

**Performance of Broiler chicken fed diets supplemented
with *Artemisia absinthium* with or without enzyme**

Dr. Shaista Saeedatu Nissa
(2018-763-D)



**Division of Livestock Production and Management
Faculty of Veterinary Sciences & Animal Husbandry
Sher-e-Kashmir University of Agricultural Sciences &
Technology of Kashmir**

2022

**Performance of Broiler chicken fed diets supplemented
with *Artemisia absinthium* with or without enzyme**

Dr. Shaista Saeedatu Nissa
(2018-763-D)



Thesis

Submitted to

**The Faculty of Veterinary Sciences & Animal Husbandry
Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
in partial fulfilment of requirements for the award of the degree of**

**Doctor of Philosophy in Veterinary Sciences
(Poultry Science)**

2022



Dedicated

*To Almighty for Blessings and Family for
their Love and Support*





Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Livestock Production and Management,
Faculty of Veterinary Sciences & Animal Husbandry, Shuhama

Certificate – I

This is to certify that the thesis entitled, **“Performance of Broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme”** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Veterinary Sciences (Poultry Science)**, to the **Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of *bona-fide* research work carried out by **Dr. Shaista Saeedatu Nissa (Regd. No. 2018-763-D)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation has duly been acknowledged.

(Prof. M.T. Bandy)
Chairman
Advisory Committee

Endorsed

Prof. & Head,
Division of Livestock Production and Management



Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Livestock Production and Management,
Faculty of Veterinary Sciences & Animal Husbandry, Shuhama

Certificate – II

We, the members of the Advisory Committee of **Dr. Shaista Saeedatu Nissa (Regd. No. 2018-763-D)** a candidate for the degree of **Doctor of Philosophy in Veterinary Sciences (Poultry Science)** have gone through the manuscript of the thesis entitled, **“Performance of Broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme”** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

Advisory Committee

Chairman

Prof. M.T. Banday
Dean, Faculty of Veterinary
Sciences and Animal Husbandry

Members

Prof. Azmat Alam Khan
OSD to Vice-Chancellor

Dr. Masood Saleem Mir
Associate Director Extension
(Animal Science)

Dr Abdul Shakoor Bhat
Professor,
Division of Veterinary
Pharmacology

Dr. Gowhar Gull Sheikh
Assistant Professor,
Division of Animal Nutrition

Dr. Asif H. Sofi
Assistant Professor, Division of Livestock Products Technology
(Dean's Nominee)



Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Livestock Production and Management,
Faculty of Veterinary Sciences & Animal Husbandry, Shuhama

Certificate – III

This is to certify that the thesis entitled, “**Performance of Broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme**” submitted by **Dr. Shaista Saeedatu Nissa (Regd. No. 2018-763-D)** to the **Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy in Veterinary Sciences (Poultry Science)** was examined and approved by the Advisory Committee and External Examiner on **16-06-2022**.

Chairman
Advisory Committee

External Examiner

Prof. & Head,
Division of Livestock Production and Management

Dean,
Faculty of Veterinary Sciences & Animal Husbandry
SKUAST-Kashmir, Shuhama



Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Livestock Production and Management,
Faculty of Veterinary Sciences & Animal Husbandry, Shuhama

Name of the student : **Dr. Shaista Saeedatu Nissa**
Registration No. : 2018-763-D
Major Subject : Poultry Science
Minor Subject : Animal Nutrition
Major Advisor : **Prof. M.T.Banday**
Professor and Dean
Faculty of Veterinary Sciences and
Animal Husbandry
Title of the Thesis : **“Performance of Broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme”**

ABSTRACT

The present study was conducted to evaluate the effect of feeding diets supplemented with *Artemisia absinthium* with or without enzyme on the performance of broilers. The study was undertaken at the Instructional Poultry farm of the Division of Livestock Production and Management, FVSc & AH, SKUAST- Kashmir, Shuhama, Alusteng (J&K). Leaves of *Artemisia absinthium* were collected, separated from the stems, washed, dried, crushed into a fine powder and stored until used. Two hundred and fifty two day old commercial broiler chicks were purchased from a reputed hatchery and reared for the first week under standard brooding conditions. On the eighth day, the chicks were randomly divided into seven treatment groups, having three replicates with twelve birds each. The groups were distributed as; group T₁-control, offered basal diet without any feed additive whereas groups T₂ T₃, T₄ were offered basal diet supplemented with *Artemisia absinthium* @ 1, 1.5 and 2% respectively on dry matter basis whereas groups T₅ T₆, T₇ were offered basal diet supplemented with *Artemisia absinthium* with cocktail enzyme @ 1, 1.5 and 2% respectively on dry

matter basis. The cocktail enzyme was used at the rate of 25g/100kg feed. *Artemisia absinthium* contained 23.56% crude protein, 4.31% ether extract, 20.30% crude fibre, 43.18% nitrogen free extract and 8.65% ash.

The results of average body weights and body weight gain (g) of broilers revealed a significant ($P \leq 0.05$) improvement in live body weight and body weight gain in the treatment groups fed diets supplemented with raw or enzyme treated *Artemisia absinthium* leaves. However, the group of birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves had significantly ($P \leq 0.05$) higher body weight when compared with the group of birds fed diets supplemented with different levels of raw *Artemisia absinthium* leaves during the growth period from 1 to 5 weeks of age. It was also observed that birds supplemented with 2% *Artemisia absinthium* with enzyme had the highest body weights ($P \leq 0.05$) and gains at the end of the growth trail when compared to birds of other treatment groups.

The cumulative feed consumption was significantly ($P \leq 0.05$) higher in group of birds fed different levels of enzyme treated *Artemisia absinthium* when compared to the birds fed diets supplemented with raw *Artemisia absinthium* leaves and the control group. Among the birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves highest feed consumption was recorded in birds fed diets supplemented with 2% *Artemisia absinthium* leaves i.e. T₇ group during the entire growth period from 1 to 5 weeks of age. Similarly, the overall feed conversion ratio (FCR) of birds fed diets supplemented with 1.5 % and 2% enzyme treated *Artemisia absinthium* leaves was significantly ($P \leq 0.05$) improved when compared to the birds fed diets supplemented with 1% enzyme treated *Artemisia absinthium* leaves or birds fed different levels of raw *Artemisia absinthium* leaves and the control diet.

Significantly ($P \leq 0.05$) higher digestibility of dry matter and crude protein (CP) was observed in treatment groups fed diets supplemented with 2% enzyme treated *Artemisia absinthium*. There was however no significant effect of feed additive supplementation on the digestibility of ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) in comparison to control.

The blood biochemicals of birds revealed significant ($p < 0.05$) differences among the various treatment groups. The mean value of glucose (mg/dl) was found to be significantly ($p < 0.05$) reduced in the group of birds fed diets supplemented with 2% *Artemisia absinthium* with enzyme supplementation when compared to other treatment groups and the control group. The serum AST levels (u/l) and serum ALT levels (u/l) were found to be within the normal range and did not differ significantly between the treatment groups. The serum creatinine levels (mg/dl) were found to be within the normal range and did not differ significantly between the treatment groups. The lipid profile including total cholesterol (mg/dl) serum triglycerides (mg/dl), high density lipoproteins (HDL) (mg/dl), and low density lipoproteins (LDL) (mg/dl) in various treatment groups varied significantly ($p < 0.05$). There was a significant ($p < 0.05$) reduction in the serum cholesterol levels

in birds fed diets supplemented with 2% *Artemisia absinthium* with enzyme supplementation when compared to other treatment groups and the control group. Significantly ($p<0.05$) highest levels of triglyceride were found in the control group and lowest in the group fed diets supplemented with 2% *Artemisia absinthium* with enzyme treatment. Similar trend was observed in the serum high density lipoproteins (HDL) and low density lipoproteins (LDL) levels.

There was no significant effect in general on the slaughter traits and carcass characteristics of birds fed supplemented with *Artemisia absinthium* with or without enzyme supplementation. Although the dressing percentage of birds fed diets supplemented with enzyme treated *Artemisia absinthium* was significantly ($p<0.05$) improved. There was no variation in histomorphology of liver and intestine of birds fed diets supplemented with *Artemisia absinthium* with or without enzyme supplementation from normal structure.

There was significant ($P\leq 0.05$) reduction in cecal viable count and caecal coliform count in the groups fed 1.5 and 2% raw and enzyme treated *Artemisia absinthium* when compared to the group of birds fed 1% raw and enzyme treated *Artemisia absinthium* and the control group. Among all the treatment groups, Significantly ($P\leq 0.05$) lower cecal viable count and cecal coliform counts was observed in treatment groups T₆ and T₇ i.e. the groups fed 1.5% and 2% enzyme treated *Artemisia absinthium*.

There was a significantly ($p<0.05$) higher total antioxidant status and lower total oxidant status and TBARS value in the treatment group T₆ and T₇ i.e. the groups fed diets supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium* when compared to other groups. Similarly the immune response measured as anti SRBC antibody titre, DNCB values was significantly ($p<0.05$) higher in the treatment group T₆ and T₇ i.e. the groups fed diets supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium* when compared to other groups. However, no significant difference was observed in the weight of immune organs between various treatment groups.

No significant difference was observed in the sensory evaluation and proximate composition of meat among various treatment groups. There was reduction in the feed cost/kg live weight gain among different treatment groups. Feed cost/kg live weight gain worked out to be highest for the birds of control group and lowest for the groups fed 1.5% and 2% *Artemisia absinthium* with enzyme.

Key words: Broiler, *Artemisia absinthium*, Performance, Blood biochemistry, Carcass parameters, Immune status, Oxidative status, Economics

Signature of Student

Dated: _____

Signature of Major Advisor

Dated: _____

ACKNOWLEDGEMENT

In the name of Allah, the most kind and the most merciful

First and foremost I thank Allah (SWT) the most beneficent, the most merciful for providing me with the gift of life to see this thesis through

I would like to express my deepest appreciation for my Major Advisor, Prof. M.T.Banday, Dean, Faculty of Veterinary Sciences and Animal Husbandry, who has the attitude and the substance of a genius; he continually and convincingly conveyed a spirit of adventure in regard to research and an excitement in regard to teaching. Without his guidance and persistent help this dissertation would not have been possible. His guidance and knowledge helped me to overcome many difficulties faced during my research programme.

I wish to humbly thank Dr. Islam ud din Sheikh, Professor and Head, Division of Livestock Production and Management who has been a source of continuous guidance and timely advice.

I am highly indebted to the other members of my advisory committee namely, Dr. Azmat Alam, (Professor & Associate Director Research), Dr. Masood Saleem Mir (Professor & Associate Director Extension), Dr. Gowher Gull (Assistant Professor, ANN), Dr. Abdul Shakoor Bhat (Professor, VPT), Dr. Asif Hassan Sofi (Assistant Professor, LPT) for their valuable inputs, continuous optimism and healthy criticism throughout the course of this study.

I owe my deepest gratitude to Dr. Asif Hassan Sofi and the members of Division of Livestock Products and Technology for their help and consistent cooperation during my research period.

I am deeply indebted to all the staff members of Division of Livestock Production and Management Dr. Sheikh Aadil, Dr. Irfan A. Baba, Dr. Rouf A. Pattoo, Dr. Henna Hamdani for their for their encouragement, help and valuable suggestions during the entire period of my study.

I acknowledge the cordial assistance provided by Division of Veterinary Biochemistry, Veterinary Microbiology and Veterinary Clinical Medicine for conducting various analysis.

