calculated from the reduction of the MTT color 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 × 100 X AT/ AB

Where, AT= Absorbance for test.

And AB= Absorbance for blank.

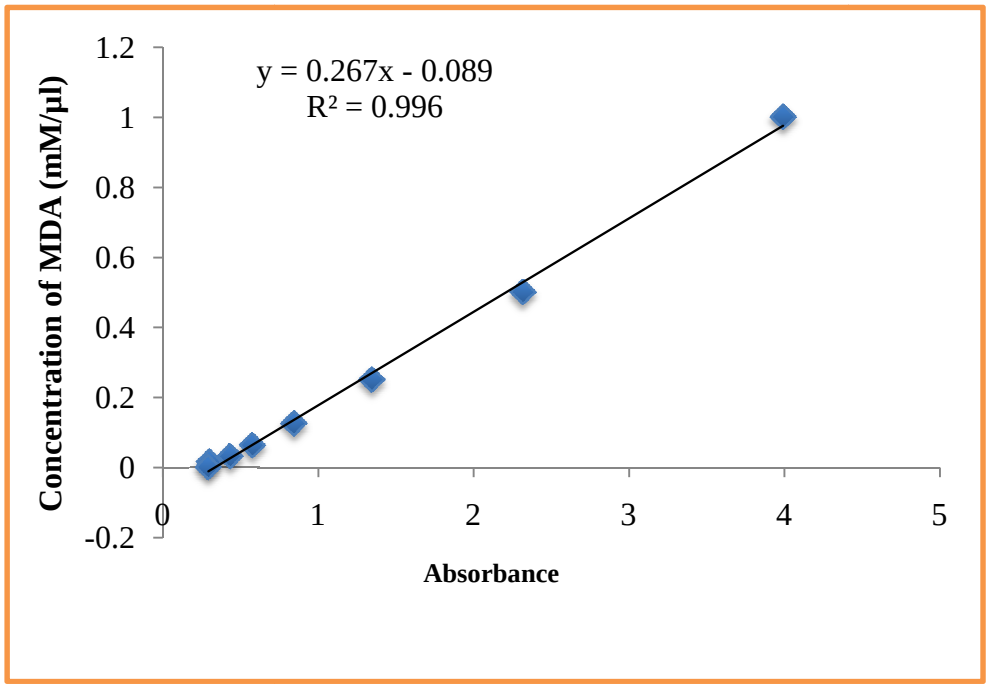
3.8.9 Lipid peroxidation/MDA (Malondialdehyde assay) estimation

Lipid peroxidation in serum was measured by reaction of thiobarbituric acid (TBA) with MDA according to Placer et al. (1966). Content of MDA was measured spectrophotometrically using a UV-VIS double beam spectrophotometer (Systronics-2203). The results were expressed as nmol MDA/ml plasma.

Protocol

1mL of test sample (100µL plasma + 900µL distilled water) was taken in a tube and then added 1mL of 10% w/v TCA (Trichloroacetic Acid) to test sample tube, mixed well and centrifuged at 2000 RPM for 10 min. One mL of supernatant was taken in separate tube and 1mL of 0.67% TBA (Thiobarbituric acid) was added to it. Then tube was heated in boiling water for 15 min. and then sample was allowed to cool and then 1mL distilled water was added. The absorbance of developed pink colour in sample was measured at 535nm. In blank sample 1mL Distilled water was used in place of test sample. A standard graph was plotted using the absorbance of different concentrations of MDA (1 to 0.015mM/µL).

Fig. 1: Best fit curve of standards for the estimation of MDA concentration



3.9 Carcass Quality Traits

At the time of slaughter, 2 male and 2 female birds from each group i.e. total 16 birds were taken after 8 weeks of age for studying various slaughter traits *viz*. pre-slaughter fasting shrinkage in live weight (%), bled weight (%), defeathered weight (%), dressed weight (%), yield after evisceration loss or ready to cook yeld (%), giblets yield (%), total ready-to-cook yield (%) and development of digestive organs. Further, percent yield of cut-of-parts (thighs, drumsticks, breast, back, neck and wing) on eviscerated carcass yield was determined.

The birds were starved for12 hours before slaughter. However, drinking water was provided *ad lib*. During the starvation period, their body weights were recorded after starvation. The birds were sacrificed by improved Kosher method, bled for 1.5 to 2 minutes and defeathered. The birds were dressed by cutting the head at atlanto-occipital joint, leg at hock joint and oil gland located at the base of the tail and weighed.

Evisceration was done by making a slit opening at the neck skin to remove oesophagus and trachea, vertical cut below the tip of breast bone to remove viscera. Heart, liver and gizzard were separated and cleaned. The internal lining of gizzard and pericardium of heart were removed before weighing them.

Further, the length and weight of different digestive organs (proventriculus, small intestine, large intestine and caecum) were measured separately at 8 weeks of age.

3.10 Chemical composition of feed, breast (*pectoralis major*) and thigh (*iliotibialis*) muscle of turkey poults after 8 weeks of age

Samples of feed, breast (*pectoralis major*) and thigh (*iliotibialis*) muscles were processed and analyzed for dry matter (DM), crude protein (CP), ether extract (EE), total ash, calcium and phosphorous (AOAC, 1990).

3.11 Evaluations of the status of macro and trace minerals in feed and meat

Evaluation of macro and trace minerals in feed and meat was done by using inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA) facility at Animal Nutrition Department, DUVASU Mathura. Values were expressed in ppb and ppm.

3.11.1 Digestion of meat sample

Meat sample was digested by microwave digestion method (Microwave digestion system: Multiwave 5000, Anton Paar, Virginia, USA) as per the procedure described by Rodushkin et al. (1999) used for ICP-OES technique. 0.5 gof feed/ meat sample was taken in perfluoroalkoxy alkane (PFA) digestion vessel Thereafter, 5ml nitric acid (additionally purified) and 0.5ml H2O2 were added. The vessel was closed and placed in the microwave oven and digested at 600 W for 1 hour. The vessel was removed from oven and cooled to room temperature. Then the digested sample was transferred to a volumetric flask and diluted with Milli-Q (MQ) water to make 50ml for ICP analysis.

3.11.2 Estimation of minerals in meat samples

The macro and trace minerals namely, calcium, phosphorus, sodium, magnesium, iron, zinc and copper were analyzed by ICP-OES. The wavelengths (nm ppm) used were 393.366for Calcium 213.618for phosphorus, 589.592 for sodium, 280.270 for magnesium, 238.204 for iron 213.857 for zinc and 327.395 for copper .The instrument conditions were 12 L/min plasma gas flow, 0.7 L/min nebulizer gas flow, 1 L/min Aux flow and the viewing mode was axial at 8 mm height for analysis of the minerals. All the samples were run in triplicate.

Standards for calcium and phosphorus used were 0.50, 1.0, 5, and 10 ppm and were prepared with ICP multi-element standard solution IV (Merck chemicals, Darmstadt, Germany). From these standards, calibration curve was prepared for Ca, P, Na, Mg, Fe, Zn and Cu by plotting the absorbance against the concentration. After plotting the calibration curve, the concentration of the minerals in the sample was calculated automatically by the system using ICP Expert software. Operating parameters for standards were similar as described for samples.

3.12 Estimation of fatty acid profile in meat sample

2 g of meat sample was taken in glass beaker in which 10 ml HCl and 5-10 ml liquid ammonia was taken. This sample was digested in water bath at 85^{o}C for 1.5 hours or till sample was completely digested and turned into brownish-black in colour. Then the sample was taken out from water bath and cooled to room temperature. The digested sample was transferred in separating funnel in which 5-10

ml ethanol and 50 ml petroleum ether were added. Samples were properly shaken to extract the fat followed by addition of water and again shaken to have two separated layers of water and petroleum ether with fat. This procedure was repeated 3-4 times for complete extraction of fat and this petroleum ether layer (pet ether) was separated and collected in a glass beaker. This pet ether was then passed through sodium sulphate to remove residual moisture using whatmann filter paper No.1 and finally evaporated in hot air oven at 75^{o}C to get pure fat. Now, minimum 50 mg fat from this pure fat was taken and dissolved in 2 ml hexane in which 0.1-1.0ml of 2N methanolic KOH was added. Then this sample was put in capped tubes and placed in vortex mixer for 1-2 minutes for proper mixing. This was followed by adding 2 ml hexane and placed in vortex mixer for 2 minutes. Finally, 1 ml of sample was taken from upper layer of tubes and evaluated for fatty acid profile in Gas chromatograph (Shimadzu GC-2014) using capillary column (SH-RTX 2560) of dimension 100 x 0.1 meter, where zero air gas, hydrogen and nitrogen gases were used for resolution of different fatty acids peaks present in the sample.

3.13 Mortality

The total mortality was recorded in the different groups during the experimental period.

3.14 Statistical analysis

The data pertaining to various parameters were analyzed statistically as per the standard procedure (Snedecor and Cochran, 1989) and difference between the treatment means were obtained by using Duncan multiple range test (Duncan, 1955).

Fig. 3.1: Mixing of oregano and thyme oil in turkey feed

Fig. 3.2: Weighing of Turkey Poults

Fig. 3.3: Injecting 1% GRBC into the wing vein of turkey poults

Fig. 3.4: Collection of blood into the wing vein of turkey poults

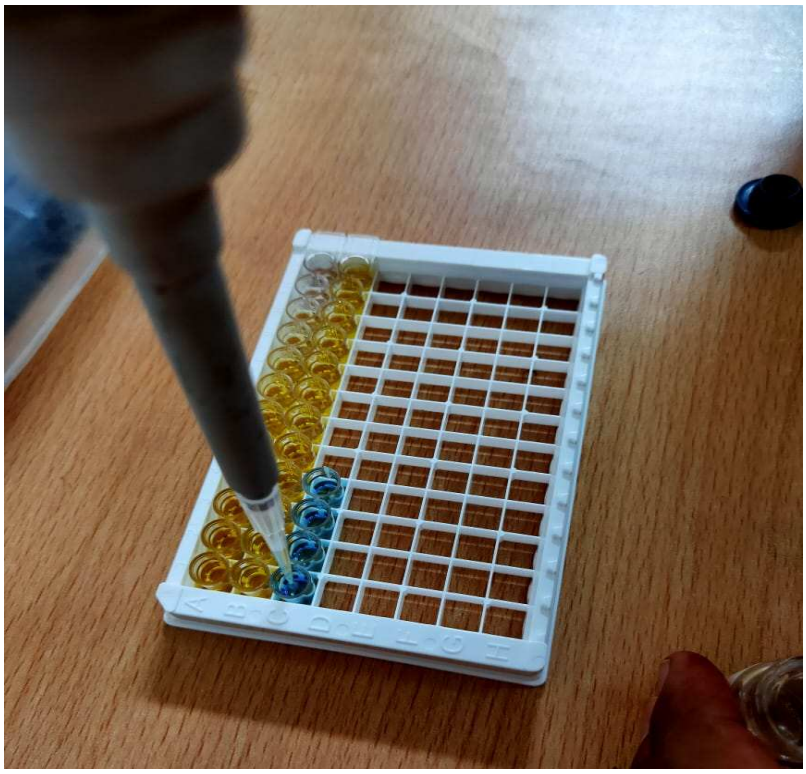


Fig. 3.5: Determination of concentration of serum cortisol, IgG and IgM antibodies through ELISA

Results

CHAPTER-4

RESULTS

The present study was conducted to assess the effect of dietary supplementation of oregano and thyme oil on performance of turkey poults. Day old turkey poults (n= 144) were distributed into four dietary treatment groups, having three replicates of 12 birds each. The study was conducted in turkey poults during 0-8 weeks of age. During the experiment, poults were fed basal ration, T1 (control), T2- basal ration supplemented with 1% oregano oil, T3- basal ration supplemented with 1g/kg thyme oil and T4- basal ration supplemented with 1% oregano oil and 1g/kg thyme oil.

The influence of various dietary treatment groups on growth, feed conversion ratio, development of digestive and lymphoid organs, immunocompetence traits, biochemical parameters, carcass quality traits and chemical composition of meat were studied.

4.1 Chemical composition of turkey starter feed

The chemical and mineral composition of turkey starter feed has been presented in Table 4.1(a), and (b). The chemical and mineral composition i.e. Moisture, DM, Total ash, Protein, EE, Ca, P, Cu, Zn, Na, Mg and Fe content were estimated as 9.5%, 90.52%, 8.01%, 24%, 2.53%, 2.01%, 1.32 %, 3.67 ppm, 91.61 ppm, 0.19%, 1724.55 ppm and 64.12 ppm respectively. Thus, the feed was adequate in all nutrients as per nutritional requirements of turkey (NRC, 1994).