I highly acknowledge the assistance rendered by all technical and non-technical staff of Division of LPM.

I cannot find words to express my gratitude to the affection and cooperation provided by colleagues, Dr. Subata Mehboob, Nahida, Mariya, Dr. Asma Qureshi, Dr. Bushra Zaffer, Dr. Shubeena, Dr. Asma and Dr. Aaliya.

I acknowledge the cordial assistance provided by Division of Animal Nutrition, Division of Veterinary Biochemistry, Veterinary Microbiology and Veterinary Clinical Medicine for conducting various analysis.

I am also indebted to the staff of Central Library, SKUAST-K, Shalimar for their cooperation and necessary help.

From the profundity of my inner heart, I take the privilege to express my deep humility and devotion to my family for their affection, faith and confidence in me, unrelenting support, blessings, encouragement and inspiration throughout my years of study. I am forever obliged to my parents, Ghulam Ahmed Malik and Fatima Malik, Dr. Abdul Rasheed and Mehmooda Rasheed, for giving me the opportunities and experiences that have made me who I am. I express my deepest and heartfelt gratitude to my brothers Naeem, Dr Shahid, sisters Farhat, Dr Nazia, Qurat, Dr Rehana, Dr Ambreen Saba for their continuous and unparalleled love, help and support. They have always been a source of encouragement to me and have bestowed me with never ending affection. Any accomplishment in my life would not be possible without them.

Finally I must express my very profound gratitude to my Husband Dr. Zahid for providing me with unfailing support and continuous encouragement. Without your unending support and love, I never would have made it through this journey or any of the tough times in my life. Thank you.

Dr. Shaista Saeedatu Nissa

Place: Shuhama, Ganderbal

Dated:

CONTENTS

Chapter	Particular	Page No.
1	INTRODUCTION	1-5
2	REVIEW OF LITERATURE	6-29
2.1	Composition	7
2.2	Growth performance	8
2.3	Nutrient digestibility	13
2.4	Antioxidant activity	14
2.5	Immune response	18
2.6	Blood biochemistry	20
2.7	Carcass characteristics	22
2.8	Microbial population	23
2.9	Histomorpholgy	26
2.10	Enzyme supplementation in poultry	27
3	MATERIALS AND METHODS	30-46
3.1	Collection and processing of <i>Artemisia</i>	30
3.2	Proximate composition	30
3.3	Blood biochemistry	34
3.4	Immune competence	34
3.5	Histopathology	38
3.6	Cecal microbial population	41
3.7	Antioxidant status	42

3.8	Slaughter and carcass characteristics	44
3.9	Sensory evaluation of meat	45
3.10	Proximate composition of meat	45
3.11	Economics	46
3.12	Statistical analysis	46
4	EXPERIMENTAL FINDINGS	47-71
4.1	Proximate analysis	47
4.2	Body weight (g)	48
4.3	Body weight gain (g)	50
4.4	Feed consumption	52
4.5	Feed conversion ratio	55
4.6	Mortality	57
4.7	Percent digestibility of nutrients (%)	58
4.8	Blood biochemical	60
4.9	Slaughter traits and carcass characteristics	63
4.10	Histomorphology	63
4.11	Cecal microbial population	65
4.12	Immune competence	65
4.13	Antioxidant assay	67
4.14	Sensory evaluation/organoleptic evaluation	69
4.15	Proximate composition of meat	69
4.16	Economics	69

5	DISCUSSION	72-95
5.1	Proximate composition	72
5.2	Body weight and weight gain	73
5.3	Feed consumption	74
5.4	Feed conversion ratio	76
5.5	Mortality	78
5.6	Digestibility of nutrients	78
5.7	Blood biochemistry	80
5.8	Slaughter traits and carcass characteristics	84
5.9	Caecal microbial population	85
5.10	Histomorphology	87
5.11	Immune competence	89
5.12	Oxidative stability	90
5.13	Sensory and organoleptic evaluation	93
5.14	Proximate composition	93
5.15	Economics	94
6	SUMMARY AND CONCLUSION	96-100
	LITERATURE CITED	i-xxviii

LIST OF TABLES

Table No.	Particular	Page No.
3.1	Proximate composition of experimental diets (DM basis)	31
3.2	Paraffin wax embedding schedule	38
4.1	Proximate composition of <i>Artemisia absinthium</i>	47
4.2	Average weekly body weight (g) of broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	49
4.3	Average weekly body weight gain (g) in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	51
4.4	Average and weekly feed consumption (g) in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	54
4.5	Average weekly feed conversion ratio in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	56
4.6	Weekly and total mortality (%) in broilers chicken fed <i>Artemisia absinthium</i> with or without enzyme	57
4.7	Average nutrient digestibility (%) in broiler fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	59
4.8	Blood biochemistry in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	62
4.9	Slaughter traits in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	64

4.10	Carcass characteristics in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	64
4.11	Cecal microbial population in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	66
4.12	Total anti SRBC antibody titre in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	66
4.13	Skin thickness (mm) in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	68
4.14	Weight of immune organs in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	68
4.15	Plasma total antioxidant status (TAS) and total oxidant status (TOS) in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	68
4.16	TBARS assay of meat of broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	70
4.17	Sensory evaluation/organoleptic parameters of meat in broilers fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	70
4.18	Proximate composition of meat in broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	71
4.19	Cost of production of broiler chicken fed diets supplemented with <i>Artemisia absinthium</i> with or without enzyme	71

LIST OF FIGURES

Fig. No.	Particular	After page No.
4.1	Average body weight (g) of different treatment groups at weekly interval	49
4.2	Average body weight gain (g) of different treatment groups at weekly interval	51
4.3	Average feed consumption (g) of different treatment groups at weekly interval	54
4.4	Feed consumption ratio of different treatment groups at weekly interval	56

LIST OF PLATES

Plate No.	Particular	After page No.
1.	Fresh <i>Artemisia absinthium</i> plant	4
2.	Finely grinded <i>A.absinthium</i> leaves	30
3.	Histological section of Liver from control group (HE×100)	64
4.	Histological section of Liver from group fed 1% <i>Artemisia absinthium</i> (HE×100)	64
5.	Histological section of Liver from group fed 1.5% <i>Artemisia absinthium</i> (HE×100)	64
6.	Histological section of Liver from group fed 2% <i>Artemisia absinthium</i> (HE×100)	64
7.	Histological section of Liver from group fed 1% <i>Artemisia absinthium</i> with enzyme (HE×100)	64
8.	Histological section of Liver from group fed 1.5% <i>Artemisia absinthium</i> with enzyme (HE×100)	64
9.	Histological section of Liver from group fed 2% <i>Artemisia absinthium</i> with enzyme (HE×100)	64
10.	Histology of duodenum from control group showing normal villus architecture and histomorphology (HE×100)	65
11.	Histology of duodenum from group fed 1% <i>Artemisia absinthium</i> showing normal villus architecture and histomorphology (HE×100)	65

12.	Histology of duodenum from group fed 1.5% <i>Artemisia absinthium</i> showing normal villus architecture and histomorphology (HE×100)	65
13.	Histology of duodenum from group fed 2% <i>Artemisia absinthium</i> showing normal villus architecture and histomorphology (HE×100)	65
14.	Histology of duodenum from group fed 1% <i>Artemisia absinthium</i> with enzyme showing normal villus architecture and histomorphology (HE×100)	65
15.	Histology of duodenum from group fed 1.5% <i>Artemisia absinthium</i> with enzyme showing normal villus architecture and histomorphology (HE×100)	65
16.	Histology of duodenum from group fed 2% <i>Artemisia absinthium</i> with enzyme showing normal villus architecture and histomorphology (HE×100)	65
17.	Bacterial growth on Brain heart infusion agar for estimation of total viable count	65
18.	Bacterial growth on MacConkey agar for estimation of total coliform count	65
19.	Anti-SRBC antibody titre on microtitreplate	65
20.	Increase in skin thickness of foot web by DNCB antigen	68

ABBREVIATIONS

%	:	Per cent
% kg BW	:	Per cent body weight
°C	:	Degree Celsius
ADF	:	Acid detergent fibre
<i>ad lib</i>	:	Ad libitum
ADG	:	Average daily gain
A: G	:	Albumin: globulin ratio
ALT	:	Alanine aminotransferase
ALP	:	Alkaline phosphatase
AM	:	Ante meridian
ANOVA	:	Analysis of variance
AST	:	Aspartate aminotransferase
AOAC	:	Association of Official Analytical Chemists
BW	:	Body weight
CF	:	Crude fibre
CP	:	Crude protein
d	:	Day
DCP	:	Digestible crude protein
DCPI	:	Digestible crude protein intake
DDM	:	Digestible dry matter
DDMI	:	Digestible dry matter intake
DE	:	Digestible energy
dL	:	Decilitre
DLC	:	Differential leucocytes count
DM	:	Dry matter
DMI	:	Dry matter intake
DNCB	:	Dinitrochlorobenzene
DOM	:	Digestible organic matter
DOMI	:	Digestible organic matter intake
EDTA	:	Ethylene di amine tetra acetic acid
EE	:	Ether extract
EFEs	:	Exogenous Fibrolytic Enzymes
EPG	:	Eggs per gram
FCR	:	Feed conversion ratio
FER	:	Feed efficiency ratio
FECR	:	Faecal egg count reduction
FI	:	Feed intake
g	:	Gram
h	:	Hour (s)
Hb	:	Haemoglobin
HDL	:	How-density lipoprotein
HI	:	Humoral Immune
IVDMD	:	<i>In-vitro</i> dry matter digestibility
IVOMD	:	<i>In-vitro</i> organic matter digestibility
IVNDFD	:	<i>In-vitro</i> neutral detergent fibre digestibility
Kcal	:	Kilocalorie
kg	:	Kilogram
L	:	Litre
LDL	:	Low-density lipoprotein
MCH	:	Mean corpuscular haemoglobin
MCHC	:	Mean corpuscular haemoglobin concentration

MCV	:	Mean corpuscular volume
ME	:	Metabolizable energy
mg	:	Milligram (s)
mL	:	Mililitre
mm	:	Milimeter
MPV	:	Mean platelet volume
N	:	Nitrogen
NDF	:	Neutral detergent fibre
NFE	:	Nitrogen free extract
NH ₃	:	Ammonia
NH ₃ -N	:	Ammonia nitrogen
NPN	:	Non protein nitrogen
NR	:	Nutritive ratio
OM	:	Organic matter
OMI	:	Organic matter intake
Pct	:	Platelet percent
PCV/Hct	:	Packed cell volume/Haematocrit
PDW	:	Platelet distribution width
RDW	:	Red cell distribution width
RBC	:	Red blood cell
ROS	:	Reactive oxygen species
PI	:	Performance index
rpm	:	Revolutions per minute
SE	:	Standard error
SRL	:	Strained rumen liquor
TA	:	Total ash
TAS	:	Total antioxidant status
T-BRAS	:	Thiobarbuturic acid reactive substances
TCA-ppt- N	:	Tri-carboxylic acid precipitable nitrogen
TDN	:	Total digestible nutrients
TEC	:	Total erythrocyte count
TOS	:	Total oxidant status
TLC	:	Total leucocytes count
TTC	:	Total thrombocyte count
TVFA	:	Total volatile fatty acids
W ^{0.75}	:	Metabolic body weight
WBC	:	White blood cell
wt.	:	Weight

Chapter 1

INTRODUCTION

The poultry industry has been steadily expanding around the world for years, with major advancements in the last fifty years, by providing globally essential supplies of animal protein. According to a report by the United States Department of Agriculture (USDA), Agricultural Marketing Services. India is the world's third-largest egg producer and fifth-largest broiler meat producer. Broiler chicken growth rates have improved dramatically during the last fifty years. Feed is a crucial input in broiler production, accounting for 70-80% of the overall cost of production. In order to economize broiler farming. Feed additives are utilized in animal feeds to enhance feed quality in addition to animal overall performance and health. Feed additives are the substances known to cause a number of desired responses such as pH shifts, growth, or metabolic modifiers. Many natural compounds have a wide spectrum of growth-promoting, immune-stimulatory, antimicrobial, antioxidants pH regulating effects, and some of them used as enzymes are the most commonly utilised feed additives in poultry diets. Antibiotics were used as growth promoters in poultry feeds as it has played a significant role in the poultry business, but the global paradigm is evolving it away from productive efficiency due to public securities issues. At present consumers demand for an antibiotic-free products has risen significantly as the World Health Organization has identified antibiotic resistance as a major public health threat all over the global. Since 2006, when the European Union banned antimicrobial growth promoters in cattle and poultry feeds. In view of this, the poultry sector has been under pressure to find viable alternatives that can increase bird performance. In this regard, a number of studies have been conducted in order to develop viable antibiotic alternatives.

Recently, phytogenic feed additives (PFAs) have been considered as potential replacements to AGPs (Wati *et al.*, 2015). These are natural growth enhancers that include herbs, spices, essential oils, and oleoresins. Herbal plants

and plant-derived products, sometimes known as herbs, have been used for therapeutic purposes for ages. They are also widely utilised in a variety of Asian, African, and other countries around the world. The use of phytogenic or plant-based feed additives in poultry diets as prospective antibiotic growth promoter alternatives has gotten a lot of interest in recent decades. Scientists, nutritionists, feed manufacturers, producers, and farmers appear to be interested in phytogenics. Phytogenic feed additives (PFA) are defined as “sensory and flavorful components, which are primarily composed of plant extracts (essential oils, oleoresins, and flavonoids) and their active principles” according to European Union legislation (EC 1831/2003, European Commission, 2003). When compared to synthetic antibiotics or inorganic chemicals, these plant-derived compounds are considered to be safe, less toxic, and residue-free feed additives in animal production. These work primarily by influencing the environment of the gastrointestinal microbiota in a positive way by controlling possible pathogens. Improved digestive capacity in the small intestines may be an unintended consequence of phytogenics maintaining gut bacteria eubiosis (Hernandez *et al.*, 2004). As a result, phytogenics help animals grow better within their genetic potential by relieving them of immune defence stress during key moments and increasing the intestinal availability of important nutrients for absorption (Windisch *et al.*, 2008).

The presence of essential oils and other bioactive compounds in phytogenic feed additives contributes to their positive attributes. They present a viable option since they improve a number of critical processes in the animal body and so can be used to improve feed quality, animal health, and animal products through the use of their specific effective compounds. Herbal plants and their derivatives, such as plant extracts or essential oils, have been studied as phytogenic feed additives in broiler diets in a number of studies. They promote poultry productivity and health by stabilising normal gut microbiota, preventing pathogen colonization and increasing the synthesis of digestive enzymes. They

also have immunological effects on the body of the bird. Gut health is a hot topic in both humans and animals, and it has an impact on the animal's overall requirements and nutrient uptake. Maintaining a healthy intestinal environment is essential for successful broiler production. It has been established that herbal plants and their products, such as extracts or essential oils, have favourable effects on poultry. As a result, these phytogetic feed additives have been suggested as potential new broiler diet choices. In ancient Indian literature many herbs are described for strengthening of body and keeping diseases away. Among them extracts of cloves, red pepper, nutmeg, curcuma, black pepper, cumin and mint have shown anti-inflammatory action. Several researchers have shown the antimicrobial effect of *Allium sativum*, *Angelica dahurica*, *Polygonum cuspidatum* etc.

Artemisia, locally known as “Tethwan” is a big and diverse genus of plants belonging to the Asteraceae family with over 500 species. *Artemisia* species are found throughout temperate North America (Mexico, the United States, and Canada), the Mediterranean, Asia (India, Pakistan, Afghanistan, Iran, and Siberia), Africa, and Australia This genus contains valuable medicinal plant species that have been used for pharmacological and culinary purposes since ancient times. From a chemical, pharmacological, and taxonomic standpoint, *Artemisia* species are well-known for treating hepatitis, cancer, inflammation, and infections caused by fungus, bacteria, and viruses. The *Artemisia* genus is known to contain a variety of bioactive chemicals, including artemisinin, which has antimalarial and cytotoxic properties against tumour cells.

The valley of Kashmir, also known as Earth's Paradise is located at an altitude range of 1500-2700 m or 5000-7000ft (Chopra, 1956) in the northwestern extremity of the Himalayan area. The region is home to a diverse diverse spectrum of wildlife as well as remarkable plant biodiversity that is both scientifically interesting and economically beneficial. *A. amygdalina*, *A. annua*, *A. absinthium*, *A. maritima*, *A. roxburghiana*, and *A. sieversiana* are among the 8-10 species of

this plant that grow in the valley's natural low-lying areas. It has a short, herbaceous perennial plant with greyish white leaves (Plate 1). The active chemicals absinthin and ana-absinthin, which are abundant in the plant, are responsible for its medical efficacy.

Traditionally genus *Artemisia* has been of noticeable ethnopharmacological noteworthiness as the dried leaves and blossoming tops are utilized as antihelminthic, antispasmodic, carminative, narcotic, stimulant and tonic (Habibi *et al.*, 2016). The essential oil of this plant contains silica, two bitter substances (absinthin and anabsinthine), resinous substances, malic acid, thujone, tannic, and succinic acid, chrysanthenyl acetate, cis-epoxyocimene, sabinene, bornyl acetate, artemisia ketone, linalool, 1,8-cineole, myrcene, myrtenol, hydrocarbon mono-terpenes, α and β -thujone, trans-sabinyl acetate, β -pinene, camphor, sesquiterpene lactones, etc. (Martinez *et al.*, 2015). The essential oil has antibacterial, antifeedant, antipyretic, fertility-inducing, cytostatic, and antimalarial properties (Kostadinovic *et al.*, 2014).

Since four decades, enzyme supplementation has been used as feed addition in poultry. Enzymes are a type of biological catalyst that are made up of amino acids, vitamins, and minerals. They cause biological processes without enduring any changes themselves. Exogenous enzyme addition in broiler diets has been studied extensively, with increases in broiler chick performance and nutritional availability well documented. The use of enzymes in poultry diets has a number of advantages, including improved bird performance and feed conversion as well as fewer environmental issues due to reduced excreta output. The addition of enzymes to chicken diets can help to eliminate anti-nutritional components and promote the utilisation of dietary energy and amino acids, resulting in better performance (Zhou *et al.*, 2009). Furthermore, the enhancement of growth performance as a result of enzyme supplementation could be related to enzyme involvement in reducing intestinal viscosity and modulating gut flora (Abdel Latef *et al.*, 2017). The release of phytochemicals and other bioactive compounds in



Plate-1: Fresh *Artemisia absinthium* plant

plants is limited by cell wall. Enzymatic treatment of plant cell walls with cellulases, pectinases, and hemicellulases, either separately or in combination, can hydrolyze and break the cell wall, allowing for the release of internal biologically active chemicals (Puri *et al.*, 2012).

Based on the worth mentioning values of *Artemisia* spp., a research study was done to investigate the potential of *Artemisia* spp. in broiler diets, with the following goal:

1. To study the effect of supplementation of broiler diets with *Artemisia absinthium* alone and in combination with enzyme on performance and nutrient utilisation in broiler chicken
2. To study the effect of supplementation of broiler diets with *Artemisia absinthium* alone and in combination with enzyme on Physiological health status of broiler chicken
3. To study the effect of supplementation of broiler diets with *Artemisia absinthium* alone and in combination with enzyme on the carcass characteristics of broiler chicken
4. To workout the economics of feeding diets supplemented with *Artemisia absinthium* alone and in combination with enzyme in broiler chicken

Chapter 2

REVIEW OF LITERATURE

The irrational use of antibiotics in animals have led to the emergence of new pathogens, cross-resistance against pathogens and residues in tissues. The growing concerns for antibiotic resistance have led to the ban of most of the antibiotic growth promoters in many countries. Plant derived products which are natural, less toxic, residue free are considered ideal feed additives in animal nutrition (Prakasita *et al.*, 2019). Phytogetic feed additives have been used as suitable alternative to the antibiotics. These are the compounds of plant origin which have anti-microbial and growth promoting effect on poultry. The beneficial effects of botanical additives in poultry (broilers, layers, broiler breeders, and layer breeders) arise from the positive effect on feed intake, increased growth performance, enhanced digestive secretions, immune stimulation, reduced incidence of disease, improved reproductive parameters, antibacterial, coccidiostatical, antiviral and anti-inflammatory activity. The active principles in the leaves, stem, seeds, roots and barks of these plants are highly effective to combat different diseases and improve the digestion.

Artemisia absinthium

Artemisia is a big and diverse genus of plants belonging to the Asteraceae family with over 500 species. This genus contains valuable medicinal plant species that have been used for pharmacological and culinary purposes since ancient times. From a chemical, pharmacological, and taxonomic standpoint, *Artemisia* species are well-known and have been used to treat ailments such as hepatitis, cancer, inflammation, and infections caused by fungi, bacteria, and viruses (Asad *et al.*, 2011). *Artemisia absinthium* L. (Asteraceae) locally known as “Tethwan,” is an aromatic, herbaceous, perennial plant commonly known as wormwood is extensively spread over Europe, North Africa, portions of Asia, and North and South America. *Artemisia absinthium* is an important medicinal plant

with a wide ethnopharmacological application (Kennedy and Deans, 2001). *A. absinthium* extracts and essential oils are used for healing various diseases. Anthelmintic, antibacterial, antifungal, insect repellent, narcotic, digestive, tonic and other bioactivities are characteristic of preparations from wormwood plants. Their stimulant property is dependent on bitter substances as artabsin (sesquiterpene lactone) and absinthin (dimmer of sesquiterpene lactones) present in plant extracts (Wright, 2002).

2.1 Composition

The different species of genus *Artemisia* have been found to be effective for use as feed additive in animals and poultry. *Artemisia* species are rich sources of sesquiterpene lactones, antioxidant phenolics, flavonoids and other biologically active compounds. All these compounds have many beneficial properties and enhance the animal performance and health

Artemisia annua leaves contain about 19.66 per cent Crude protein, 14.4 per cent Crude fibre, 5.73 per cent Crude fat, 10.26 per cent Ash content, 49.94 per cent Digestible carbohydrates, 0.46, 2.10, 1.60, 1, 0.011, 0.072, 0.03 per cent P, K, Ca, Mg, Zn, Fe and Na respectively (Brisbie *et al.*, 2008).

The phytoconstituents of *Artemisia absinthium* consist of essential oil, sesquiterpene lactones, tannins, carotenoids, lignans, glucosides, phenolic compounds, flavonoids and some bitter principles (Bora and Sharma, 2010)

Iqbal *et al.* (2012) reported that the chemical composition of *Artemisia annua* leaves comprised of 7.5 per cent Ash, 8.3 per cent Carbohydrate, 6.07 per cent Fat, 14.2 per cent Fibre, 11.4 per cent Moisture and 24.37 mg/100 g Protein on dry matter basis.