4.2 Growth performance

4.2.1 Body Weight

The body weight of turkey poults from 0-8 weeks have been presented in Table 4.2 and graphically in Fig 4.1. The initial body weight (day old) in T1, T2, T3 and T4 groups were recorded as 47.33g, 47.11g, 47.44g and 47.22g respectively whereas, final body weight (8 week) were recorded as 911.76g, 869.28g, 933.56g and 851.82g respectively. Data on body weight showed that T1 (80.83g) and T3 (78.06g) poults had significantly higher ($P<0.05$) body weight than T4 (75.11g) treatment group at 1st week of age. At 2nd week of age, T1 (141.45g) and T3 (135.33) poults had

significantly higher (P<0.01) body weight than T2 and T4 (128.06g and 118.62g) treatment groups. At 3rd week of age, T1 (209.36g) and T3 (208.83g) poults had significantly higher (P<0.01) body weight than T2 and T4 (199.89g and 189.43g) treatment groups. At 4th week of age, T1 (294.92g) and T3 (299.03g) poults had significantly higher (P<0.05) body weight than T4 (276.56g) treatment groups. At 5th week of age, T1 (401.43g) and T3 (415.44g) poults had significantly higher (P<0.01) body weight than T4 (374.54g) treatment groups. At 6th week of age, T1 (556.97 g) and T3 (570.78g) poults had significantly higher (P<0.01) body weight than T2 and T4 (531.89g and 529.76g) treatment groups. At 7th week of age, T1 (747.40g) and T3 (758.61g) poults had significantly higher (P<0.01) body weight than T2 and T4 (694.97g and 676.66g) treatment groups. At 8th week of age, T1 (911.76g) and T3 (933.56g) poults had significantly higher (P<0.01) body weight than T2 and T4 (869.28g and 851.82g) treatment groups Further, weekly body weight was apparently higher in T1 poults as compared to poults of other treatment groups from 1st to 3rd weeks of age. Thereafter, the weekly body weight was apparently higher in T3 as compared to other treatment groups. This trend has also been presented graphically in Fig 4.1.

4.2.2 Body Weight Gain

The body weight gain of turkey poults from 0-8 weeks have been presented in Table 4.3 and graphically in Fig 4.2. The initial average weekly body weight gain at 1st week of T1, T2, T3 and T4 were recorded as 33.50g, 28.00 g, 30.61g and 25.39 g respectively, while final body weight gain at 8th week of age were found as,164.36g, 174.30 g, 174.94g and 175.16 g respectively. There was no significant difference in the average weekly body weight gain of birds among various treatment groups throughout the experimental period except at 1st, 2nd, 6th and 7th weeks of age. Weekly body weight gain was significantly higher (P<0.05) in T1 (33.50g) than T4 (25.39 g) at 1st week of age. At 2nd week of age, weekly body weight gain was significantly higher (P<0.01) in T1 (60.62g) and T3 (57.28g) than T4 (46.01g). At 6thweek of age, weekly body weight gain was significantly higher (P<0.01) in T1 (155.53g) and T3 (155.33g) than T2 (139.17g). At 7th week of age, weekly body weight gain was significantly higher (P<0.01) in T1 (190.43g) and T3 (187.83g) than T2 and T4 (163.09g and 146.89g).

4.2.3 Phase wise body weight gain

The body weight gain of turkey poults at different growth phases (0-4th week, 4-8th week and 0-8th week) have been tabulated in Table 4.4 and portrayed graphically in Fig 4.2. Body weight gain of T1, T2, T3 and T4 groups were recorded during 0-4th week as 247.59g, 239.94g, 251.58g and 229.33g, during 4–8th week as 616.84 g, 582.22g, 634.53 g and 575.26 g while 0-8th week as 864.43g, 822.17g, 886.11 g and 804.60g respectively. Phase wise body weight gain was significantly higher ($P<0.05$) in T1 (247.59 g) and T3 (251.58g) than T4 (229.33g) during 0 to 4 weeks. During 4 to 8 weeks, phase wise body weight gain was significantly higher ($P<0.05$) in T3 (634.53 g) than T2 and T4 (582.22 g and 575.26 g). In addition, phase wise body weight gain was significantly higher ($P<0.01$) in T1 (864.43 g) and T3 (886.11g) than T2 and T4 (822.17g and 804.60g) during 0 to 8 weeks. Further, phase wise body weight gain was apparently higher in T3 as compared to other treatment groups during 0-4 weeks, 4-8 weeks and 0-8 weeks of age. This trend has also been presented graphically in Fig 4.3.

4.2.4 Feed intake

The average weekly feed intake of turkey poults during 0-8 weeks have been tabulated in Table 4.5. Weekly average feed intake during 1st week of T1, T2, T3 and T4 were recorded as 66.67g, 66.81 g, 68.92g and 65.00g respectively whereas, weekly average feed intake of the treatment groups at 8th week were recorded as 509.53g, 528.76g, 537.22g and 439.01g respectively. The results indicated that there was no significant difference in the average weekly feed intake of birds among various treatment groups throughout the experimental period except at 1st, 2nd, and 8th week of age. Weekly feed intake was significantly higher ($P<0.05$) in the poults of T3 as compared to T4 treatment groups at 1st week of age (68.92g *vs*. 65.00g). Weekly feed intake was significantly higher ($P<0.01$) in poults of T1, T2 and T3 treatment groups than T4 at 2nd week of age (104.83g 101.31g and 99.28*vs*. 78.07g). Weekly feed intake was significantly higher ($P<0.05$) in poults of T1, T2 and T3 as compared to, T4 at 8th week of age (509.53, 528.76 g and 537.22g *vs*. 439.01g).

4.2.5 Phase wise feed intake

Phase wise feed intake of turkey poults during 0-4 weeks, 4-8 weeks and 0-8 weeks of growth phase have been tabulated in Table 4.6. Phase wise feed intake of

poults of T1, T2, T3 and T4 groups were recorded during 0-4 weeks of growth phase as 423.43g, 414.97g, 418.08g and 377.48g, during 4–8 weeks of growth phase as 1603.89g, 1529.26g, 1639.72g and 1450.77g while 0-8 weeks of growth phase as 2027.32g, 1944.23g, 2057.81g and 1828.25g respectively. Phase wise feed intake during 0-4 weeks was significantly higher ($P<0.01$) in T1, T2 and T3 than T4 (423.43g, 414.97g and 418.08g *vs.*, 377.48g). Further, during 0-8 weeks, phase wise feed intake was significantly higher ($P<0.05$) in T1 and T3 than T4 (2027.32 and 2057.81g *vs*., 1828.25g). However, there was no significance difference observed in feed intake of the poults among the different treatment groups during 4- 8 weeks of age.

4.2.6 Feed Conversion Ratio

Weekly feed conversion ratio (FCR) of turkey poults from 0-8 weeks have been presented in Table 4.7. The initial FCR (1st week) of the poults of T1, T2, T3 and T4 groups were recorded as 1.99, 2.39, 2.29 and 2.57 respectively. Final FCR at 8th week were recorded as3.11, 3.04, 3.07 and 2.51 respectively. Results indicated that there was no significant difference in FCR of birds during the entire experimental period except at 2nd, 3rd, 7th and 8th week. At 2nd week of age, FCR of poults of T1 (1.68), T3 (1.73) and T4 (1.70) were significantly better ($P<0.05$) than T2 (1.70) (1.98) and comparatively better hand. At 3rd week of age, FCR of poults of T2 (1.50), T3 (1.51) and T4 (1.51) were significantly better ($P<0.01$) than T1 (1.73). At 7th week of age, FCR of poults of T1 (2.65), T2 (2.73) andT3 (2.66) were significantly better ($P<0.05$) than T4 (3.13). At 8th week of age, FCR of T4 poults (2.51) was significantly better ($P<0.01$) than T1 (3.11), T2 (3.04) and T3 (3.07).

4.2.7 Phase-wise Feed Conversion Ratio

Feed Conversion Ratio (FCR) of turkey poults at different growth phases (0-4 weeks, 4-8 weeks and 0-8 weeks) have been tabulated in Table 4.8. FCR of T1, T2, T3 and T4 poults were recorded during 0-4 weeks of growth phase as 1.71, 1.73, 1.66 and 1.65, during 4–8 weeks of growth phase as 2.60, 2.62, 2.58 and 2.52 while during 0-8 weeks of growth phase as 2.35, 2.36, 2.32 and 2.27 respectively. Results indicated that there was no significant difference in FCR of birds among the different treatment groups during different phases of growth.

4.3 Immuno Competence Traits

4.3.1 Humoral Immune Response

The humoral immune response (response to 1% GRBC HA titre) of the poults of treatment groups at 8 weeks of age has been compiled in Table 4.9 and graphically in Fig 4.3. The total haemagglutination titre of T1, T2, T3 and T4 poults were observed as 7.80, 6.83, 6.33 and 6.67 while IgG and IgM titre values were recorded as 2.60, 2.33, 1.50, 1.83 and 5.20, 4.50, 4.83, 4.83 respectively. The statistical analysis revealed that there was no significant difference in total immunoglobulins, IgG and IgM values among the different treatment groups.

4.3.2 Cell-Mediated Immune Response

Cell mediated immune response (CMI) to PHA-P determined as Foot web index of the poults of T1, T2, T3 and T4 at 8 weeks of age has been tabulated in Table 4.9 and graphically portrayed in Fig 4.4. The foot web index of the poults of T1, T2, T3 and T4 were recorded as 0.36, 0.62, 0.70 and 0.70 respectively. CMI response indicated that T2, T3 and T4 poults (0.62, 0.70 and 0.70) had significantly higher ($P<0.01$) foot web index compared to T1 poults (0.36).

4.3.3 Cortisol

The concentration of serum cortisol of poults of different treatment groups measured at 8^{th} weeks of age has been presented in Table 4.10. The concentration of cortisol recorded in T1, T2, T3, T4 poults were 2.68 mg/ml, 2.97 mg/ml, 2.75mg/ml and 2.88 mg/ml respectively. Statistical analysis revealed that there was no significant difference in the serum cortisol values among different treatment groups.

4.3.4 IgG

The concentration of serum IgG measured at 8^{th} weeks of age has been presented in Table 4.10 and graphically portrayed in Fig 4.5. The concentration of IgG recorded in T1, T2, T3, T4 were 6.95mg/ml, 16.99mg/ml, 8.60mg/ml and 9.89mg/ml respectively. Statistical analysis revealed that T2 group poults had significantly higher ($P<0.05$) IgG value as compared to other treatment groups. Further, the serum IgG values of all the oil supplemented groups were apparently higher than the control group.

4.3.5 IgM

The concentration of serum IgM measured at 8th weeks of age have been shown in Table 4.10 and graphically portrayed in Fig 4.5. The values recorded in T1, T2, T3, T4 were 2.55mg/ml, 4.03mg/ml, 3.04mg/ml and 4.58mg/ml respectively. Statistical analysis revealed that there was no significant difference among treatment groups. However, the serum IgM values of all the oil supplemented groups were apparently higher than the control group.

4.4 Blood biochemical parameters

Effect of oregano and thyme oil on plasma protein, uric acid, ALT, AST, ALP, cholesterol, HDL (high density lipoprotein), SOD and LPO have been presented in Table 4.11.

4.4.1 Total Plasma Protein

Total plasma protein values (g/dl) of T1, T2, T3 and T4 poults were 4.42, 4.37, 4.31 and 4.97g/dl. Statistical analysis of data revealed that T4 poults (4.97g/dl) had significantly higher ($P<0.05$) total plasma protein value as compared to T1 (4.42), T2 (4.37) and T3 (4.31).

4.4.2 Total Plasma Uric Acid

The recorded values of plasma uric acid in T1, T2, T3 and T4 poults were 4.36mg/dl, 4.21 mg/dl, 5.19 mg/dl and 4.33 mg/dl respectively. There were no significant difference observed in the uric acid values among the poults of different treatment groups.

4.4.3 Plasma ALT (Alanine aminotransferase)

The recorded ALT values (IU/L) of T1, T2, T3 and T4 groups were 13.79, 16.62, 16.97 and 10.61 IU/L respectively. Statistical analysis of the data revealed that T2 and T3 poults had significantly higher ($P<0.05$) ALT values as compared to T4 poults and comparatively higher ALT values as compared to T1.

4.4.4 Plasma AST (Aspartate amino transferase)

The recorded AST values (IU/L) of T1, T2, T3 and T4 poults were 117.13, 141.44, 140.73 and 137.90 IU/L respectively. Results revealed that there were no significant difference in AST values of the poults among the treatment groups.

4.4.5 Plasma ALP (Plasma Alkaline Phosphatase)

The ALP values (IU/L) of T1, T2, T3 and T4 poults were recorded as 781.05, 625.61, 800.04 and 899.30 IU/L respectively. Results indicated that there were no significant difference in the total plasma ALP values of the poults among the treatment groups.

4.4.6 Total Plasma Cholesterol

Total cholesterol values (mg/dl) of T1, T2, T3 and T4 treatment groups were 157.82, 209.33, 252.14 and 244.77 mg/dl respectively. Statistical analysis of the data revealed that T3 (252.14) and T4 (244.77) poults had significantly higher ($P<0.05$) plasma cholesterol values as compared to T1 poults (252.14) and apparently higher values as compared to T2 (209.33) poults.

4.4.7 HDL (High Density Lipoprotein)

The recorded HDL (mg/dl) values in poults of T1, T2, T3 and T4 were 108.72, 131.24, 128.67 and 108.32mg/dl respectively. Statistical analysis of the data revealed that T2 (131.24) and T3 (128.67) poults had significantly higher ($P<0.01$) HDL values as compared to T1 (108.72) and T4 (108.32) poults.

4.4.8 SOD (Super oxide dismutase)

The tabulated SOD (units/ml) values of T1, T2, T3 and T4 poults were 59.87, 55.39, 63.52 and 66.67 units/ml respectively. Results indicated that there were no significant difference in plasma SOD values among the poults of different treatment groups.

4.4.10 LPO (Lipid per-oxidation)

The calculated LPO values (nM/ml) of T1, T2, T3, and T4 poults were 0.10, 0.13, 0.13and 0.10nM/ml respectively. There were no significant difference in the LPO values of the poults among the treatment groups.

4.5 Development of digestive organs

The effect of dietary supplementation of oregano and thyme oil on the development of the digestive organs of turkey poults at 8 weeks of age has been presented in Table 4.12.

4.5.1 Proventriculus

The weight of proventriculus (g/100g) of T1, T2, T3 and T4 were 0.67, 0.55, 0.48 and 0.54 % respectively. There were no significant difference in proventriculus (%) of the poults among the different treatment groups.

4.5.2 Small intestine (SI) weight

The SI weight (g/100g) of T1, T2, T3 and T4 were 3.44, 2.92, 3.16 and 3.39 g/100g respectively. Results indicated that there were no significant difference in SI weight of the poults among the different treatment groups.

4.5.3 Large intestine (LI) weight

The LI weight (g/100g) of T1, T2, T3 and T4 were 0.32, 0.34, 0.26, and 0.30g/100g respectively. There were no significance difference in LI weight of the poults among the treatment groups.

4.5.4 Cecal weight

The cecal weight (g/100g) of T1, T2, T3 and T4 were 0.82, 0.64, 0.70 and 0.90 g/100g respectively. No significant difference were recorded in cecal weight of the poults among the different treatment groups.

4.5.5 SI length

The SI length (cm/100g) of T1, T2, T3 and T4 were13.00, 11.99, 12.38 and 13.02 cm/100g respectively. There were no significant difference in SI length of the poults among the treatment groups.

4.5.6 LI length

The LI length (cm/100g) of T1, T2, T3 and T4 were 0.87, 0.83, 0.79 and 0.81cm/100g respectively. Results revealed that there were no significant difference in LI length of the poults among the treatment groups.

4.5.7 Cecal length

The cecal length (cm/100g) of T1, T2, T3 and T4 were1.98, 1.77, 1.80 and 1.86cm/100g respectively. Results indicated that there were no significant difference in cecal length of the poults among the treatment groups.

4.6 Development of lymphoid organs

The effect of dietary supplementation of oregano and thyme oil on the development of the lymphoid organs (spleen, thymus and bursa) at 8 weeks of age has been presented in Table 4.13.

4.6.1 Spleen

The weight of spleen (%) T1, T2, T3 and T4 were 0.08, 0.10, 0.08 and 0.10 % respectively. Result indicated that there were no significant difference in % value of spleen among the treatment groups.

4.6.2 Thymus

The weight of thymus (%) of T1, T2, T3 and T4 were 0.09, 0.07, 0.14 and 0.07 % respectively. There were no significant difference in % value of thymus among the treatment groups.

4.6.3 Bursa

The weight of bursa (%) of T1, T2, T3 and T4 were 0.21, 0.23, 0.18 and 0.19 % respectively. No significant difference were recorded in % value of bursa among the treatment groups.

4.7 Carcass quality traits and yield of giblet

The effect of dietary supplementation of oregano and thyme oil on carcass quality traits and yield of giblet (heart, liver and gizzard) at 8 weeks of age (% live weight) has been presented in Table 4.14 and Table 4.15 respectively.

4.7.1 Shrinkage

The shrinkage (%) of T1, T2, T3 and T4 were 9.43, 7.91, 9.99 and 8.24 % respectively. No significant differences were recorded in value of shrinkage % of poults among the different treatment groups.

4.7.1 Bled weight

The bleding weight (%) of T1, T2, T3 and T4 were 96.27, 97.21, 97.55 and 95.59 % respectively. No significant differences were recorded in values of bled weight % of poults among the different treatment groups.

4.7.1 Defeathered weight

The defeathered weight (%) of T1, T2, T3 and T4 were 82.07, 82.40, 81.78 and 81.34 % respectively. No significant differences were recorded in values of defeathered weight % of poults among the treatment groups.

4.7.2 Dressing %

The values of dressing (%) of T1, T2, T3 and T4 were 72.59, 73.38, 72.80 and 72.54 % respectively. There were no significant differences in % values of ready to cook yield of poults among the treatment groups.

4.7.3 Ready to cook yield

Ready to cook yield (%) of T1, T2, T3 and T4 were 54.05, 59.47, 57.94 and 55.90 % respectively. Statistical analysis revealed that T2 (59.47%) had significantly higher ($P<0.05$) ready to cook yield (%) as compared to T1 (54.05%) and apparently higher than T3 (57.94%) and T4 (55.90%).

4.7.4 Heart

Heart (%) of T1, T2, T3 and T4 were 0.42, 0.46, 0.43 and 0.45 % respectively. Results indicated that there were no significant differences in heart % of poults among the different treatment groups.

4.7.5 Liver

Liver (%) of T1, T2, T3 and T4 poults were 1.64, 1.76, 1.71 and 1.99 % respectively. No significant differences were recorded in liver % among the different treatment groups.

4.7.6 Gizzard

Gizzard (%) of T1, T2, T3 and T4 were 4.76, 3.42, 3.32 and 4.02 % respectively. No significant differences were recorded in gizzard % of the poults among the treatment groups.

4.8 Yield of cut-up-parts

Results pertaining to yield of cut-up-parts of the carcass at 8 weeks of age have been expressed as percent yield of ready to cook yield in Table 4.16.

4.8.1 Breast

Breast (%) of T1, T2, T3 and T4 poults were 28.17, 28.52, 28.52 and 28.12 % respectively. No significant differences were recorded in breast % of the poults among the different treatment groups.

4.8.2 Back

The back (%) of T1, T2, T3 and T4 poults were 21.03, 20.38, 21.66 and 20.63 % respectively. There were no significant differences in back % of the poults among the different treatment groups.

4.8.3 Wings

The wings (%) of T1, T2, T3 and T4 poults were13.93, 14.49, 13.48 and 14.07 % respectively. There were no significant differences in wing % of the poults among the different treatment groups.

4.8.4 Neck

The neck (%) of T1, T2, T3 and T4 poults were 5.38, 4.81, 4.52 and 4.91 % respectively. Results indicated that there were no significant differences in neck % of the poults among the different treatment groups.

4.8.5 Drumstick

The drumstick (%) of T1, T2, T3 and T4 poults were 16.24, 15.37, 16.06 and 16.29 % respectively. No significant differences were recorded in drumstick % of poults among the different treatment groups.

4.8.6 Thighs

The thighs (%) of T1, T2, T3 and T4 poults were 15.26, 16.44, 15.77 and 15.98 % respectively. Results indicated that there were no significant differences in thigh % of the poults among the different treatment groups.

4.9 Chemical and mineral composition of breast (*pectoralis major*) muscle

Results pertaining to yield of chemical and mineral composition of breast (*pectoralis major*) muscle of the carcass at 8 weeks of age have been presented in Table 4.17(a) and Table 4.17(b).

4.9.1 Moisture

The moisture (%) of breast muscle of T1, T2, T3 and T4 poults were 72.20, 72.30, 71.60 and 72.40 % respectively. No significant differences were recorded in moisture % among the treatment groups.

4.9.2 Dry matter (DM)

The dry matter (%) of breast muscle of T1, T2, T3 and T4 poults were 27.80, 27.70, 28.40 were and 27.60 % respectively. There were no significant differences in dry matter % among the treatment groups.

4.9.3 Crude protein (CP)

The crude protein (%) of breast muscle of T1, T2, T3 and T4 poults were 24.75, 24.63, 24.13 and 25.13 % respectively. There were no significant differences in crude protein % among the treatment groups.

4.9.4 Ether extract (EE)

The EE (%) of T1, T2, T3 and T4 were 1.05, 1.00, 0.91 and 0.96% respectively. Results indicated that there were no significant differences in EE % among the various treatment groups.

4.9.5 Total ash

The total ash (%) of T1, T2, T3 and T4 were 1.00, 1.15, 1.05 and 1.04 % respectively. There were no significant differences in total ash % among the various treatment groups.

4.9.6 Calcium

Ca (mg/100g) of T1, T2, T3 and T4 were 2.59, 2.49, 3.31 and 2.62 mg/100g respectively. Statistical analysis of data revealed that there were no significant differences in Ca (mg/100g) among different treatment groups.

4.9.7 Phosphorus

P (mg/100g) of T1, T2, T3 and T4 were 362.40, 367.60, 219.78 and 242.12 mg/100g respectively. Results revealed that there were no significant differences in P (mg/100g) among different treatment groups.

4.9.8 Iron

Fe (ppm) content of breast muscle of T1, T2, T3 and T4 poults were 33.81, 19.95, 21.48 and 17.79ppm respectively. Results revealed that there were no significant differences in Fe (ppm) content among different treatment groups.

4.9.9 Copper

Cu (mg/100g) content of breast muscle of T1, T2, T3 and T4 poults were 1.75, 1.42, 3.16 and 2.87mg/100g respectively. Results revealed that there were no significant differences in Cu (mg/100g) content among the different treatment groups.

4.9.10 Sodium

Na (mg/100g) contents of breast muscle of T1, T2, T3 and T4 were 1000.83, 1011.88, 885.56 and 790.33mg /100g respectively. Results revealed that there were no significant differences in Na (mg/100g) among the different treatment groups.

4.9.11 Zinc

Zn (ppm) content of breast muscle of T1, T2, T3 and T4 were 3.40, 3.10, 3.89 and 5.76ppm respectively. Results revealed that there were no significant differences in Zn (ppm) among the different treatment groups.

4.9.12 Magnesium

Mg (mg/100g) content of breast muscle of T1, T2, T3 and T4 were 31.47, 38.23, 39.79 and 36.66mg/100g respectively. Statistical analysis of data revealed that T2 (38.23mg/100g) T3 (39.79mg/100g) poults had significantly higher ($P<0.05$) deposition of Mg in breast muscle as compared to T1 (31.47mg/ 100g). Further, all the oil supplemented groups had apparently higher deposition of Mg in breast muscle as compared to the control group at 8 weeks of age.

4.10 Chemical and mineral composition of thigh (*iliotibialis*) muscle

Results pertaining to yield of chemical and mineral composition of thigh (*iliotibialis*) muscle of the carcass at 8 weeks of age have been presented in Table 4.18 (a) and Table 4.18 (b).

4.10.1 Moisture

The moisture (%) of thigh muscle of T1, T2, T3 and T4 poults were 74.15, 73.40, 72.40 and 72.70 % respectively. No significant differences were recorded in moisture % of the thigh muscle among the different treatments groups.

4.10.2 Dry matter (DM)

The dry matter (%) of thigh muscle of T1, T2, T3 and T4 poults were 25.85, 26.60, 27.60 and 27.30 % respectively. There were no significant differences in dry matter % of the thigh muscle among the different treatment groups.

4.10.3 Crude protein (CP)

The crude protein (%) of thigh muscle of T1, T2, T3 and T4 poults were 22.75, 22.50, 22.88, and 23 % respectively. There were no significant differences in crude protein % among the treatment groups.

4.10.4 Ether extract (EE)

The EE (%) of thigh muscle of T1, T2, T3 and T4 poults were 1.33, 1.35,1.30 and 1.47% respectively. Results indicated that there were no significant differences in EE % among the different treatment groups.

4.10.5 Total ash

The total ash (%) of thigh muscle of T1, T2, T3 and T4 poults were 1.41, 1.52, 1.44 and 1.28 % respectively. There were no significant differences in total ash % among the different treatment groups.

4.10.6 Calcium

Ca (mg/100g) of thigh muscle of T1, T2, T3 and T4 poults were 2.22, 2.54, 2.63 and 2.33 mg/100g respectively. However statistical analysis of data revealed that there were no significant differences in Ca (mg/100g) content among different treatment groups.

4.10.7 Phosphorus

P (mg/100g) of thigh muscle of T1, T2, T3 and T4 poults were 326.92, 278.09, 288.50 and 195.58 mg/100g respectively. Results revealed that there were no significant differences in P (mg/100g) content among different treatment groups.

4.10.8 Iron

Fe (ppm) of thigh muscle of T1, T2, T3 and T4 were 29.06, 22.85, 31.03 and 27.52ppm respectively. Results revealed that there were no significant differences in Fe (ppm) content among the different treatment groups.

4.10.8 Copper

Cu (mg/100g) of thigh muscle of T1, T2, T3 and T4 were 1.49, 2.64, 2.04 and 2.55mg/100g respectively. Results revealed that there were no significant differences in Cu (mg/100g) contents among different treatment groups.

4.10.8 Sodium

Na (mg/100g) of thigh muscle of T1, T2, T3 and T4 poults were 1060.99, 748.57, 1335.47 and 807.24mg/100g respectively. Results revealed that there were no significant differences in Na (mg/100g) content among different treatment groups.