Artemisia vulgaris comprised of 13.87 per cent moisture, 15.80 per cent total ash, 12.85 per cent starch, 8.66 per cent sugar, 0.21 per cent tannin and 2.16 per cent phenol. The hexane and methanolic extracts of *Artemisia vulgaris* revealed the presence of saponins, phytosterols, carbohydrates, proteins & amino

acid, and flavonoids, ascorbic acid and phenolics both of which are powerful antioxidants (Ashok and Upadhyaya, 2013)

Monica and Kaur (2014) found that the important constituents present in *Artemisia absinthium* plant extract were flavonoids, phenols, saponins, tannins, alkaloids, and quinones. Through HPLC analysis the important flavonoids present in plant extract were Quercetin, Rutin, Kaempferol, and Gallic acid due to which the plant possess antimicrobial, antioxidant and antimalarial activity.

The constituents of essential oil from *Artemisia absinthium* include chrysanthenyl acetate, cis-epoxyocimene, sabinene, bornyl acetate, Artemisia ketone, linalool, sesquiterpene lactones, 1,8-cineole, myrcene, myrtenol, α and β -thujone, trans-sabinyl acetate, β -pinene, camphor, hydrocarbon monoterpenes etc. (Martínez-Díaz *et al.*, 2015).

Artemisia herba-alba contain 29.6 per cent crude fibre, 11 per cent crude protein which consists of 72% of amino acids and 16.5 per cent total nitrogen content. The mineral composition consists of Ca, P, K, Na and Mg as 10.6, 3.3, 35.5, 0.4 and 2.5g/kg respectively (Ait-Kaki *et al.*, 2018).

2.2 Growth performance

The effect of phytogenic feed additives on the growth performance of poultry is mainly due to their effects of improving gut microbiota, nutrient digestibility and antioxidant properties which leads to improvement in body weight, body weight gain, feed intake, FCR, carcass characteristics. They influence the digestive secretions and enhance the activities of digestive enzymes. The intestinal activities of trypsin, lipase and amylase in broilers is enhanced by the phytogenic compounds (Lee *et al.*, 2004).

Brisbie *et al.* (2008a) reported that the broilers supplemented with 10% and 20% of dried *Artemisia annua* leaves in the commercial mash had significantly ($P < 0.05$) higher live weight gain and better feed conversion ratio compared to control group.

In laying hens supplementation of the feeds with up to 20% of *Artemisia* leaves had a significant effect ($P < 0.05$) on weekly egg production rate and the mean egg size. The intensity of yolk colour was also higher in *Artemisia* fed birds compared to the control treatment (Brisbie *et al.*, 2008b)

Dragan *et al.* (2010) found that *Artemisia annua* oil extract significantly improved weight gain and feed conversion in broilers compared to control group.

Addition of 1% *Artemisia sieberi* leaves did not affect body weight and feed conversion ratio but decreased feed intake significantly up to 21 d of age ($P \leq 0.01$) in broilers (Khalaji *et al.*, 2011).

Supplementation of diets with *Artemisia annua* significantly improved weight gain in broilers (De Almeida *et al.*, 2012).

Chu and Song (2012) reported that supplementation of basal diet with 0.5% *Artemisia montana* did not significantly affect the feed intake, feed efficiency and growth performance in fattening pigs.

Engberg *et al.* (2012) investigated the effect of increasing dietary concentrations of dried *A. annua* leaves (0, 5, 10 and 20 g/kg) and n-hexane extract from fresh *A. annua* leaves (0, 125, 250 and 500 mg/kg) on broiler performance. They found that the supplementation resulted in dose dependent decrease in body weight and feed consumption and an improved feed conversion ratio.

Supplementation of *Artemisia vulgaris* L. powder and *Pinus densiflora* powder at 1 and 2% in broilers diets had no effect on growth performance (Kim *et al.*, 2012a).

Kim *et al.* (2012b) observed the effect of dietary supplementation of 2.5 and 5.0 g/kg of *Lactobacillus*-fermented *Artemisia princeps* (LFA) on growth performance in Hy-line Brown male chickens and observed that increasing inclusion level of LFA in diets significantly ($P < 0.05$) improved body weight gain and feed efficiency.

Cherian *et al.* (2013) reported that there was no significant difference on body weight, body weight gain and feed efficiency of broilers fed diets supplemented with 0, 2 and 4% *Artemisia annua* leaf powder compared to control group.

Supplementation of 2000, 4000 ppm methanolic extract and 0.5%, 1% and 1.5% leaf powder of *Artemisia annua* significantly ($P<0.05$) increased daily weight gain, reduced daily feed intake and feed conversion ratio in broiler chicken (Gholamrezaie *et al.*, 2013).

The inclusion of 100g, 150g and 200g/kg of *Artemisia absinthium* in broiler diets significantly ($P<0.05$) improved body weight gain and lowered feed efficiency in treatment groups compared to control group. The inclusion level of 200g/kg of *Artemisia absinthium* was found to have significantly ($P<0.05$) highest body weight gain and lowest feed efficiency compared to other treatment groups. (Kostadinovic *et al.*, 2015).

Supplementation of broiler diets with 100, 200 and 300 ppm of Wormwood (*Artemisia absinthium*) and Cumin (*Cuminum cyminum*) essential oil had no significant effects on growth performance of broiler chicken (Habibi *et al.*, 2016b).

Wan *et al.* (2016) reported no significant effect of the supplementation of broiler diets with enzymatically treated *Artemisia annua* L. on the body weight, average daily feed intake and feed efficiency of broilers.

Beigh *et al.* (2017) assessed the effect of supplementation of *Artemisia absinthium* L. as feed additive, alone and in combination with exogenous fibrolytic enzymes cocktail on nutritional profile in lambs. It was observed that the supplementation significantly ($P<0.01$) improved the mean apparent digestibility values, net weight gain and average daily gain (ADG) in all feed additives supplemented groups in comparison to control.

Bhat *et al.* (2017) evaluated the effects of feeding phytogenic feed additive, *Artemisia absinthium* on nutrient intake, nutrient digestibility, utilization and performance of sheep. The authors found statistically higher ($P<0.05$) digestible dry matter intake, organic matter intake, crude protein intake and total digestible nutrient intake in treatment groups as compared to control groups

The addition of 200 g/kg of *Artemisia absinthium* (wormwood) in rabbit diet led to the highest body weight as well to the lowest feed conversion ratio with significant differences compared to the control treatment (Popovic *et al.*, 2017).

Dietary supplementation of 1g/kg enzymatically treated *Artemisia annua* increased ($P<0.05$) body weight gain and feed intake in heat stressed broilers compared to the control group thus alleviating the heat-stress-induced compromised growth performance in broilers (Song *et al.*, 2018).

It was found that the supplementation of *Artemisia absinthium* (wormwood) herb alone as feed additive in complete diets in lambs enhanced the growth rates. A significantly ($P<0.05$) higher nutrient intake, digestibility, total body weight gain and average daily gain in lambs supplemented with *Artemisia absinthium* was observed (Beigh *et al.*, 2018).

Addition of 0.5% *Artemisia herba* powder significantly ($P<0.05$) increased the egg production, feed conversion ratio and egg mass while decreasing the feed consumption in laying hens (Multag *et al.*, 2018).

Popovic *et al.* (2018) found no significant difference in the body weight and feed efficiency of broilers fed diets supplemented with medicinal plants mixtures (*Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum*).

Supplementation of a dietary herb mixture significantly increased feed consumption, body weight gain, feed conversion ratio and breast muscle weight in broilers (Saleh *et al.*, 2018)

It has been reported that under heat stress broilers fed a mixture of 1% *Artemisia annua* powder and 0.005% oil had significantly ($P<0.05$) higher body weight and average daily feed intake compared to control (Saracila *et al.*, 2018).

Artemisia annua leaves significantly ($P<0.05$) reduced the feed consumption ratio, increased yolk color index and shell thickness and decreased egg yolk cholesterol in laying hens compared to the control (Baghban Kanani *et al.*, 2018).

Diets supplemented with *Artemisia apiacea* Hance at 25, 50, and 75 g/kg significantly ($P<0.05$) improved feed intake, body weight gain, and feed efficiency in rabbits compared to control group with more pronounced effects at 50 and 75 g/kg doses as compared to 25 g/kg dose (Ding *et al.*, 2019).

Oso *et al.* (2019) observed the effects of dietary supplementation with phytogenic blend (PB) of *Aerva lanata*, *Piper betle*, *Cynodon dactylon* and *Piper nigrum* on growth performance of broilers and found that the diets supplemented with 1% phyogenic blend resulted in highest body weight gain and FCR as compared to the control.

Wang *et al.* (2019) investigated the effect of *Artemisia vulgaris* meal supplementation on the growth performance of rabbits and found that diets supplemented with *A. vulgaris* meal improved final body weight, feed intake, body weight gain and decreased feed conversion ratio of rabbits.

Dietary supplementation with 2 g/kg enzymatically treated *Artemisia annua* L. significantly ($p<0.05$) increased growth performance, nutrient digestibility and decreased the incidence of diarrhoea in weaned pigs (Yu Niu *et al.*, 2020).

The effect of *Artemisia annua* leaves extract (ALE) on the growth performance of common carp (*Cyprinus carpio*) was studied and a significant improvement was observed in the growth performance and feed conversion of fish compared to control group (Sarhadi *et al.*, 2020).

Dietary inclusion of *Artemisia annua* L. aqueous extract (AAE) significantly improved the average daily gain, feed conversion ratio and decreased the feed intake of broilers (Guo *et al.*, 2020).

Yang *et al.* (2021) studied the effects of *Artemisia argyi* flavonoids (AAF) supplemented in diets on the growth performance of broiler chickens challenged with lipopolysaccharide (LPS). They found that dietary AAF significantly ($p<0.05$) improved the body weight and alleviated the decrease of average daily gain in broilers challenged with LPS on d 21 and d 35 compared to the control.

2.3 Nutrient digestibility

Amad *et al.* (2011) studied the effects of a phytogenic feed additive (PFA) containing essential oils of thyme and star anise on the apparent ileal nutrient digestibility in broiler chickens. They found that the means of the ileal digestibility of crude ash, crude protein, crude fat, and the net absorption of calcium and phosphorus were significantly ($P<0.05$) higher in the supplemented groups compared with those of the control and birds fed with the highest additive concentration (1,500 mg/kg of PFA) showed the highest apparent ileal nutrient digestibility of nutrients.

Khodambashi Emami *et al.* (2012) reported that feeding broilers a diet supplemented with 200 mg/kg peppermint essential oil significantly ($P<0.01$) increased the digestibility of crude protein.

Murugesan *et al.* (2015) conducted an experiment to evaluate the effects of phytogenic feed additives as an alternative to antibiotic growth promoters on nutrient digestibility of broilers and found that supplementation of phytogenic feed additives to the basal diet significantly ($P<0.05$) increased the apparent total tract digestibility of crude protein and ether extract when compared to the control group.

Dietary supplementation with 1 and 2 per cent phytogenic blend (PB) of *Aerva lanata*, *Piper betle*, *Cynodon dactylon* and *Piper nigrum* increased the

apparent ileal digestibility of organic matter, serine, arginine, threonine and tryptophan in broilers with highest apparent ileal digestibility of tryptophan at 1% level as compared to the control (Oso *et al.*, 2019).