4.10.8 Zinc

Zn (ppm) of thigh muscle of T1, T2, T3 and T4 poultswere4.35, 4.12, 5.28and 4.60ppm respectively. Results revealed that there were no significant differences in Zn (ppm) among the different treatment groups.

4.10.8Magnesium

Mg (mg/100g) of thigh muscle of T1, T2, T3 and T4 poults were 32.46, 33.61, 38.89and 37.20mg/100g respectively. Results revealed that there were no significant differences in Mg (mg/100g) content among different treatment groups.

4.11 Fatty acid profile of turkey meat (breast and thigh meat)

The fatty acid profile of turkey meat has been presented in Table 4.19. The fatty acid profile i.e. saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid and unidentified peaks were recorded.

4.11.1 Saturated fatty acid

Saturated fatty acid (%) of turkey meat of T1, T2, T3 and T4 poults were 11.62, 11.19, 9.94 and 9.42% respectively. Results revealed that there were no significant differences in saturated fatty acid contents among different treatment groups. However, the saturated fatty acid content of the turkey meat were

comparatively less in the meat of turkey poults supplemented with oregano and thyme oil than the poults of the control group.

4.11.2 Monounsaturated fatty acid

Monounsaturated fatty acid (%) of turkey meat of T1, T2, T3 and T4 poults were 43.62, 33.95, 32.86 and 33.48% respectively. Results revealed that mono-unsaturated fatty acid were significantly higher ($P<0.01$) in T1 as compared to other treatment groups.

4.11.3 Polyunsaturated fatty acid

Polyunsaturated fatty acid (%) of turkey meat of T1, T2, T3 and T4 poults were 42.23, 53.87, 57.23 and 56.07% respectively. Results revealed that poly unsaturated fatty acid were significantly higher ($P<0.01$) in all the oil fed groups as compared to the control group.

4.12 Mortality

The total mortality in the different groups during the experimental period was between 0-4 percent considered as normal house mortality in turkeys.

Table 4.1(a): Chemical composition of Turkey starter feed

Category	Moisture %	DM %	Total Ash %	CP%	EE%
Turkey starter feed	9.5	90.52	8.01	24	2.53

Table 4.1(b): Mineral composition of Turkey starter feed

Category	Ca%	P%	Cu (ppm)	Fe (ppm)	Na%	Zn (ppm)	Mg(ppm)
Turkey starter feed	2.01	1.32	3.67	64.12	0.19	91.61	1724.55

Table 4.2: Effect of dietary supplementation of oregano and thyme oil on average weekly body weight (g) of turkey poults during 0-8 weeks of age

Treatment	0 day	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T1	47.33	80.83^{c}	141.45^{c}	209.36^{c}	294.92^{b}	401.43^{bc}	556.97^{b}	747.40^{b}	911.76^{b}
T2	47.11	75.11^{ab}	128.06^{b}	199.89^{b}	287.06^{ab}	392.72^{ab}	531.89^{a}	694.97^{a}	869.28^{a}
T3	47.44	78.06^{bc}	135.33^{c}	208.83^{c}	299.03^{b}	415.44^{c}	570.78^{b}	758.61^{b}	933.56^{b}
T4	47.22	72.61^{a}	118.62^{a}	189.43^{a}	276.56^{a}	374.54^{a}	529.76^{a}	676.66^{a}	851.82^{a}
Pooled SEM	0.27	1.14	2.71	2.67	3.07	5.24	6.01	11.71	11.13
Sig. Level	NS	P<0.05	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01

Means bearing different superscripts within a column differ significantly (P<0.05)

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.3: Effect of dietary supplementation of oregano and thyme oil on average weekly body weight gain (g) of turkey poults during 0-8 weeks of age

Treatment	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T1	33.50[b]	60.62[c]	67.91	85.56	106.51	155.53[b]	190.43[b]	164.36
T2	28.00[ab]	52.94[b]	71.83	87.17	105.67	139.17[a]	163.09[a]	174.30
T3	30.61[ab]	57.28[bc]	73.50	90.19	116.42	155.33[b]	187.83[b]	174.94
T4	25.39[a]	46.01[a]	70.81	87.13	97.99	155.22[b]	146.89[a]	175.16
Pooled SEM	1.14	1.81	0.98	1.50	2.73	2.40	6.28	3.87
Sig Level	P<0.05	P<0.01	NS	NS	NS	P<0.01	P<0.01	NS

Means bearing different superscripts within a column differ significantly (P<0.05)

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.4: Effect of dietary supplementation of oregano and thyme oil on average body weight gain (g) of turkey poults at different phases of growth during 0-8 weeks of age

Treatment	0-4 weeks	4-8 weeks	0-8 weeks
T1	247.59^{b}	616.84^{bc}	864.43^{b}
T2	239.94^{ab}	582.22^{ab}	822.17^{a}
T3	251.58^{b}	634.53^{c}	886.11^{b}
T4	229.33^{a}	575.26^{a}	804.60^{a}
Pooled SEM	3.1	8.76	11.13
Sig Level	P<0.05	P<0.05	P<0.01

Means bearing different superscripts within a column differ significantly (P<0.05)

Table 4.5: Effect of dietary supplementation of oregano and thyme oil on average weekly feed intake (g) of turkey poults during 0-8 weeks of age

Treatment	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T1	66.67^{ab}	101.31^{b}	117.60	137.85	212.67	375.94	505.75	509.53^{b}
T2	66.81^{ab}	104.83^{b}	108.06	135.28	205.28	350.11	445.11	528.76^{b}
T3	68.92^{b}	99.28^{b}	110.72	139.17	224.39	381.11	497.00	537.22^{b}
T4	65.00^{a}	78.07^{a}	107.05	127.36	201.59	351.14	459.03	439.01^{a}
Pooled SEM	0.51	3.38	1.82	2.65	4.31	10.71	12.51	14.34
Sig Level	P<0.05	P<0.01	NS	NS	NS	NS	NS	P<0.05

Means bearing different superscripts within a column differ significantly (P<0.05)

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.6: Effect of dietary supplementation of oregano and thyme oil on average feed intake (g) at different phases of growth of turkey poults during 0-8 weeks of age

Treatment	0-4 weeks	4-8 weeks	0-8 weeks
T1	423.43[b]	1603.89	2027.32[b]
T2	414.97[b]	1529.26	1944.23[ab]
T3	418.08[b]	1639.72	2057.81[b]
T4	377.48[a]	1450.77	1828.25[a]
Pooled SEM	6.09	30.06	34.38
Sig. level	P<0.01	NS	P<0.05

Means bearing different superscripts within a column differ significantly (P<0.05)
NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.7: Effect of dietary supplementation of oregano and thyme oil on weekly feed conversion ratio of turkey poults during 0-8 weeks of age

Treatment	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T1	1.99	1.68^{a}	1.73^{b}	1.61	2.00	2.42	2.65^{a}	3.11^{b}
T2	2.39	1.98^{b}	1.50^{a}	1.56	1.94	2.52	2.73^{a}	3.04^{b}
T3	2.29	1.73^{a}	1.51^{a}	1.55	1.93	2.46	2.66^{a}	3.07^{b}
T4	2.57	1.70^{a}	1.51^{a}	1.46	2.06	2.27	3.13^{b}	2.51^{a}
Pooled SEM	0.08	0.05	0.03	0.04	0.03	0.08	0.08	0 .08
Sig. Level	NS	P<0.05	P<0.01	NS	NS	NS	P<0.05	P<0.01

Means bearing different superscripts within a column differ significantly (P<0.05)
NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.8: Effect of dietary supplementation of oregano and thyme oil on feed conversion ratio at different phases of growth of turkey poults during 0-8 weeks of age

Treatment	0-4 weeks	4-8 weeks	0-8 weeks
T1	1.71	2.60	2.35
T2	1.73	2.62	2.36
T3	1.66	2.58	2.32
T4	1.65	2.52	2.27
Pooled SEM	0.02	0.03	0.02
Sig. level	NS	NS	NS

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.9: Effect of dietary supplementation of oregano and thyme oil on humoral response [antibody titre (log2) values] to 1% GRBC and cell mediated immune response to (PHA-P) in turkey poults after 8 weeks of age

Treatment	HA	IgG	IgM	FWI
T1	7.80	2.60	5.20	0.36^{a}
T2	6.83	2.33	4.50	0.62^{b}
T3	6.33	1.50	4.83	0.70^{b}
T4	6.67	1.83	4.83	0.70^{b}
Pooled SEM	0.22	0.16	0.14	0.04
Sig. level	NS	NS	NS	P<0.01

Means bearing different superscripts within a column differ significantly (P<0.05)
NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.10: Effect of dietary supplementation of oregano and thyme oil on concentration of serum cortisol, IgG and IgM antibodies of turkey poults after 8 weeks of age

Treatment	Cortisol (mg/ml)	IgG (mg/ml)	IgM (mg/ml)
T1	2.68	6.95[a]	2.55
T2	2.97	16.99[b]	4.03
T3	2.75	8.60[a]	3.04
T4	2.88	9.89[a]	4.58
Pooled SEM	0.09	1.27	0.41
Sig. level	NS	P<0.05	NS

Means bearing different superscripts within a column differ significantly (P<0.05)
NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.11: Effect of dietary supplementation of oregano and thyme oil on blood biochemical parameters of turkey poults after 8 weeks of age

Treatment	Protein (g/dl)	Uric acid (mg/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	SOD (units/ml)	LPO (nM/ml)
T1	4.42^{a}	4.36	13.79^{ab}	117.13	781.05	157.82^{a}	108.72^{a}	59.87	0.10
T2	4.37^{a}	4.21	16.62^{b}	141.44	625.61	209.33^{ab}	131.24^{b}	55.39	0.13
T3	4.31^{a}	5.19	16.97^{b}	140.73	800.04	252.14^{b}	128.67^{b}	63.52	0.13
T4	4.97^{b}	4.33	10.61^{a}	137.90	899.30	244.77^{b}	108.32^{a}	66.67	0.10
Pooled SEM	0.09	0.16	0.89	4.55	51.48	12.51	3.23	2.73	0.01
Sig. Level	P<0.05	NS	P<0.05	NS	NS	P<0.05	P<0.01	NS	NS

Means bearing different superscripts within a column differ significantly (P<0.05)
NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.12: Effect of dietary supplementation oforegano and thyme oil on development of digestive organs of turkey poults after 8 weeks of age

Treatment	Proventriculus (g/100g)	SI weight (g/100g)	LI weight (g/100g)	Cecal Wt. (g/100g)	SI length (cm/100g)	LI length (cm/100g)	Cecal length (cm/100g)
T1	0.67	3.44	0.32	0.82	13.00	0.87	1.98
T2	0.55	2.92	0.34	0.64	11.99	0.83	1.77
T3	0.48	3.16	0.26	0.70	12.38	0.79	1.80
T4	0.54	3.39	0.30	0.90	13.02	0.81	1.86
Pooled SEM	0.03	0.11	0.03	0.05	0.24	0.05	0.07
Sig. Level	NS	NS	NS	NS	NS	NS	NS

NS: Not Significant (P>0.05) SEM: Standard Error of Mean

Table 4.13: Effect of dietary supplementation of oregano and thyme oil on development of lymphoid organs of turkey poults after 8 weeks of age

Treatment	Spleen %	Thymus %	Bursa %
T1	0.08	0.09	0.21
T2	0.10	0.07	0.23
T3	0.08	0.14	0.18
T4	0.10	0.07	0.19
Pooled SEM	0.01	0.01	0.01
Sig. Level	NS	NS	NS

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.14: Effect of dietary supplementation of oregano and thyme oil on carcass quality traits of turkey poults after 8 weeks of age (% live weight)

Treatment	Shrinkage %	Bled weight %	Defeathered weight %	Dressing %	Ready to cook yield%
T1	9.43	96.27	82.07	72.59	54.05^{a}
T2	7.91	97.21	82.40	73.38	59.47^{b}
T3	9.99	97.55	81.78	72.80	57.94^{ab}
T4	8.24	95.59	81.34	72.54	55.90^{ab}
Pooled SEM	0.45	0.30	0.27	0.24	0.77
Sig. level	NS	NS	NS	NS	P<0.05

Means bearing different superscripts within a column differ significantly (P<0.05)
NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.15: Effect of dietary supplementation of oregano and thyme oil on yield of giblet (heart, liver, gizzard) of turkey poults after 8 weeks of age (% live weight)

Treatment	Heart %	Liver %	Gizzard %
T1	0.42	1.64	4.76
T2	0.46	1.76	3.42
T3	0.43	1.71	3.32
T4	0.45	1.99	4.02
Pooled SEM	0.01	0.05	0.22
Sig. Level	NS	NS	NS

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.16: Effect of dietary supplementation of oregano and thyme oil on cut up parts of turkey poults after 8 weeks of age (% ready to cook yield)

Treatment	Breast %	Back %	Wings %	Neck %	Drumstick %	Thigh %
T1	28.17	21.03	13.93	5.38	16.24	15.26
T2	28.52	20.38	14.49	4.81	15.37	16.44
T3	28.52	21.66	13.48	4.52	16.06	15.77
T4	28.12	20.63	14.07	4.91	16.29	15.98
Pooled SEM	0.37	0.27	0.25	0.16	0.18	0.28
Sig. level	NS	NS	NS	NS	NS	NS