Supplementation of exogenous protease significantly ($p < 0.01$) increased the digestibility of crude protein, crude fat, nitrogen-free extract and calcium in broilers compared to the control (Hafeez *et al.*, 2021).

Mohebodini *et al.* (2021) found that the apparent ileal digestibility of organic matter and ether extract increased significantly in broiler chickens fed diets supplemented with 0 to 1,000 mg/kg eucalyptus essential oil ($P < 0.05$). However, they observed no significant effects on apparent dry matter and crude protein digestibility.

Wang *et al.* (2021) conducted an experiment to evaluate the effect of a phytogenic feed additive (PFA) on nutrient digestibility of broilers fed corn and soybean meal-based diets containing two different levels of crude protein. They found that the PFA addition did not cause significant differences in the digestibility of dry matter, crude protein, and ileal digestible energy among the treatments at 21 and 42 days of age.

2.4 Antioxidant activity

Aromatic and medicinal plants are an edible and easily accessible source of natural antioxidants. The antioxidant effect of plant products is mainly attributed to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes. These compounds can be used as electron or hydrogen suppliers to reduce free radical formation and scavenging free radicals which is responsible for their antioxidant capacity (Pietta 2000). Phenolic compounds prevent radical formation, scavenge free-radicals, chelate metal ions and improve the antioxidant endogenous system and therefore act as powerful antioxidants (Qingming *et al.*, 2010).

The antioxidant activity of *Artemisia* spp. could be attributed to its flavonoidal content. They are rich sources of sesquiterpene lactones, antioxidant phenolics, flavonoids and other biologically active compounds. The flavonoids such as quercetin, rutin and other flavonoid glycosides and phenolic acids such as chlorogenic, syringic, coumaric, salicylic and vanillic acids are probably involved in the mechanism of free-radical scavenging activity (Rice Evans *et al.*, 1996). These flavonoids act as scavengers of various oxidizing species and they also act as quenchers of singlet oxygen. Phenols are also very important plant constituents which possess antioxidant activity. Phenolic compounds are effective hydrogen donors and their hydroxyl group have the scavenging abilities. Therefore, there is a highly positive relationship between total phenols and antioxidant activity of many plant species.

Aerial parts of *A. absinthium* have been shown to possess potent antioxidant and free radical scavenging activity (Braca *et al.*, 2003).

Jasna *et al.* (2005) reported the free radical scavenging activity of *Artemisia absinthium* Linn. Extract by measuring their ability to scavenge stable 2,2-diphenyl-1-picrylhydrazyl free radical and reactive hydroxyl radical during the Fenton reaction trapped by 5,5-dimethyl-1-pyrroline-N-oxide, using electron spin resonance spectroscopy.

A. absinthium has been reported to contain flavonoids, thymol, carvacrol as well as other phenolic compounds (Kordali *et al.*, 2006).

Birds fed diets supplemented with *Artemisia annua* leaf powder had significantly lower malondialdehyde content in the breast and thigh muscles compared with the control birds (Cherian *et al.*, 2013).

The diversified phenolic compounds and flavonoids in *A.annua* are potent scavenger of free radicals (Gouveia and Castilho, 2013).

Addition of *Artemisia princeps* Pamp extract delayed the lipid oxidation of raw chicken patties stored for 12 days under 4°C in refrigeration, which may extend the shelf life of chicken patties (Hwang *et al.*, 2013).

Kostadinovic *et al.* (2015) reported that supplementation of broiler diets with 200g/kg *A. absinthium* significantly ($P<0.05$) reduced the malondialdehyde activity in blood, plasma and liver and increased the blood levels of glutathione peroxidase as compared to control group. They also found significantly ($P<0.05$) higher oxidative stability of broiler meat in groups fed 200g/kg *A. absinthium* compared to other treatment groups (100 and 150g/kg) and the control group.

Chu *et al.* (2015) reported that broilers supplemented with 1,000 mg/kg *Artemisia argyi* aqueous extract (AAE) improved the serum superoxide dismutase (SOD) activity and decreased the serum malondialdehyde (MDA) content.

Supplementation of 500, 1000 and 2000 mg/kg *Artemisia argyi* aqueous extract to the basal diet increased the antioxidant capabilities of small intestine tissue in broilers. At 21 days of age, supplementation of 500 mg/kg AAE improved the intestinal tissue antioxidant enzymes activity, especially the activity of Catalase. While at 42 days of age, the antioxidant function of small intestinal in 1,000 mg/kg group had the best promotion effect (Zhao *et al.*, 2016).

Wan *et al.* (2016a) conducted an experiment to evaluate the effects of including enzymatically treated *Artemisia annua* L. (EA) in broiler diets on the oxidative stability of breast and thigh muscles. They found that the diets supplemented with various levels of EA decreased the malondialdehyde concentrations and increased free radical scavenging activities of breast and thigh muscles in broilers, indicating that EA supplementation improved the antioxidant capacity of meat.

In other experiment (Wan *et al.*, 2017) found that the dietary addition of enzymatically treated *Artemisia annua* L. (EA) enhanced ($P<0.05$) the activities of total superoxide dismutase and glutathione peroxidase and reduced ($P<0.05$) malondialdehyde concentration in serum and liver of broilers at 21 d and 42 d and increased ($P<0.05$) activity of catalase at 42 d in liver of broilers compared to control.

Popovic *et al.* (2017) reported that feeding 100g/kg dried *Artemisia absinthium* plant to rabbits significantly ($P<0.05$) reduced the levels of malondialdehyde in liver homogenate while increasing the levels of glutathione peroxidase, superoxide-dismutase and glutathione reductase.

Wan *et al.* (2017) conducted an experiment to study the effect of dietary enzymatically treated *Artemisia annua* (EA) on, antioxidant capacity of breast muscle in heat stressed broilers. They found that EA reduced the reactive oxygen metabolites and thiobarbituric acid reactive substances, increased the antioxidant enzyme activities and reduced the free radical scavenging abilities of breast muscle in heat stressed broilers

Artemisia annua leaf extract has been found to possess immunomodulatory and antioxidative properties due to the monomer polyphenolic compounds which may help in improving the health status and the growth performance of animals (Koul *et al.*, 2017).

Song *et al.* (2018) found that the diets supplemented with enzymatically treated *Artemisia annua* (EA) significantly ($P<0.05$) increased the jejunal total superoxide dismutase activity and glutathione peroxidase content and decreased the jejunal malondialdehyde concentration in the heat stressed broilers compared with the control group.

Baghban Kanani *et al.* (2019) performed a study to investigate the effects of using *Artemisia annua* leaves on the antioxidant status of laying hens and found significantly higher glutathione peroxidase level and lower and malondialdehyde levels in hens fed 7.5% *A. annua* leaves compared to other treatments (i.e. 2.5% and 5% *A. annua* leaves) and control.

A. absinthium contains various flavonoids and phenolic compounds that are possibly involved in the mechanism of free radical scavenging activity (Javed Ahmed *et al.*, 2019)

Artemisia dracunculus (Tarragon) supplementation significantly reduced the malondialdehyde levels of the serum, liver and yolk, decreased the plasma corticosterone (CORT) and serum total oxidant (TOS) levels induced by high stocking density in laying hens (Kaya *et al.*, 2020).

Sarhadi *et al.* (2020) investigated the effect of *Artemisia annua* leaves extract on the immune antioxidant capacity of common carp (*Cyprinus carpio*) and found increased levels of catalase enzyme and decreased levels of malondialdehyde (MDA) in fish fed *Artemisia annua* leaf extract.

Shiwei Guo *et al.* (2020) studied the effects of *Artemisia annua* L. aqueous extract on antioxidant capacity of broilers and found that *Artemisia annua* L. aqueous extract elevated superoxide dismutase, glutathione peroxidase and catalase activities and reduced malondialdehyde concentration in serum, liver and spleen. They also found that *Artemisia annua* L. aqueous extract improved the gene expression of antioxidant enzymes in the liver and spleen hence, increasing the activity of the body's antioxidant enzymes and improving the body's antioxidant abilities.

Yanli *et al.* (2021) observed that dietary inclusion *Artemisia argyi* flavonoids in broilers ameliorated the antioxidative status when stimulated by lipopolysaccharide (LPS) and alleviated the negative effects of immune stress by regulating the production of pro-inflammatory cytokines in serum of broilers.

2.5 Immune response

Dietary supplementation of enzyme and oregano essential oil at two levels, alone and in combination had no significant effect on immune response of broilers (Malayoglu *et al.*, 2010).

Khalaji *et al.* (2011) studied the effects of using black cumin seeds, *Artemisia* leaves and *Camellia* L. plant extract in the diets of broiler chicks and found that *Artemisia* leaves did not affect primary or secondary antibody response against the sheep red blood cells.

Chu and Song (2012) reported that the addition of 0.5% dried powder of *A. Montana* to basal diet of fattening pigs significantly ($p<0.05$) increased the immunoglobulins M and G and interferon- gamma of blood cells hence improving the immune response.

Gholamrezaie Sani *et al.* (2013) studied the effects of methanolic extract and leaf powder of *Artemisia annua* on cellular and humoral immunity in broiler chicks. They found that the extract and leaf powder of *Artemisia annua* significantly ($P<0.05$) increased cellular immunity on PHA-P injection after 24 and 48 h, total anti-SRBC and IgG titer in experimental groups compared to control group. Thymus and bursa of fabricius weights were also significantly ($P<0.05$) increased in treatment groups.

Addition of 200 ppm *Artemisia absinthium* essential oil to broiler diets significantly ($P<0.05$) increased haemagglutination titre against sheep red blood cells (SRBC) in broilers after day 7 and 14 of injection. Also the weight of bursa of fabricius was significantly ($P<0.05$) increased in the treatment groups compared to control group (Habibi *et al.*, 2016).

The supplementation with enzymatically treated *Artemisia annua* (EA) significantly ($P<0.05$) increased the concentrations of Intestinal secretory immunoglobulin A (SIgA) and immunoglobulin G (IgG) concentrations in heat stressed broilers compared with the control (Song *et al.*, 2018).

Saleh *et al.* (2018) conducted an experiment to study that effect of phytogenics (herbal mixture) supplementation as alternative to antibiotics on the immune response of broilers and found that plasma levels of total protein and antibodies titers for Newcastle disease virus before and after the infection were significantly improved in treatments fed the herb mixture supplemented diet compared with the control treatment.

Aroche *et al.* (2018) studied the effect of the mixed powder of medicinal plant leaves on immunoglobulin concentration of broiler chickens and found that a

small amount of powder inclusion (0.5%) significantly exerted beneficial immune effect, through an increase in the IgG concentration.

Wang *et al.* (2019) investigated the effect of *A. vulgaris* meal supplementation on the immune function in rabbits and found significantly ($P<0.05$) increased blood levels of IgA and IgM, lymphocytes of bursale, thymus, CD4 and CD8 hence improving the immunity in rabbits.

Yu Niu *et al.* (2020) found that enzymatically treated *Artemisia annua* L. (EA) significantly ($p<.05$) decreased the concentrations of IL-1 β , IL-6 and TNF-alpha in the jejunum, and increased ($p<.05$) the concentrations of IL-10, sIgA and IgG in the ileum of weaned pigs compared with those fed the basal diet hence reducing the inflammation and enhancing the immunity of weaned pigs.

2.6 Blood biochemistry

Malayoglu *et al.* (2010) conducted a study to determine the effects of dietary supplementation of enzyme and oregano essential oil at two levels, alone or together on lipid metabolism of broilers. They found that the high supplementation (500 mg/kg) of essential oil with and without enzyme significantly ($P<0.05$) increased the serum total cholesterol but there was no significant effect of dietary treatments on serum triglyceride and HDL cholesterol.

Supplementation of *Artemisia sieberi* leaves in the diet of broilers did not significantly alter plasma cholesterol, triglyceride, HDL, LDL, VLDL, LDL: HDL ratio, hematocrit, hemoglobin, and differential counting of lymphocytes, eosinophils and basophils (Khalaji *et al.*, 2011).

It has been reported that the addition of 0.5% dried powder of *A. Montana* to basal diet of fattening pigs concentration did not significantly affect the erythrocytes, hemoglobin, MCV, MCH, MCHC, platelets, total protein, albumin, total cholesterol, triglyceride, albumin/globulin, HDL-cholesterol and LDL-cholesterol. However, it significantly ($p<0.05$) decreased leukocytes concentration, lactate dehydronase, blood urea nitrogen and plasma cortisol while increasing the hematocrit concentration (Chu and Song, 2012).

Inclusion of *Artemisia vulgaris* L. and *Pinus densiflora* powder in broiler diets significantly ($P < 0.05$) decreased the levels of total serum cholesterol and LDL-cholesterol and increased the levels of HDL-cholesterol (Kim *et al.*, 2012a).

Khattak *et al.* (2013) observed the effect of a novel commercial preparation of natural blend of essential oils from basil, caraway, laurel, lemon, oregano, sage, tea and thyme (Tecnaroma Herbal Mix PL) on blood biochemistry of broilers and found no differences ($P > 0.05$) between treatments in blood biochemistry parameters.

Hosseinzadeh and Farhoomand (2014) investigated the effects of different levels (0.125%, 0.25% and 0.5%) of *Artemisia dracunculus* powder on the blood biochemical profile of broilers and found no significant difference in the levels of calcium, phosphorus, glucose, cholesterol, triglycerides, VLDL, LDL and HDL between different treatments.

Popovic *et al.* (2018) observed the influence of medicinal plants mixtures (*Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum*) in broiler diets on their blood biochemical status. They found no significant ($P > 0.05$) difference on the levels of triglycerides and cholesterol ($p > 0.05$) in the treatment group. However the medicinal plants mixture statistically ($p < 0.05$) increased the HDL-cholesterol and decreased LDL cholesterol concentration in treatment group compared to control group.

Supplementation of broiler diets with phytogenics (herb mixture) significantly ($P < 0.05$) reduced the plasma total cholesterol, glucose and triglyceride while increasing the plasma high-density lipoprotein compared to the control group (Saleh *et al.*, 2018).

Supplementation of *Artemisia annua* leaves significantly reduced the plasma LDL and cholesterol but did not affect the triglycerides and HDL levels in laying hens (Baghban Kanani *et al.*, 2018).

Abdul hafeez *et al.* (2020) evaluated the effect of diet supplemented with phytochemicals and protease enzyme in broiler diets and found no significant difference in blood metabolites between the control and treatment groups.

2.7 Carcass characteristics

In terms of quality, the dietary intake of phytoadditive compounds has shown beneficial effects on stored meat quality, an effect related to their antioxidant properties in the case of the reduction or delaying of lipid oxidation (Puvaca *et al.*, 2013). Lipid oxidation is one of the major causes of the deterioration in meat quality during storage as the poultry meat is rich in polyunsaturated fatty acids (Grashorn, 2007). Methods that are safe, effective and consumer-friendly for limiting lipid peroxidation are extremely important to the poultry meat industry. Research has indicated that feeding diets rich in phenolics and flavonoids to broilers can reduce lipid oxidation products in meat and enhance meat lipid stability during storage (Aziza *et al.*, 2010). It is widely acknowledged that the addition of phytoadditive compounds such as herbs, spices or essential oils in foods of animal origin contribute to food microbiological safety and quality upon food storage in the raw or cooked stages through their antimicrobial and antioxidant functions (Soltan *et al.*, 2008).

Dietary supplementation of *Artemisia sieberi* leaves in broilers did not affect the relative weight and length of different parts of the carcass and gastrointestinal tract (Khalaji *et al.*, 2011).

Kim *et al.* (2012a) studied the proximate composition of thigh muscle of broilers fed diets supplemented with *Artemisia princeps* and *Pinus densiflora* powder and found no significant difference in moisture, crude protein and crude ash content. However, they found that inclusion of *Artemisia princeps* (1% or 2%) and *Pinus densiflora* powder (1% or 2%) in the diets significantly ($P < 0.05$) decreased crude fat contents of chicken thigh meat compared with the control groups.

Dietary supplementation of *Lactobacillus*-fermented *Artemisia princeps* (LFA) reduced thiobarbituric acid-reactive substance (TBARS) values in breast and thigh meat during 15 days of storage (Kim *et al.*, 2012b).

Hosseinzadeh and Farhoomand (2014) found no statistically significant effect on the relative weight of heart, liver, spleen, bursa, cook carcasses and abdominal fat of broilers fed different levels (0.125%, 0.25% and 0.5%) of *Artemisia dracunculus* powder.

Kostadinovic *et al.* (2015) investigated the effect of dietary supplementation of *Artemisia absinthium* on the carcass quality of broilers and found that birds fed with 200 g/kg of *A. absinthium* of feed had higher protein content and significantly ($P<0.05$) lower fat content as compared to the control.

Wan *et al.* (2016) studied the effects of enzymatically treated *Artemisia annua* (EA) supplementation on meat quality of breast and thigh muscles in broilers. They found that the broilers fed diets supplemented with EA significantly ($P<0.05$) reduced the drip loss cooking loss and shearing force of breast and thigh muscle.

The different levels of cumin and wormwood EOs in diets did not have a significant effect on carcass yield and relative weights of liver, intestine, heart, gizzard, thigh, breast and abdominal fat in broiler chicks at 42 days of age (Habibi *et al.*, 2016).

Saracila *et al.* (2018) observed that under heat stress broilers fed diets supplemented with *Artemisa annua* oil and powder did not significantly affected the carcass and organ weights.

2.8 Microbial population

Lopes-Lutz *et al.* (2008) reported that oils of different species of *Artemisia* had varying degrees of growth inhibition against microorganisms such as *Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Khalaji *et al.* (2011) observed that the supplementation of broiler diets with *Artemisia sieberi* leaves significantly ($P<0.05$) reduced coliform and *E. coli* populations in cecum of birds but had no effect on *Lactobacillus* population.

Kim *et al.* (2012b) reported that dietary supplementation of *Lactobacillus*-fermented *Artemisia princeps* (LFA) significantly increased the concentrations of *Lactobacillus spp.* and reduced the concentrations of *Salmonella spp.* in the ileal content of birds. However the concentrations of *Clostridium perfringens* and *Escherichia coli* in the ileum content were not affected.

Supplementation of 0.2% Korean pine extract along with 2% citric acid significantly ($P<0.05$) increased fecal *lactobacillus* counts in weaning pigs (Zhang *et al.*, 2012).

Supplementation of phytogetic feed additives significantly ($P<0.05$) decreased the total coliform count, total anaerobic count and total *Clostridium* and increased the number of cecal *Lactobacillus* compared to the control group (Murugesan *et al.*, 2015).

Kim *et al.* (2015) studied the antimicrobial effects of Artemisinin extracts from *Artemisia annua* L. extracts and found that water, methanol, ethanol and acetone extracts of *Artemisia annua* L. had antimicrobial activity against the periodontopathic microorganisms like *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* subsp. *animalis*, *Fusobacterium nucleatum* subsp. *polymorphum* and *Prevotella intermedia*.

Wati *et al.* (2015) observed that supplementation of broiler diets with 150 mg/kg phytogetic feed additive containing fennel, lemon balm, peppermint, anise, oak, clove and thyme significantly ($P<0.05$) lowered the counts of *Salmonella*, *E. coli* and *Clostridium* and increased the counts of *Lactobacillus* in *Salmonella enteritidis* and *E.coli* challenged broilers.

Ahsan *et al.* (2018) conducted a study to investigate the influence of different levels of dietary phytogetic feed additive (PFA) on the caecal microbiota

of broilers. They found no difference in the total aerobic bacteria, *Escherichia coli*, coliforms and *Lactobacilli* count among the different treatments groups.

Dietary supplementation of oregano essential oil (OEO) and a commercial blend of phytogenic (CBP) significantly ($P<0.01$) reduced the ileal *E. coli* count but did not affect the *Lactobacillus* count in broilers (Maziar Mohiti-Asli and Moein Ghanaatparast-Rashti, 2018).

Panaite *et al.* (2018) observed that the supplementation of *Artemisia annua* powder in heat stressed broilers significantly ($P<0.05$) reduced the number of intestinal *Enterobacteriaceae* and increased the count of lactobacilli.

Under heat stress feeding broilers diets supplemented with *Artemisia annua* powder and oil significantly reduced the number of *Enterobacteriaceae* and increased the *lactobacilli* count in the intestinal content of broilers compared to control (Saracila *et al.*, 2018)

Ding *et al.* (2019) investigated the effect of *Artemisia apiacea* Hance supplementation on cecal opportunistic bacteria of rabbits and found that compared with the control treatment, the herb significantly ($P<0.05$) decreased cecal population of *Clostridium perfringens*, Gram-negative bacteria.

Dietary supplementation with phytogenic blend (PB) of *Aerva lanata*, *Piper betle*, *Cynodon dactylon* and *Piper nigrum* did not significantly affect the total aerobic count, *Clostridium* count, *Lactobacillus* count, *Bifidobacterium* count and Coliform count in broilers (Oso *et al.*, 2019).

Adding *A. vulgaris* meal at 3.0, 6.0 or 9.0% significantly ($P<0.05$) increased the gut beneficial populations of *Lactobacilli* and *Bifidobacteria* and decreased the opportunistic pathogenic bacteria, including *E. coli*, *C. perfringens*, *Salmonella* and Gram negative bacteria of Rex rabbits (Wang *et al.*, 2019).

Supplementation of broiler diets with essential oils of thyme and star anise did not affect the intestinal bacterial count and *Lactobacillus* population but significantly increased the *Enterococcus* counts in treatment groups compared to the control group ((Saeed El-Ashram and Gamal A. Abdelhafez, 2020).

Mohebodini *et al.* (2021) conducted a study to evaluate the effects of different dietary inclusion (0, 250, 500, 750 and 1,000 mg/kg) of eucalyptus essential oil (EEO) on cecal microflora of broilers and found that the *Lactobacillus* counts increased and the *E. coli* population decreased with the increasing dietary EEO supplementation.

2.9 Histomorphology

Peric *et al.* (2010) investigated the effects of phytogetic additives on gut morphology of broilers and found that the phytogetic additive had no effect ($P>0.05$) on villus height or villus surface area, but reduced ($P<0.005$) the villus/crypt ratio.

Addition of the composite containing the three herbal extracts (carvacrol, cinnamaldehyde and capsaicin) significantly ($P<0.005$) improved the intestinal villus height but did not affect the crypt depth and villus/crypt ratio (Petroli *et al.*, 2012).

Supplementation of phytogetic feed additives significantly ($P<0.05$) increased the villus height in the duodenum, jejunum and ileum, lowered the crypt depth in jejunum and lowered the mucosal thickness in the duodenum and ileum in comparison to the control group (Murugesan *et al.*, 2015).