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.17(a): Effect of dietary supplementation of oregano and thyme oil on chemical composition of breast muscle (*pectoralis major*) of turkey poults after 8 weeks of age

Treatment	Moisture%	DM%	CP%	EE %	Total Ash %
T1	72.20	27.80	24.75	1.05	1.00
T2	72.30	27.70	24.63	1.00	1.15
T3	71.60	28.40	24.13	0.91	1.05
T4	72.40	27.60	25.13	0.96	1.04
Pooled SEM	0.32	0.32	0.22	0.02	0.03
Sig. Level	NS	NS	NS	NS	NS

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.17(b): Effect of dietary supplementation of oregano and thyme oil on mineral composition of breast muscle (*pectoralis major*) of turkey poults after 8 weeks of age

Treatment	Ca (mg/100g)	P (mg/100g)	Cu(mg/100g)	Fe(ppm)	Na(mg/100g)	Zn (ppm)	Mg(mg/100g)
T1	2.59	362.40	1.75	33.81	1000.83	3.40	31.47^{a}
T2	2.49	367.60	1.42	19.95	1011.88	3.10	38.23^{b}
T3	3.31	219.78	3.16	21.48	885.56	3.89	39.79^{b}
T4	2.62	242.12	2.87	17.79	790.33	5.76	36.66^{ab}
Pooled SEM	0.32	28.66	0.38	3.21	98.05	0.48	1.16
Sig. Level	NS	NS	NS	NS	NS	NS	P<0.05

Means bearing different superscripts within a column differ significantly (P<0.05)

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.18 (a): Effect of dietary supplementation of oregano and thyme oil on chemical composition of thigh muscle (*iliotibialis*) of turkey poults after 8 weeks of age

Treatment	Moisture %	DM %	CP %	EE %	Total Ash %
T1	74.15	25.85	22.75	1.33	1.41
T2	73.40	26.60	22.50	1.35	1.52
T3	72.40	27.60	22.88	1.30	1.44
T4	72.70	27.30	23	1.47	1.28
Pooled SEM	0.32	0.32	0.14	0.04	0.05
Sig. Level	NS	NS	NS	NS	NS

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.18 (b): Effect of dietary supplementation of oregano and thyme oil on mineral composition of Thigh muscle (*iliotibialis*) of turkey poults after 8 weeks of age

Treatment	Ca (mg/100g)	P (mg/100g)	Cu(mg/100g)	Fe (ppm)	Na(mg/100g)	Zn(ppm)	Mg(mg/100g)
T1	2.22	326.92	1.49	29.06	1060.99	4.35	32.46
T2	2.54	278.09	2.64	22.85	748.57	4.12	33.61
T3	2.63	288.50	2.04	31.03	1335.47	5.28	38.89
T4	2.33	195.58	2.55	27.52	807.24	4.60	37.20
Pooled SEM	0.07	33.27	0.34	1.93	155.22	0.58	1.70
Sig. Level	NS	NS	NS	NS	NS	NS	NS

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Table 4.19: Effect of dietary supplementation of oregano and thyme oil on fatty acid profile of meat of turkey poults after 8 weeks of age

Treatment	Saturated fatty acid (%)	Monounsaturated fatty acid (%)	Polyunsaturated fatty acid (%)	Unidentified peaks (%)
T1	11.62	43.62^{b}	42.23^{a}	2.53^{c}
T2	11.19	33.95^{a}	53.87^{b}	1.03^{b}
T3	9.94	32.86^{a}	57.23^{b}	0.01^{a}
T4	9.42	33.48^{a}	56.07^{b}	1.03^{b}
Pooled SEM	0.35	1.44	1.92	0.27
Sig. Level	NS	P<0.01	P<0.01	P<0.01

Means bearing different superscripts within a column differ significantly (P<0.05)

NS: Not Significant (P>0.05) SEM: Standard Error of Means

Fig. 4.1: Effect of oregano and thyme oil supplementation on the average weekly body weight (g) of turkey poults during 0-8 weeks of age

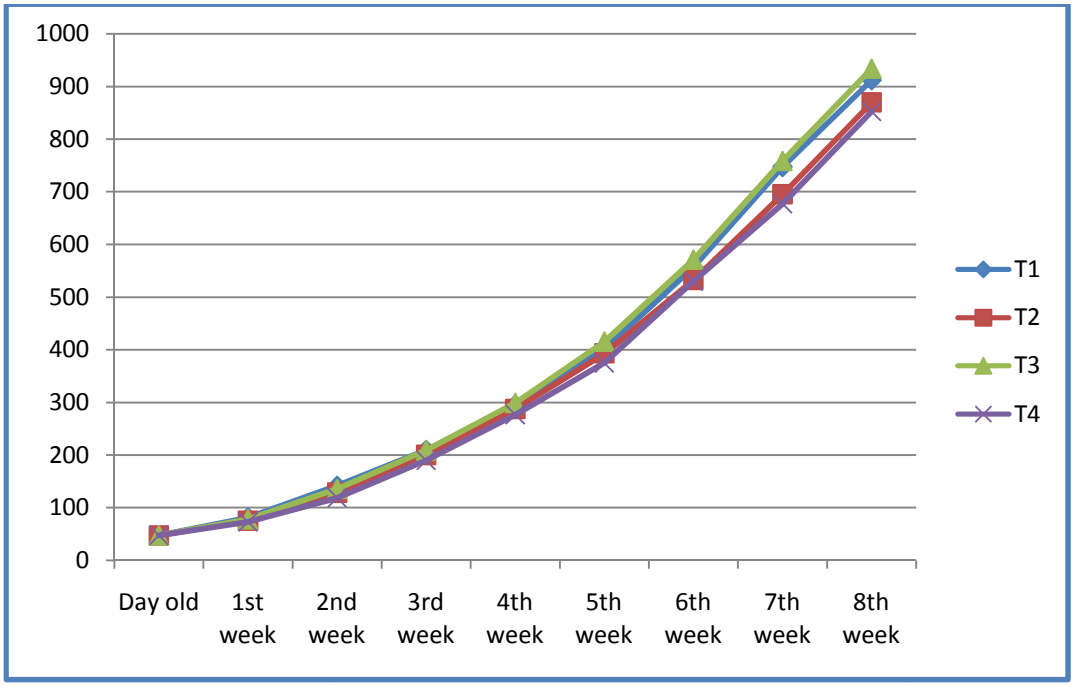


Fig 4.2: Effect of oregano and thyme oil supplementation on the average weekly body weight gain (g) of turkey poults during 0-8 weeks of age

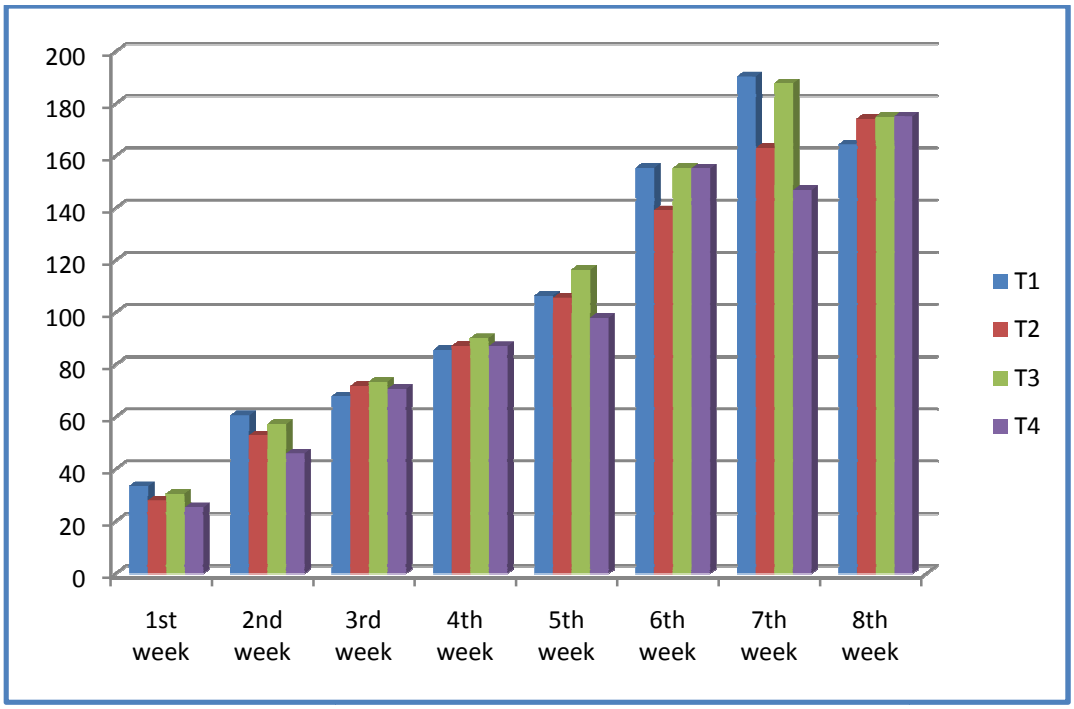


Fig 4.3: Effect of oregano and thyme oil supplementation on body weight gain (g) of turkey poults at different phases of growth during 0-8 weeks of age

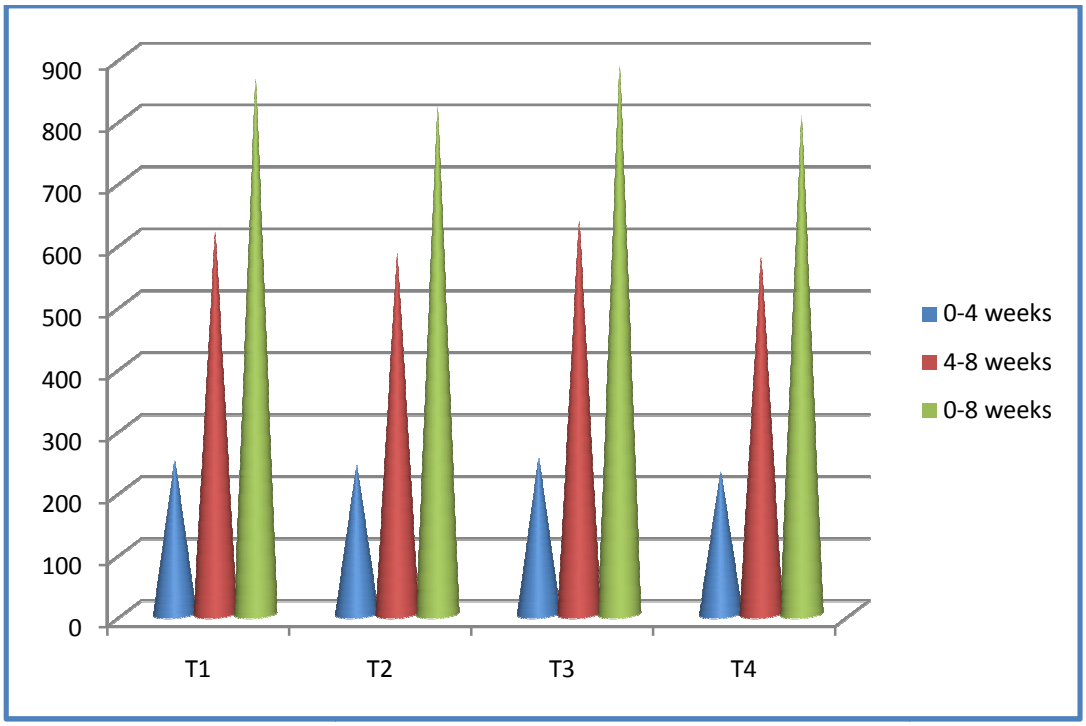


Fig 4.4: Effect of oregano and thyme oil supplementation on the cell mediated immune response to PHAP-P in turkey poults after 8 weeks of age.