Supplementation of broiler diets with phytogetic feed additive significantly ($P<0.05$) increased the villus height, villus width, muscularis thickness and reduced the crypt depth and goblet cell number per villus in broilers compared to those fed control diets and hence improved the jejunal morphometry of broilers (Ahsan *et al.*, 2018).

Mounia *et al.* (2018) studied the effect of phytogetic products on the gut morpho-histology of broilers and found highest villus height of duodenum, jejunum and ileum in the control group compared to the experimental group. However, the depths of the duodenal crypt were found to be significantly ($P<0.05$) deeper in birds supplemented with phytogetic products and shallower for broilers in the control group.

Inclusion of 300 ppm dietary oregano essential oil in broiler diets significantly ($P<0.05$) increased villus height, villus height to crypt depth ratio, villus surface area and decreased crypt depth in the jejunum and increased villus height in the ileum compared with broilers fed control diet (Maziar and Moein Ghanaatparast, 2018).

Song *et al.* (2018) conducted a study to investigate the effects of dietary supplementation with enzymatically treated *Artemisia annua* (EA) on intestinal morphology of broilers challenged with heat stress. They found that EA supplementation significantly increased ($P<0.05$) the villus height and ratio of villus height to crypt depth and decreased the crypt depth compared with the heat stress group.

Oso *et al.* (2019) observed that the broilers fed diets supplemented with 1 and 2 % of phytochemical blend of *Aerva lanata*, *Piper betle*, *Cynodon dactylon* and *Piper nigrum* had longer villi height in duodenum and jejunum, reduced crypt depth and higher villus/crypt ratio as compared to the control.

Tunc *et al.* (2019) studied the effect of addition of tarragon (*Artemisia dracunculus*) powder at three different levels i.e. 0.1%, 0.2% and 0.5% in the diet of broiler chickens on the histopathology of internal organs. They found no changes in the appearance of the liver, kidney and intestinal tissues obtained from the experimental groups and increased villus length in the group fed 0.2% tarragon powder.

Supplementation of broiler diets with phenolic rich onion extract significantly ($P<0.05$) increased the villus height and width, mucosal thickness and goblet cell numbers in different sections of the small intestine compared to control (Omar *et al.*, 2020).

2.10 Enzyme supplementation in poultry

In the poultry industry, the importance of dietary exogenous enzymes in increasing development and feed utilisation efficiency is well understood. The

plant cell wall restricts the release of phytochemicals, which is unfavourable for the use of natural herbs (Puri *et al.*, 2012). Enzymatic treatment with cellulases, pectinases and hemicellulases, either alone or in combination, can hydrolyze and disintegrate the plant's cell wall, allowing intracellular bioactive chemicals to be released more easily (Puri *et al.*, 2012). Plant cell wall integrity is disrupted by enzymes, resulting in the release of nutrients enclosed by the cell wall (Ravindran, 2013).

Khan *et al.* (2006) reported that the supplementation of enzyme@ 500g per ton corn-sunflower diet significantly improved productive performance of birds. Birds fed the enzyme supplemented diet had significantly higher feed consumption, better feed conversion ratio when compared to the control group.

Attia *et al.* (2008) observed that adding multienzyme to poultry feeds significantly increased body weight gain, packed cell volume (PCV), hemoglobin (Hgb), lymphocytes, and monocytes and weight of immune organs and decreased the concentration of liver enzymes, malondialdehyde and cost of diets compared to the control.

Enzyme supplementation @500mg/kg of feed in broiler diets significantly improved the body weight gain, feed to gain ratio, relative growth, energy and protein efficiency when compared with the control group (Hajati *et al.*, 2009).

Addition of enzyme Yemzim B (containing 300 IU of xylanase/g, 20 IU of β -glucanase/g, 20 IU of hemicellulase/g and 260 IU of amylase/g) to wheat-soybean meal based mash with or without essential oil in the diet of broiler chicken containing enzyme, essential oil (250 mg/kg) and enzymes plus essential oil (250 mg/kg) and observed significantly ($p \leq 0.05$) higher weight gain when compared to the control group from day 0 to 7 (Basmaciog *et al.*, 2010).

Supplementation of maize-soya based diets with glucanase and xylanase significantly improved feed conversion ratio and ileal nutrient digestibility. Both

the enzymes added simultaneously resulted in a synergistic effect and hence greater benefits were observed (Cowieson *et al.*, 2010).

Shirmohammad and Mehri (2011) observed that the supplementation with multi-enzyme improved the nutritive value of corn-soybean diet in broiler chicks.

Zou *et al.* (2013) reported that the supplementation of cocktail enzyme@0.05 % level in the diet of broiler chicken significantly improved body weight and feed conversion ratio when compared with the control group.

Goli and and Shahryar (2015) studied the effect of adding enzyme on growth performance of quail and found that supplementary multi-enzyme improved body weight gain significantly.

Multienzyme supplementation, such as amylase, xylanase, and protease promote energy utilization in corn- soybean meal diets and sorghum- soybean meal diets by stimulating disintegration of starch, cell walls and endogenous proteins (Aftab and Bedford, 2018).

β -mannanase has the potential to improve the growth performance, total tract nutrient retention and gut health of broiler chickens fed a corn and soybean meal (SBM) - based diet (Hosseindoust *et al.*, 2019).

Chapter-3

MATERIALS AND METHODS

The present experiment was planned to evaluate the effect of supplementing broiler diets with *Artemisia absinthium* alone and in combination with enzyme on the performance of broiler chicken reared for five weeks. The research was carried out at Instructional Poultry Farm of the Livestock Production and Management Division, FVSc & AH.

3.1 Collection and processing of *Artemisia*

The plant material of *Artemisia* was collected and the leaves were stripped, washed and dried. The dried leaves were pulverized in a hammer mill and kept in air tight containers for further use (Plate 2).

3.1.1 Proximate composition

The dried leaves of *Artemisia absinthium* and the feed ingredients used in the study were subjected to proximate analysis in accordance with standard protocol. The per cent dry matter, crude protein, total ash, ether extract, and crude fibre content of the leaves were analyzed using the Association of Official Analytical Chemists' standard protocol (AOAC, 2000).

3.2 Experimental plan

A biological trial was carried out at Instructional Poultry Farm of the Division of Livestock Production and Management, FVSc & AH.

Two hundred and fifty two one-day old commercial broiler chicks were purchased from a reputable hatchery and reared for the first week under typical brooding conditions. On day eight, the chicks were split into seven treatment



Plate-2: Finely grinded *A.absinthium* leaves

Table-3.1: Proximate composition of experimental diets (DM basis)

Ingredients	Diet		
	Pre-starter	Starter	Finisher
Maize (yellow)	45.00	44.00	48.12
Soybean meal	38.00	29.00	23.73
Wheat bran	7.50	18.68	20.00
Vegetable oil	5.00	4.50	4.50
Lime stone powder (LSP)	1.97	1.70	1.60
Dicalcium phosphate (DCP)	1.33	1.00	0.95
Salt	0.29	0.29	0.29
Mineral premix +Vitamins*	0.75	0.75	0.75
Methionine	0.16	0.08	0.06
Total	100.00	100.00	100.00
Proximate composition (%)			
Dry matter (DM)	91.00	92.30	90.32
Crude protein (CP)	23.20	20.16	18.00
Crude Fibre (CF)	2.33	3.22	4.28
Ether extract (EE)	9.06	10.80	11.99
Ash	3.87	4.04	4.16
ME (Kcal/kg) Calculated	3,000	3,100	3,191

*Supplied/kg of diet: vitamin D₃-3300IU; vitamin A-8800IU; vitamin E-1100IU; Riboflavin-9mg; Biotin-0.30mg; Thiamine-4mg; Pantothenic acid-11mg; vitamin B₁₂- 13µg; Niacin-30mg; Choline-950mg; Vitamin K-1.5mg; Folic acid-1.5mg; Ethoxyquin-130mg; Manganese-50mg; copper 5.5mg iron 30mg and selenium 0.1mg.

groups at random, each containing three replicates of twelve birds. The treatment groups were as follows:

Treatment-1: Commercial diet without *Artemisia absinthium* leaf powder (Control group)

Treatment-2: Commercial diet supplemented with 1% *Artemisia absinthium* leaf powder

Treatment-3: Commercial diet supplemented with 1.5% *Artemisia absinthium* leaf powder

Treatment-4: Commercial diet supplemented with 2% *Artemisia absinthium* leaf powder

Treatment-5: Commercial diet supplemented with 1% *Artemisia absinthium* leaf powder and enzyme

Treatment-6: Commercial diet supplemented with 1.5% *Artemisia absinthium* leaf powder and enzyme

Treatment-7: Commercial diet supplemented with 2% *Artemisia absinthium* leaf powder and enzyme

The cocktail enzyme was used at the rate of 25g/tonne of feed.

The cocktail enzyme composition was: Cellulase 150000 CMCU, Arabinase 2120 U, beta galactosidase 25000 U, Xylanase 28000 U, Protease 70800 U, alpha-Amylase 70000 U, Lipase 15000 U, Phytase 2500 FTU, *Lactobacillus acidophilus* 6×10^9 , *Saccharomyces boulardii* 6×10^9

The birds were raised in cages upto five weeks of age under standard managemental conditions with *ad lib* feeding and watering. All birds were vaccinated and assessed twice daily for mortality, if any.

3.2.1 Data/Observations recorded

The following observations were recorded during the experimental period:

3.2.1.1 Live body weight

The body weight of birds was recorded using a digital weighing balance in the morning hours before feeding at weekly intervals.

3.2.1.2 Body weight gain

The body weight gain in birds was computed on a weekly basis using the body weights of broilers recorded at various intervals.

3.2.1.3 Feed consumption

Each replicate's feed bins were filled with a weighed amount of feed and feed was supplied ad libitum from those feed bins. The leftover feed in the individual bins and feeders was weighed again at weekly intervals to calculate how much feed was consumed by each replication that week.

3.2.1.4 Feed conversion ratio

The feed conversion ratio of experimental birds was calculated weekly for the duration of the study using the following formula

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed (kg)}}{\text{Body weight gain (kg)}}$$

3.2.1.5 Mortality

Throughout the study, individual pens were examined daily, and any mortality was reported on a regular basis.

3.2.1.6 Balance trial

At the end of the experiment, three randomly selected birds from each treatment group were used in a three-day balancing trial to evaluate the percent digestibility of dry matter (DM), ether extract (EE), crude fibre (CF) and crude protein (CP). The birds were maintained in individual battery cages for seven days, including adaption period of four days and collection period of three days. A weighed amount of feed and excreta voided by each bird was collected and weighed individually throughout a 24-hour period for three days. The Excreta

collected for three days was pooled and stored in individual air tight polythene pouches. Excreta was homogenised for DM estimation, and around 1/20th of the entire excreta was placed in a moisture cup and dried in an oven at 800°C until a consistent weight was reached. 1/10th of the total excreta was put into a wide mouthed well-stoppered specimen bottle and rapidly examined for nitrogen measurement. Chemical analysis was also performed on representative feed samples. The samples were subjected to proximate analysis as per AOAC (2005a).

3.3 Blood biochemistry

Routine blood biochemical analysis were done at the end of the experiment period to determine the effect of diets fed with *Artemisia absinthium* alone and in combination with enzyme on the blood biochemical parameters of broiler chicken.