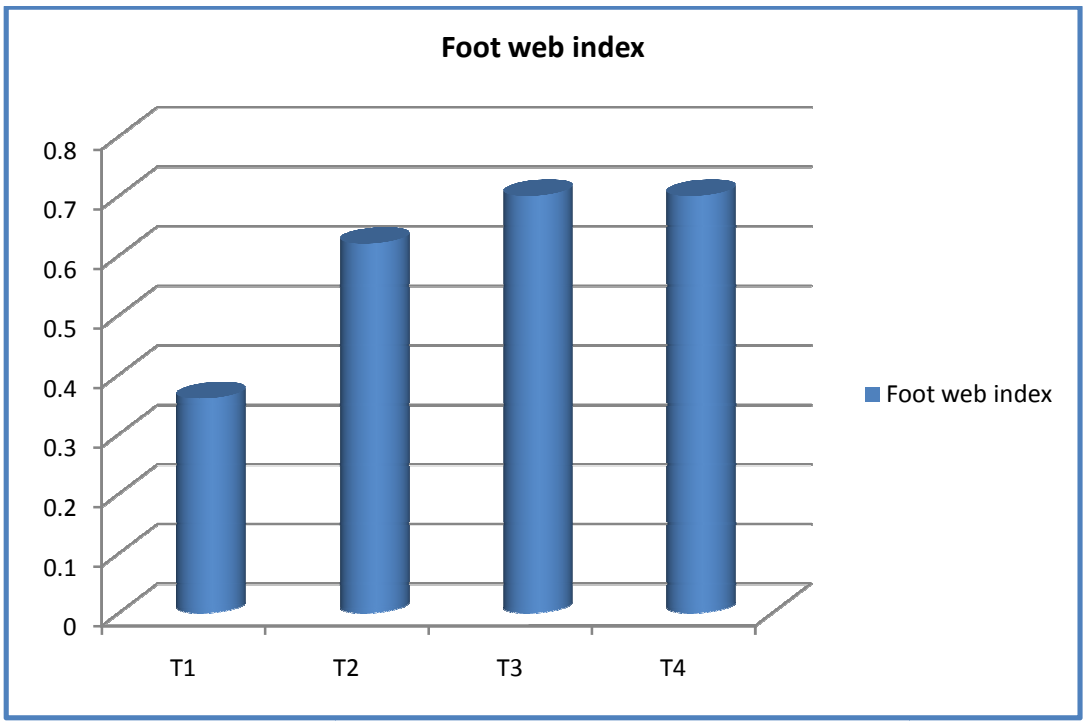
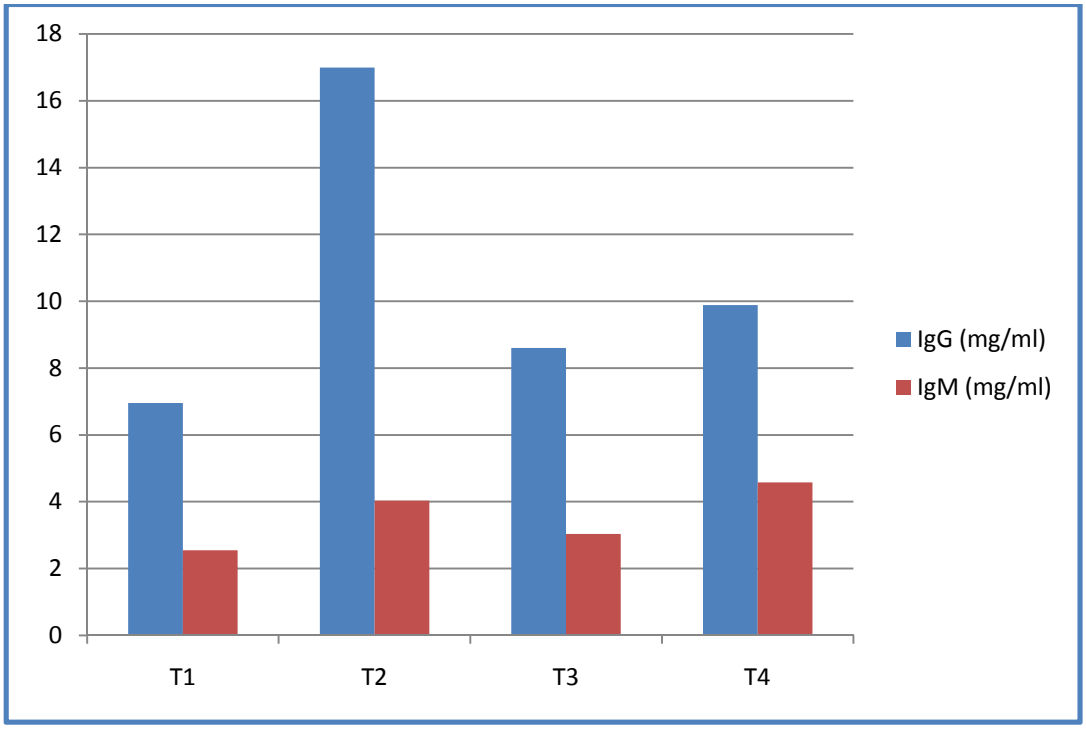


Fig 4.5: Effect of oregano and thyme oil supplementation on concentration of IgG and IgM antibodies of turkey poults after 8 weeks of age



Discussion

CHAPTER-5

DISCUSSION

5.1 Growth parameters

5.1.1 Body Weight

The data on body weight showed that Tl and T3 poults had significantly higher (P<0.05) body weight than T4 treatment group at 1st week of age. At 2nd week of age, T1 and T3 poults had significantly higher (P<0.01) body weight than T2 and T4 treatment group. At 3rd week of age, T1 and T3 poults had significantly higher (P<0.01) body weight than T2 and T4 treatment groups. At 4th week of age, Tl and T3 poults had significantly higher (P<0.05) body weight than T4 treatment groups. At 5th week of age, Tl and T3 poults had significantly higher (P<0.01) body weight than T4 treatment group. At 6th week of age, TI and T3 poults had significantly higher (P<0.01) body weight than T2 and T4 treatment group. At 7th week of age, T1 and T3 poults had significantly higher (P<0.01) body weight than T2 and T4 treatment group. At 8th week of age, Tl and T3 poults had significantly higher (P<0.01) body weight than T2 and T4 treatment group. Further, weekly body weight was apparently higher in Tl poults as compared to poults of other treatment groups from 1st to 3rd week of age. Thereafter, the weekly body weight was apparently higher in T3 as compared to other treatment groups.

Studies on effect of supplementation of essential oil in turkeys are scanty and most of the studies have been conducted in chickens. Fotea et al. (2004) reported that addition of oregano oil 0.3%, 0.7% and 1% in broiler diets improved daily live weight by approximately 1.5%, 4.1% and 5% as compared to the control group respectively. Similarly, Peng et al. (2016) observed that oregano oil @ 300 mg/kg and oregano oil 600 mg/kg resulted in improvement in body weight as the supplementation significantly decreased the crypt depth and increased the villus height to crypt depth ratio. It has also been reported that supplementation of oregano oil @ 50 mg/ kg diet in quail significantly increased body weight compared to control group (Badiri and Saber, 2016). Supplementation of thyme oil have also resulted in better growth performance in broilers as reported in different studies (Cross et al. 2007; Al-Kassie et

al., 2009; Alali et al., 2013; Attia et al., 2017 and Wade et al., 2018a). Oregano oil contains different bioactive substances viz. carvacrol, linalool, borneol etc. Similarly, thyme oil contains bioactive substances viz. thymol, carvacrol etc. which are considered as digestion stimulating factors, in addition to their antimicrobial activity against bacteria found in the intestine (Alçiçek et al., 2003; Feizi et al., 2014). Langhout (2000) reported that thyme oil added in the diet increases digestive enzymes and improves nutrient utilization through the enhanced liver function (Safa and AL-Beitawi, 2009). Thus, the bio active substances in oregano and thyme oil stimulate digestion, modulates the rate of feed passage in gut (Lee et al., 2003; 2004) and thereby results in improved growth in birds. Other factors which might have contributed to the beneficial effects on the growth performance of birds, were their probable antioxidant and antibacterial effects in the intestine (Nascimento et al., 2000). However, in the present study, there was a decrease in body weight in oregano oil supplemented group though this decrease was not consistent throughout the experimental period. The decrease in the oregano oil supplemented group might have been due to higher level of supplementation of oregano oil unlike other experiments where higher body weight was observed with oregano oil supplementation. Secondly, the experiment was done in turkeys unlike other studies which were limited to chickens. Turkeys are reluctant to consume feed at the earliest and are sometimes even subjected to forced feeding. So, a higher level of oregano oil supplementation might have resulted in decreased nutrient utilization. However, in the present study, the results were better in thyme oil supplemented group because the level of supplementation of thyme oil was lower as compared to oregano oil supplementation in the experiment. In spite of the lower level of supplementation of thyme oil in the present experiment, weekly body weight were apparently higher in control group poults as compared to poults of other treatment groups from 1^{st} to 3^{rd} week of age. The weekly body weight were apparently higher in the thyme oil supplemented group as compared to other treatment groups only after 3^{rd} week of age which reflects that in the neonatal turkey poults, the decrease in body weight in the oil fed groups may be due to poor nutrient utilization as the GIT is not well developed during this period. The decrease in body weight was well pronounced in the group where both oregano oil and thyme oil were supplemented as there was decreased feed consumption in this group through the experimental period. This decrease in feed consumption may be associated with palatability as has been reflected in another study where decrease in

feed consumption was noted in weaner pigs fed diets supplemented with oregano and thyme oil as compared to control in choice feeding system (Chizzola et al., 2006). Thus, further studies are required to be carried out with different levels of supplementations of oregano and thyme oil in turkeys to understand their effect on the body weight.

5.1.2 Body weight gain

There was no significant difference in the average weekly body weight gain of birds among various treatment groups throughout the experimental period except at 1st, 2nd, 6th and 7th week of age. Weekly body weight gain was significantly higher ($P<0.05$) in T1 than T4 at 1st week of age. At 2nd week of age, weekly body weight gain was significantly higher ($P<0.01$) in T1 and T3 than T4. At 6th week of age, weekly body weight gain was significantly higher ($P<0.01$) in T1 and T3 than T2. At 7th week of age, weekly body weight gain was significantly higher ($P<0.01$) in T1 and T3 than T2 and T4. Phase wise body weight gain was significantly higher ($P<0.05$) in T1 and T3 than T4 during 0 to 4 weeks. During 4 to 8 weeks, phase wise body weight gain was significantly higher ($P<0.05$) in T3 than T2 and T4. In addition, phase wise body weight gain was significantly higher ($P<0.01$) in T1 and T3 than T2 and T4 during 0 to 8 weeks. Further, phase wise body weight gain was apparently higher in T3 as compared to other treatment groups during 0-4 weeks, 4-8 weeks and 0-8 weeks of age. Several studies point out that supplementation of oregano and thyme oil resulted in significant increase in body weight gain as compared to the control group in chicken (Fotea et al., 2004; Al-Kassie et al., 2009; Roofchaee et al., 2011; Abdel-Wareth et al., 2012; Alali et al., 2013; Khattak et al., 2014; Peng et al., 2016; Behboudi et al., 2016, Hafeez et al., 2016; Wade et al., 2018a). In contrast with these studies, dietary supplementation of oregano essential oil to broilers (Botsoglou et al., 2002) at 50 and 100, 150, 300, and 1,000 mg/kg had no beneficial effect on growth performance. Cross et al. (2003) indicated that the inclusion of thyme oil had no effect on body weight gain of broilers. Lee et al. (2003) pointed out that 200 mg/kg of thymol in diet did not affect the body weight gain, feed intake, and FCR of female broilers. In the present study too, there was no such effect on body weight gain of turkey poults. Weekly body weight gain was higher in T1 poults as compared to poults of other treatment groups during 1st and 2nd week of age. Thereafter, the weekly body weight gain was higher in T3 as compared to other treatment groups. The reason

for the lack of effects of oregano and thyme oil on performance in different studies may be due to composition of the basal diet and environmental conditions (Lee et al., 2003). Further, as mentioned in the discussion on body weight, all the studies done were on chickens. So, further studies are needed at different levels of oregano and thyme on turkeys to understand their effect on body weight gain.

5.1.3 Feed intake

The results indicated that there were no significant difference in the average weekly feed intake of birds among various treatment groups throughout the experimental period except at 1st, 2nd and 8th week of age. Weekly feed intake was significantly higher ($P<0.05$) in the poults of T3 as compared to T4 treatment group at 1st week of age. Weekly feed intake was significantly higher ($P<0.01$) in poults of T1, T2 and T3 treatment groups than T4 at 2nd week of age. Weekly feed intake was significantly higher ($P<0.05$) in poults of T1, T2 and T3 as compared to T4 at 8th week of age. Phase wise feed intake during 0-4 weeks was significantly higher ($P<0.01$) in T1, T2 and T3 than T4. Further, during 0-8 weeks, phase wise feed intake was significantly higher ($P<0.05$) in T1 and T3 than T4. However, there was no significant difference observed in feed intake of the poults among the different treatment groups during 4-8 weeks of age. Thus, the phase wise feed consumption reflected that feed consumption was significantly higher in the poults of control group as compared to the poults subjected to oregano and thyme supplementation only during the initial phase of growth. This might have been due to the palatability of the feed. It has already been report that there was a decrease in feed consumption in weaner pigs fed diets supplemented with oregano and thyme oil as compared to control in choice feeding system (Chizzola et al., 2006). Further, studies on the effect of supplementation of oregano and thyme oil on feed consumption in chicken are contradictory. While some studies point out that there was an increased feed consumption after dietary supplementation of oregano and thyme oil (Al-Kassie et al., 2009; Abdel-Wareth et al., 2012; Peng et al., 2016), a few other studies state that there was no effect of dietary supplementation of oregano and thyme oil on the feed consumption of chicken (Lee et al., 2003, Hernandez et al., 2004, Behboudi et al., 2016). Thus, as mentioned in the previous sections, further studies are needed to be carried out in turkeys at different levels of supplementation to understand their effect on feed consumption.

5.1.4 Feed Conversion Ratio

Results indicated that at 2nd week of age, FCR of poults of T1, T3 and T4 were significantly better (P<0.05) than T2. At 3rd week of age, FCR of poults of T2, T3 and T4 were significantly better (P<0.01) than T1. At 7th week of age, FCR of poults of T1, T2 and T3 were significantly better (P<0.05) than T4. At 8th week of age, FCR of T4 poults were significantly better (P<0.01) than T1, T2 and T3. Thus, there was no clear trend observed in the weekly FCR when oregano and thyme were supplemented in the diet of turkey poults. There are several studies where it has been reported that FCR was improved after dietary supplementation of oregano and thyme oil in chicken (Bampidis et al., 2005; Al-Kassie et al., 2009; Roofchaee et al., 2011; Abdel-Wareth et al., 2012; Alali et al., 2013; Khattak et al., 2014; Attia et al., 2017; Wade et al., 2018a). In the present study too, it was found that dietary supplementation of both oregano and thyme oil was comparatively better than the control group though the results were not significant. As mentioned in the discussion on body weight gain and feed intakes, further studies in this area on turkeys are necessary.

5.2 Immuno Competence Traits

5.2.1 Humoral Immune Response

There was no significant difference in total immunoglobulins, IgG and IgM titre values in primary response to 1% GRBC among treatment groups. There are few studies that under the line of present study. Hong et al. (2012) also reported that supplementation of 125 ppm essential oil (derived from oregano, anis and citrus peel) resulted in no significant difference in IgG and total antibody values. However, Asli and Rashti (2017) reported that broilers fed 300 ppm oregano essential oil produced higher secondary total antibody titre against sheep red blood cell and their immunoglobulin G titre was higher than those fed control even though there was no significant difference in the primary response among the treatment groups. In addition, Attia et al. (2017) reported that thyme oil supplemented @ 1g/kg in broilers did not exhibit any significant effect to New Castle disease virus (NDV) and Infectious bursal disease virus (IBDV) among the treatment groups but when supplementation was @ 2g/kg of thyme oil, a higher antibody titre was observed in the antibody titre to only IBDV in broilers reared under hot climate. Contradictory to the results of aforesaid studies, Hashemipour et al. (2013) reported that broiler diet

supplemented with thymol and carvacrol @ 60, 100 and 200 mg/kg linearly increased both the primary (P <0.01) and secondary response (P < 0.05) against SRBC antigen and IgG. Saleh et al. (2014) reported that there was a significant increase in antibody titers against both NDV and IBDV in broiler chicken supplemented with 200 mg/kg thyme oil as compared to the control group. Thus, it is evident that further studies are needed in turkeys in normal and stress conditions at various levels of essential oils to understand the effect of essential oils in the humoral immune response to different antigens.

5.2.2 Cell-Mediated Immune Response

CMI response to PHA-P at 8 weeks of age indicated that T2, T3 and T4 poults had significantly higher (P<0.01) foot web index compared to T1 poults. Further, all the oil supplemented groups had apparently higher CMI response than the control group poults. Similar results were reported in other studies. Hong et al., (2012) Delayed type hypersensitivity (DTH) test showed increased (P < 0.05) values when broilers were supplemented with essential oil @ 125 ppm (derived from oregano, anis and citrus peel) as compared to the control group. Similarly, Hashemipour et al. (2013) reported that toe web thickness of broilers linearly increased within 24 and 48 h after injection with dietary supplementation of thymol and carvacrol @ 0, 60, 100 and 200 mg/kg of diet as compared to the control group. In addition, Acamovic and Brooker (2005) also reported that polyphenol fraction of thymol and oregano essential oil possess immunostimulating activity pertaining to cellular immunity. It has already been reported that herbs rich in flavonoids such as thyme extend the activity of vitamin C, act as antioxidants, and thus may elicit immunity (Acamovic and Brooker, 2005). As oregano and thyme are rich in carvacrol and thymol respectively and it has already been reported that these essential oils possess antibacterial, antiviral and antioxidant activities, an increase in immune responses of chicks is anticipated (Botsoglou et al., 2002).

5.2.3 Serum cortisol, IgG, IgM

There was no significant difference in the serum concentration of cortisol and IgM except IgG among the treatment groups. Studies on the effect of dietary supplementation of essential oil in turkey are limited. Hosseini et al. (2018) observed that high stocking density increased corticosterone in broilers when compared to low

stocking density but the effects of the former were in consistent with dietary supplementation of blend of essential oils (cinnamaldehyde and thymol). Thus, studies in this area are warranted. In the present study, T2 group poults had significantly higher ($P<0.05$) IgG value as compared to other treatment groups. Further, the serum IgG and IgM values of all the oil supplemented groups were apparently higher than the control group. Our results pertaining to IgG and IgM corroborate with the findings of several other studies (Doaa et al., 2017; Hosseini et al., 2018). Essential oils positively influence the avian immune system, since they promote production of immunoglobulins, enhance lymphocytic activity, and boost interferon-y release (Awaad et al., 2010). Acamovic and Brooker (2005) noted the immunostimulating activity of polyphenol fraction of thymol and oregano essential oil with respect to the system of mononuclear phagocyte system and humoral immunity. Placha (2014) noted that thyme oil stimulated phagocytic activity in blood, improved intestinal barrier integrity in broiler chicken. Further, it has already been mentioned in the discussion pertaining to cellular immunity that oregano and thyme are rich in carvacrol and thymol respectively. These essential oils possess antibacterial, antiviral and antioxidant activities and thus an increase in immune responses of chicks is anticipated (Botsoglou et al., 2002). Carvacrol, can lower the expression of COX, 2 mRNA and proteins induced by lipopolysac charides. Because it is an inhibitor of PPAR-α, γ-regulator and COX-2, it has various effects, including an anti-inflammatory action (Hotta et al., 2010). Burt et al. (2007) suggested that the components of thyme essential oil can induce bacterial HSP60 and inhibit the synthesis of 0157:H7 flagellin of *Escherichia coli*. Thyme essential oil significantly suppresses the proliferation of aspergillus, ten bacteria and eight yeast strains. Zu et al. (2010) investigated ten essential oils and suggested that thyme, cinnamon and rose essential oils display the best antibacterial activity against propionibacterium acnes and inhibit its diameter respectively by 40 mm, 33.5 and 16.5 mm. Schnitzler et al. (2007) indicated that plant essential oils, like thyme essential oil, have a more powerful antiviral activity against acyclovir-sensitive strain KOS, and acyclovir-resistant herpes simplex virus.

5.3 Blood Biochemical parameters

There was no significant difference in plasma uric acid, AST, ALP, SOD and LPO among different treatment groups except plasma total proteins, plasma

cholesterol, plasma HDL and ALT values. The results showed that there was no deleterious effect of dietary supplementation of oils at different levels in turkey poults. Further, the results of the present study pertaining to plasma uric acid and AST corroborate with the results of the findings of other studies (Khattak et al., 2014). There are contradictory reports pertaining to the effect of dietary supplementation of essential oil on serum sop level in chicken. Doaa et al. (2017) reported that supplementation of thyme leaves powder in the broiler's diet significantly reduced serum SOD level in chicken. On the other hand, Roofchaee et al., (2011) reported that supplementation of oregano oil in chicken resulted in no significant difference in the serum SOD levels. In the presence study too, no significant difference in serum SOD level among the different treatment groups were observed. T4 poults had significantly higher ($P<0.05$) total plasma protein value as compared to T1, T2 and T3. There are contradictory reports on effect of essential oils on serum protein in chicken. While studies point out that there was an increase in serum protein after feeding of essential oils (Al-Kassie et al., 2009; Attia et al., 2017; Zhu et al., 2016; El Ghousein and Al-Beitawi 2009; Toghyani et al., 2010; Köksal and kucukersan, 2012) reported that there has been a decrease in serum protein of chicken reared on diets supplemented with essential oil. Serum total proteins consist of albumin and globulin. Their content can effectively reflect protein metabolism, feed condition and growth of animals. γ-globulins are responsible for humoral immunity. The A/G ratio can reflect spleen functions and partly the immune and physiological status of animals (Xie et al., 2010). In the present study, the increase in serum protein may be attributed to increase in the components of immunomodulatory activity. The increased levels also suggested the capacity of essential oils to improve digestion and absorption of proteins as previously reported by Bento et al. (2013). Carvacrol and thymol present in oregano and thyme oil respectively, possess potent antioxidant properties and consequently, elevate immune modulators of the immune system. Though there was no desirable effect in growth in T4 birds as there was decreased feed consumption, there was better cell mediated immune response and increase in IgG and IgM level in T4 poults compared to control. However, as pointed out earlier, further studies are needed to be carried out in this area in turkey poults. T2 and T3 poults had significantly higher ($P<0.05$) ALT values as compared to T3 poults and comparatively higher ALT values as compared to T1. Very few studies have been conducted on the effect of essential oils on serum ALT in chicken (Khattak et al., 2014; Tekce and Gul 2017, Doaa et al., 2017).

Further, the results in these studies are variable. T3 and T4 poults had significantly higher (P<0.05) plasma cholesterol values as compared to T1 poults and apparently higher values as compared to T2 poults. The results in present study fall in line with the results of Tekce and Gul (2017) who also observed an increase in serum cholesterol in broilers after supplementation of oregano essential oil in broilers. However, contradictory, to this, there are a few studies in Japanese quails and chicken who have reported a decrease in cholesterol level after supplementation of oregano and thyme oil (Lee et al., 2004; Khaksar et al., 2012; Hong et al., 2012; Doaa et al., 2017; Al-Kassie et al., 2009). The contradictory reports may be due to the variation in the levels of supplementation under different environment in different species of poultry T2 and T3 poults had significantly higher (P<0.01) HDL values as compared to T1 and T4 poults. The results in present study fall in line with several other studies. (Tekce and Gul., 2017; Bölükbaşı et al., 2006; Lee et al., 2004; Hong et al., 2012).

5.4 Development of digestive and lymphoid organs

No significant difference was recorded in development of the digestive and lymphoid organs among the treatment groups. The results in present study fall in line with several other studies (Jang et al., 2007; Hashemipour et al., 2013; Ocak et al., 2008; Rahimi 2011; Hernandez et al., 2004).

5.5 Carcass quality traits, yield of giblet and cut-up parts

No significant differences were recorded in carcass quality traits, yield of giblet and cut up parts among the treatment groups except percent ready-to-cook yield. Several studies also reported no significant differences in the carcass quality traits with dietary supplementation of essential oils in broilers, turkey and quail as compared to the control group (Alcicek et al., 2003; Jang et al., 2007; Ocak et al., 2008; Hernandez et al., 2004; Küçükyilmaz et al., 2017; Denli et al., 2004). T2 poults had significantly higher (P<0.05) ready to cook yield % as compared to T1. Further, the percent ready to cook yield was apparently higher in all the oil supplemented groups as compared to the control group. The results fall in line with several other studies in chicken (Peng et al., 2016; Wade 2018b; Abdulkarimi et al., 2011; Amouzmehr et al., 2012; AL-Kassie 2009; Ragaa et al., 2016). The increase in percent ready to cook yield may be due to enhancement of the

metabolism of oil, carbohydrates and proteins in the major organs resulting in increase in growth rate of these organs (Mellor, 2000a)

5.6 Chemical and mineral composition of Breast (*pectoralis major*) muscle and Thigh (*ilio tibialis*) muscle

No significant difference was observed in the chemical and mineral composition of breast (*pectoralis major*) and thigh (*ilio tibialis*) meat of turkey poults at 8 weeks of age. Studies in this aspect is extremely limited. Giannena et al. (2016) also reported that there was no significant difference in the chemical and mineral composition of breast and thigh meat of chicken supplemented with oregano and laurel essential oils as compared to birds reared on a control diet. However, in the present experiment, T2 and T3 poults had significantly higher ($P<0.05$) deposition of Mg in breast muscle as compared to T1. Further, all the oil supplemented groups had apparently higher deposition of Mg in breast muscle as compared to the control group at 8^{th} weeks of age. The present study is the first reported study on the chemical and mineral composition of breast and thigh meat of turkey poults supplemented with oregano and thyme oil.

5.7 Fatty acid profile of turkey meat (breast and thigh meat)

There was no significant difference in saturated fatty acid (SFA) contents of breast and thigh meat among the different treatment groups. Percent monounsaturated fatty acid (MUFA) was significantly higher ($P<0.01$) in T1 as compared to other treatment groups. Further, percent polyunsaturated fatty acid (PUFA) was significantly higher ($P<0.01$) in all the oil fed groups as compared to the control group. In addition, the percent saturated fatty acids was numerically lower in all the oil fed groups as compared to the control group. The present study is the first study to report on the effect of dietary supplementation of oregano and thyme oil on the lipid profile of turkey meat. However, a few studies have been carried out in chicken on this aspect and the results of the studies collaborate with the results of the present study pertaining to the SFA and PUFA levels in meat of chicken. An increased level of PUFA and decrease level of SFA was observed with dietary supplementation of essential oil in chicken in the aforesaid studies (Omidi et al., 2020; Hashemipour et al., 2013; Ciftci et al., 2010; Ertas et al., 2005). In the present study, the high level of PUFA in the meat of turkey poults of T2, T3 and T4

might be due to the antioxidant activity of carvacrol and thymol present in oregano and thyme respectively as they blocked the lipid peroxidation of meat lipids, especially PUFA (Hashemipour et al., 2013).

Summary and Conclusions

CHAPTER-6

SUMMARY AND CONCLUSIONS

Phytogenic feed additives are plant-based feed additives that are used to elicit performance in poultry. The beneficial properties of these phytogenic compounds are due to their bioactive molecules. Many of these bioactive molecules are essential oils. Many studies have already depicted that dietary essential oil supplementation has a positive effect on the growth performance and immunity in poultry. Many herbal oils are rich in essential oils. Oregano and thyme are rich in essential oil. Few studies have been conducted on dietary supplementation of oregano and thyme oil in chicken. However, limited work has been done in turkeys. Hence, the present study has been designed with the objectives- To study the effect of supplementation of oregano and thyme oil on growth performance, immunocompetence traits, blood biochemical attributes and carcass quality of turkey poults. A total no. of (n=144), straight run day old turkey poults were divided into four treatment groups, having three replicates of 12 birds each. The study was conducted in turkey poults during 0-8 weeks of age. The birds of the control group (T1) were fed a basal diet, while T2 group was supplemented with oregano oil @ 1% basal diet, T3 group was supplemented with and thyme oil @ 1g/kg basal diet and T4 group was supplemented with oregano oil @ 1% and thyme oil @ 1g/kg basal diet.

Turkey poults showed that T1 and T3 had significantly higher ($P<0.05$) body weight than T4 treatment group at 1st week of age. At 2nd week of age, T1 and T3 poults had significantly higher ($P<0.01$) body weight than T2 and T4 treatment groups. At 3rd week of age, T1 and T3 poults had significantly higher ($P<0.01$) body weight than T2 and T4 treatment groups. At 4th week of age, T1 and T3 poults had significantly higher ($P<0.05$) body weight than T4 treatment groups. At 5th week of age, T1 and T3 poults had significantly higher ($P<0.01$) body weight than T4 treatment groups. At 6th week of age, T1 and T3 poults had significantly higher ($P<0.01$) body weight than T2 and T4 treatment groups . At 7th week of age, T1 and T3 poults had significantly higher ($P<0.01$) body weight than T2 and T4 treatment groups. At 8th week of age, T1 and T3 poults had significantly higher ($P<0.01$) body weight than T2 and treatment groups. Further, weekly body weight was apparently

higher in T1 poults as compared to poults of other treatment groups from 1st to 3rd week of age. Thereafter, the weekly body weight was apparently higher in T3 as compared to other treatment groups.

There were no significant differences in the average weekly body weight gain of birds among various treatment groups throughout the experimental period except at 1st, 2nd, 6th and 7th week of age. Weekly body weight gain were significantly higher ($P<0.05$) in T1 than T4 at 1st week of age. At 2nd week of age, weekly body weight gain was significantly higher ($P<0.01$) in T1 and T3 than T4. At 6th week of age, weekly body weight gain were significantly higher ($P<0.01$) in T1 and T3 than T2. At 7th week of age, weekly body weight gain were significantly higher ($P<0.01$) in T1 and T3 than T2 and T4.

Phase wise body weight gain were significantly higher ($P<0.05$) in T1 and T3 than T4 during 0 to 4 weeks. During 4 to 8 weeks, phase wise body weight gain were significantly higher ($P<0.05$) in T3 than T2 and T4. In addition, phase wise body weight gain was significantly higher ($P<0.01$) in T1 and T3 than T2 and T4 during 0 to 8 weeks. Further, phase wise body weight gain was apparently higher in T3 as compared to other treatment groups during 0-4 weeks, 4-8 weeks and 0-8 weeks of age.

There were no significant differences in the average weekly feed intake of birds among various treatment groups throughout the experimental period except at 1st, 2nd and 8th week of age. Weekly feed intake was significantly higher ($P<0.05$) in the poults of T3 as compared to T4 treatment groups at 1st week of age. Weekly feed intake was significantly higher ($P<0.01$) in poults of T1, T2 and T3 treatment groups than T4 at 2nd week of age. Weekly feed intake was significantly higher ($P<0.05$) in poults of T1, T2 and T3 as compared to T4 at 8th week of age.

Phase wise feed intake during 0-4 weeks was significantly higher ($P<0.01$) in T1, T2 and T3 than T4 .Further, during 0-8 weeks, phase wise feed intake was significantly higher ($P<0.05$) in T1 and T3 than T4. However, there was no significant difference recorded in feed intake of the poults among the different treatment groups during 4- 8 weeks of age.

There was no significant difference in FCR of birds during the entire experimental period except at 2nd, 3rd, 7th and 8th week. At 2nd week of age, FCR of

poults of T1, T3 and T4 were significantly better (P<0.05) than T2. At 3rd week of age, FCR of poults of T2, T3 and T4 were significantly better (P<0.01) than T1. At 7th week of age, FCR of poults of T1, T2 and T3 were significantly better (P<0.05) than T4. At 8th week of age, FCR of T4 poults were significantly better (P<0.01) than T1, T2 and T3. However, there was no significant difference in the phase wise FCR among the different treatment groups

There was no significant difference in total haemagglutination titre values in response to 1% GRBC as well as IgG and IgM titre values among the different treatment groups. CMI response to PHA-P after 8 weeks of age indicated that T2, T3 and T4 poults had significantly higher (P<0.01) foot web index compared to T1 poults.

There was no significant difference among the treatment groups in serum cortisol and IgM levels except IgG. T2 group poults had significantly higher (P<0.05) IgG value as compared to other treatment groups. Further, the serum IgG and IgM values of all the oil supplemented groups were apparently higher than the control group.

There was no significant difference in uric acid, AST, ALP, SOD, LPO values among different treatment groups except plasma total proteins, plasma ALT, plasma cholesterol, plasma HDL.T4 poults had significantly higher (P<0.05) total plasma protein value as compared to T1 , T2 and T3. T2 and T3 poults had significantly higher (P<0.05) ALT values as compared to T3 poults and comparatively higher ALT values as compared to T1. T3 and T4 poults had significantly higher (P<0.05) plasma cholesterol values as compared toT1 poults and apparently higher values as compared to T2 poults. T2 and T3 poults had significantly higher (P<0.01) HDL values as compared to T1 and T4 poults

No significant difference was recorded in development of the digestive and lymphoid organs among the treatment groups. No significant difference were recorded in carcass quality traits, yield of giblet and cut up parts among the treatment groups except ready to cook yield %. T2 poults had significantly higher (P<0.05) ready to cook yield % as compared to T1 poults and apparently higher than poults of T3 and T4

The data obtained on chemical composition of breast (*pectoralis major*) and thigh (*ilio tibialis*) muscle showed that there was no significant difference in the chemical composition of breast and thigh meat except for Mg. T2 and T3 poults had significantly higher ($P<0.05$) deposition of Mg in breast muscle as compared to T1. Further, all the oil supplemented groups had apparently higher deposition of Mg in breast muscle as compared to the control group at 8 weeks of age.

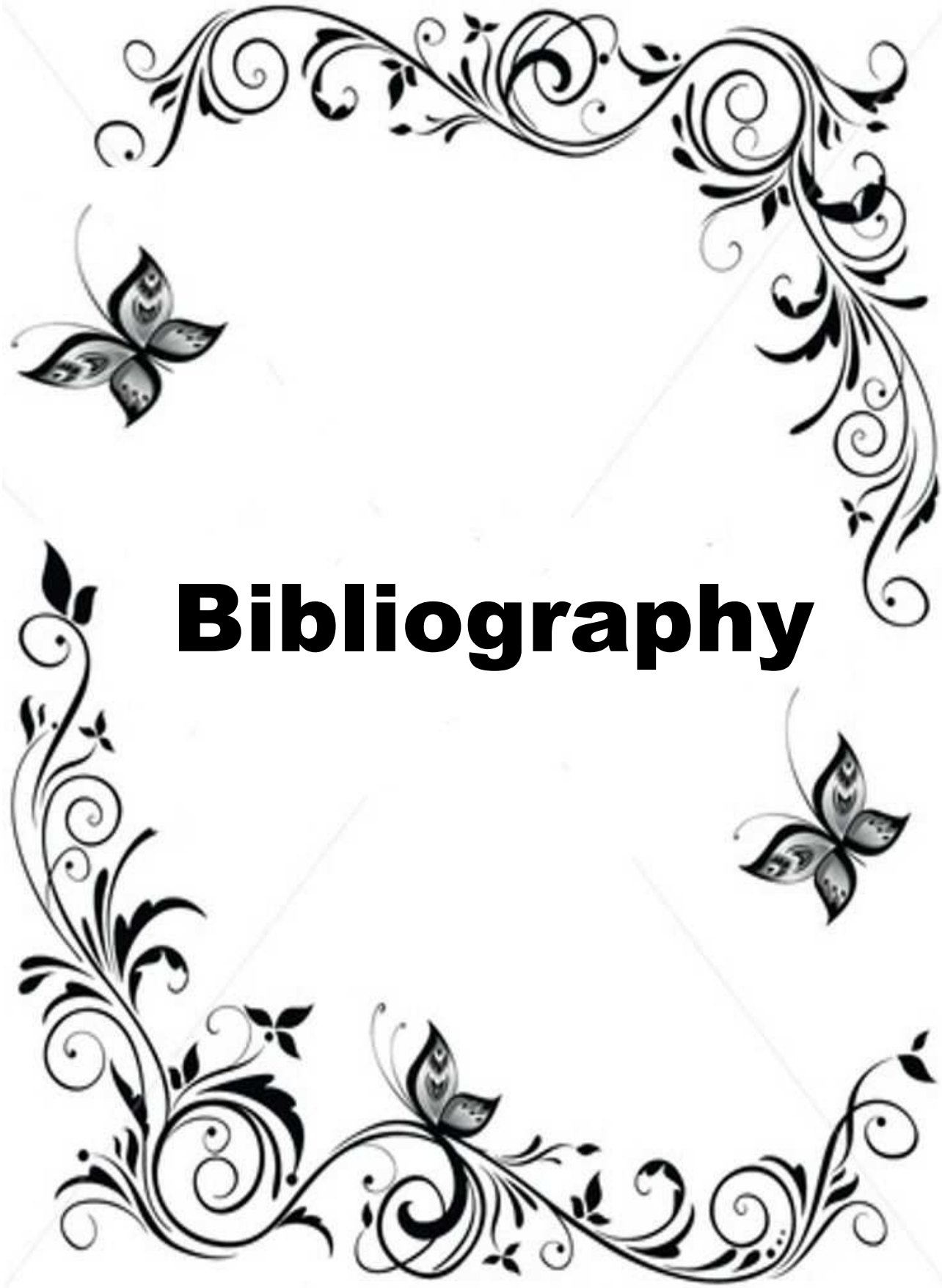
The data obtained on fatty acid profile of breast and thigh meat showed that there was no significant difference in the saturated fatty acid of breast and thigh meat. Monounsaturated fatty acid was significantly higher ($P<0.01$) in T1 as compared to other treatment groups. Polyunsaturated fatty acid was significantly higher ($P<0.01$) in all the oil fed groups as compared to the control group.

From the present study it may be concluded that:

1. Dietary supplementation of thyme oil @ 1g/kg feed and the control group resulted in better growth performance as compared to other treatment groups, During the first 3 weeks of age, the control group poults showed a numerically better growth performance as compared to the thyme oil supplemented group poults. Thereafter, the thyme oil supplemented group poults depicted numerically a better growth performance as compared to the control group.
2. Supplementation of oregano oil @ 1% and thyme oil @ 1g/kg feed resulted in better immunity
3. Further, dietary supplementation of oregano @ 1% and thyme oil @ 1g/kg feed resulted in increased percent ready to cook yield and higher deposition of magnesium in breast meat.
4. Dietary supplementation of oregano and thyme oil did not result in any adverse effect on development of digestive and lymphoid organs, carcass quality traits, yield of giblet and chemical composition of breast (*pectoralis major*) and thigh (*ilio tibialis*) muscle of turkey poults at 8 weeks of age.

Further, dietary supplementation of oregano and thyme oil resulted in increased PUFA levels in meat of turkey poults.

Thus, thyme oil @ 1g/kg of diet may be supplemented to elicit growth performance, immunity, percent ready to cook yield, Mg deposition in breast meat cut and PUFA in meat of turkey poults. However, studies may be done at different levels of oregano and thyme in different environment to assess their effect on performance, blood biochemical and haematological profile, carcass quality, chemical composition, mineral composition and lipid profile in meat of turkey poults. Further, studies may also be carried out to assess the effect of dietary supplementation of oregano and thyme oil on the genetic expression of genes for growth, immunity and genes associated with stress markers in turkey poults.



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EXTRA-CURRICULAR ACTIVITIES:

- Received 2nd prize in Oral presentation in Annual Conference of VIPM held during 8-9th November, 2019 at DUVASU, Mathura, India
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- Member of winning team of Annual Cultural Programme Group Dance Competition held during 2017 at NDVSU, Mhow, India
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Signature

UNDERTAKING OF COPY RIGHT

I, **Abhilasha Rai,** Enrollment No. **V-2126/17** undertake that I give copyright to the DUVASU, Mathura of my thesis entitled, **"Effect of dietary supplementation of oregano and thyme oil on the performance of turkey poults".**

I also undertake that patent, if any, arising out of research work conducted during the programme shall be filed by me only with due permission of the competent authority of U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura (UP).

Signature of the Student