3.3.1 Collection of blood

The blood from three birds per treatment was randomly collected on 35th day in blood collection tubes and stored in an inclined position. The tubes were then incubated at 37°C for an hour. The clots of blood were then disintegrated and tubes were centrifuged for 30 minutes at 3000 rpm. The blood serum was collected into tiny tubes and kept frozen (at -20°C) until biochemical investigation. The parameters i.e. glucose, cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, alanine aminotransferase, aspartate transaminase and creatinine below were calculated from serum samples using an auto analyzer and the appropriate biochemical kits.

3.4 Immune competence

3.4.1 Haemagglutination Titre to Sheep Red Blood Cells (HA titre to SRBC)

The red blood cells of the sheep (SRBC) in the Alsever solution were centrifuged three times in a phosphate buffered isotonic saline solution (PBS: pH 7.2), calibrated to a 1 percent suspension (v/v) and kept at 4°C before use. One ml of SRBC suspension was given intravenously to three birds from each treatment at

twenty eight days of age. Five days later, 2 ml blood sample (2 ml) was collected from the jugular vein of each chick. For serum collection, the blood samples were allowed to coagulate, and the serum samples were stored at -20°C until analysis. A conventional haemagglutination assay was used to assess the antibody response to SRBC (Siegel and Gross, 1980). The end point of titre was the reciprocal of the maximum dilution displaying unambiguous agglutination, and the data were represented as log 2.

Reagent preparation

Phosphate buffer saline (PBS) and Alsever solution were made according to the following formulas:

Phosphate buffer saline (PBS)

- Sodium chloride (NaCl) - 8.0 g
- Potassium chloride (KCl) - 0.20 g
- Potassium Dihydrogen phosphate (KH₂PO₄) - 0.20 g
- Disodium hydrogen phosphate (Na₂HPO₄.2H₂O) - 1.44 g
- Distilled water - 1000ml

The solution pH was calibrated to 7.2

Alsever solution

- Dextrose - 2.05 g
- Tri sodium citrate dihydrate - 0.80 g
- Sodium chloride - 0.42 g
- Citric acid - 0.055g
- Distilled water - 100ml

The solution pH was calibrated to 6.5 by addition of citric acid and kept in refrigerator at 4° C.

Sheep red blood cell (SRBC) suspension preparation: Alsever solution was used to collect blood from healthy sheep's jugular veins. For around 10 minutes, centrifugation of the blood sample was done at 2500 rpm. The red blood cells were washed three times in Phosphate buffer saline after the supernatant was removed. The SRBC suspension (1 percent v/v) in Phosphate buffer saline was produced and placed in refrigerator at 4° C till further use.

Immunization and immune serum collection: A 1.0 ml suspension of SRBC was given in the brachial vein of 3 birds from each treatment group on the 28th day after hatching to evaluate the primary antibody response to SRBC. 2 ml blood was taken from the brachial vein on the 33rd day (5 days after vaccination). The blood samples were allowed to coagulate, the sera samples were collected, and were frozen (-20° C) until the antibody titres to SRBC were determined.

Haemagglutination test (HA test): HA techniques were used to determine the antibody titre to SRBC (Siegel and Gross, 1980). The plates utilised were microtitre plates with a U bottom.

Procedure:

- 50 µl of PBS was filled in each well of the micro titter plate
- 50 µl of serum was filled in the first well,.
- Up to row 11, two fold serial dilutions were made, with row 12 serving as a control.
- In each well, 50 µl of 1% SRBC was added and gently tapped to mix.
- For incubation, the plates were covered and kept at 37°C for 1 hour.
- The plates were read in direct sunlight.

The reciprocal of highest dilution showing clear agglutination was the ends titter. The titres were expressed as log₂

3.4.2 Cell mediated immunity using DNCB (1-Chloro Di-nitro Benzene)

Preparation of DNCB

- As a vehicle, a 2 percent solution of DNCB was produced using a 4:1 ratio of acetone to olive oil.
- To generate a final volume of 20 ml, 16 ml of acetone and 4 ml of olive oil were combined appropriately.

DNCB Injection

- With the use of an insulin syringe, 0.25 ml of DNCB solution was infused intra-dermally into 3 birds in each replicate at the gap between the third and fourth digit of the right leg.
- 0.25 ml NSS was administered into the same birds of each replicate at the same position of the cleaned out limb that was utilised as a control.
- The skin thickness at the site of injection in both legs was measured with a Vernier calliper before and after DNCB injection in all replicates.

The cell-mediated immune response (CMIR), also known as the Foot Web Index, was calculated using the formula below:

$$FWI = (R_2 - R_1) - (L_2 - L_1)$$

Where,

R_2 - is the thickness after 24 hours of DNCB injection

R_1 - is the thickness before injection of DNCB injection

L_2 - is the thickness after 24 hours of NSS injection

L_1 - is the thickness before injection of NSS injection

3.4.3 Immune organ weight

The Halal technique was used to sacrifice three birds of average body weight from each group. Organs such the thymus, bursa of fabricius, and spleen were taken and weighed immediately after bleeding.

3.5 Histopathology

Representative small intestine and liver samples were collected into sterile containers containing 10% formal saline for further processing according to the procedure outlined.

3.5.1 Tissue preparation for microsectioning

Tissues were processed for microsectioning using the paraffin embedding procedure. After fixation, the tissues were sliced into 2-3 mm thick pieces and rinsed in water for several hours before being dehydrated in increasing degrees of alcohol. Tissues were cleared in benzene before being embedded in paraffin.

Table 3.2: Paraffin wax embedding schedule

S. No.	Solution	Time
1.	Washing	1-2 hours
2.	70%alcohol	Overnight
3.	80%alcohol	60 minutes
4.	90% alcohol	60 minutes
5.	Absolute alcohol-I	60 minutes
6.	Absolute alcohol-II	60 minutes
7.	Absolute alcohol-III	60 minutes
8.	Benzene-I	60 minutes
9.	Benzene-II	60 minutes
10.	Paraffin-I (58 °C)	Overnight
11.	Paraffin-II (58 °C)	60 minutes
L-molds (two L-shaped pieces) were used to cast the blocks, making it easier to manipulate the size as needed		

3.5.2 Micro sectioning

For cutting paraffin sections, a rotary microtome was employed. The blocks were trimmed properly, and pieces were cut at a thickness of 5 mm. The material was cut into continuous ribbons (6-7 inches long) and set on top of a constant temperature water bath (around 55° C). After the portions had spread entirely, they were separated with a hot scalpel.

Mayer's egg albumin was used as a section glue to mount the cut sections on clean glass slides. The mounted slides were labelled for identification and dried for one hour in a paraffin oven at 60°C.

Mayer's Egg Albumin

Composition:

- Egg white 50 ml
- Glycerine 50 ml

The contents were thoroughly combined and filtered through coarse filter paper, with a thymol crystal added for preservation.

3.5.3 Staining of paraffin sections

Harris Haematoxylin and eosin staining technique was used to stain the tissue sections (Lunas, 1968). Xylene was used to deparaffinize the sections before being hydrated with distilled water via graded alcohol. Staining was carried out as per usual method, followed by dehydration in increasing alcohol concentrations. Xylene was used for clearing, while DPX was used for mounting.

Staining Schedule

- I. Deparaffinization
 - Xylene -I 15 min
 - Xylene -II 15 min
 - Xylene-III 15 min

II. Hydration

Absolute alcohol-I 5 min

Absolute alcohol-II 5 min

90% Alcohol 5 min

80% Alcohol 5 min

70% Alcohol 5 min

50% Alcohol 5 min

Distilled water 15 min

III. Staining

Harris Haematoxylin 7 min

Rinsed in distilled water

Acid Alcohol (1%) One dip

0.1% ammonia water Until optimum bluing of section

Washed in running water

Rinsed in distilled water

Eosin stain (alcoholic) 4 min

IV. Dehydration

50% Alcohol 30 sec.

70% Alcohol 30 sec.

80% Alcohol 30 sec.

95% Alcohol 30 sec.

Absolute Alcohol-I 30 sec.

Absolute Alcohol-II 30 sec.

Absolute Alcohol-III 30 sec.

V. Clearing

Xylene-I 30 min.

Xylene-II 30 min.

Xylene-III 60 min.

VI. Mounting

After removing the slide from the Xylene, a drop of DPX mountant was put to a cover slip, and the slide's section was pushed into it. The slide was flipped and the cover slip pressed with a glass rod to eliminate any trapped air bubbles.

3.6 Cecal microbial population

Microbiological investigation of cecal contents from representative birds was performed to estimate total viable count and coliform counts using standard procedures.

3.6.1 Sample preparation and serial dilution

One gram of cecal contents was weighed and transferred to appendroff tubes in an aseptic manner. To obtain a 10^{-1} dilution, 1ml of 0.9 percent sterile normal saline was placed in the tubes and carefully mixed with the help of a vortex. For the 10^{-2} dilution, 0.1 mL of this diluted solution was transferred to another tube holding 2 mL of sterile 0.9 percent normal saline. For 10^{-3} dilution, the procedure was repeated by transferring 0.1 ml from the 10^{-2} dilution to a new tube containing 2 ml sterilized 0.9 percent normal saline. It was kept going until the needed dilution was achieved. The entire process was performed in a laminar airflow. Air flow near the flame creates sterile conditions that are nearly foolproof.

3.6.2 Total viable count

The total viable count was calculated based on the American Public Health

Association's approach (1992). 15.6g Brain heart infusion agar (processed by Micro Master Laboratories Pvt. Ltd.) was dissolved in 1000ml distilled water, then sterilised for 15 minutes at 15 lb pressure (121°C) in an autoclave. In petri plates, around 15-20 ml of sterile medium kept at 45°C was poured. 0.1 ml inoculums from dilutions of 10^{-4} , 10^{-5} and 10^{-6} were inoculated into a double set of sterilized petri plates and disseminated with sterilized glass beads. The plates were incubated for 24 hours at 37°C. From each plate, colonies were counted. The colony forming unit per gram was derived by multiplying the average number of colonies by the reciprocal of the dilution factor and expressed as log cfu/g of contents.

3.6.3 Coliform count

The coliform count was calculated using the Cowan and Steele technique (1993). 15.3 g McConkey Agar was dissolved in 1000 mL distilled water, then sterilised in a 15 lb pressure autoclave (121° C) for 15 minutes. Using the pour plate technique, roughly 15-20 ml of McConkey Agar was poured onto petri plates at a temperature of about 45°C. A 0.1 ml aliquot from 10^{-4} , 10^{-5} and 10^{-6} dilution was used to inoculate duplicate sets of petridishes aseptically. The plates were incubated for 24 hours at 37°C. The number of selected coliform bacteria-specific colonies (pink colonies) was counted. The reciprocal of the dilution factor was multiplied by the average number of colonies, and the result was expressed as log cfu/g sample.

3.7 Antioxidant status

3.7.1 Total oxidant status

The automated colorimetric approach was used to determine the total oxidant status (TOS) (Erel, 2005). To 35 µl of serum solution, 225 µl of Reagent 1 (xylenol orange 11.4 mg, 818 mg sodium chloride, and 10 mL glycerol in 90 ML H₂SO₄ 25mM solution, pH 1.75) was added. After Reagent 1, 11 µl of Reagent 2 was added (0.196 g ferrous ammonium sulphate, 0.317 g o-dianisidine

dihydrochloride in 100 mL H₂SO₄ 25mM solution). In deionized water, a 20 mM stock solution of H₂O₂ was prepared as a standard. At 560nm, the first absorbance (A1) was measured before the mixing of R1 and R2 (as a sample blank), and the last absorbance (A2) was measured 3-4 minutes later (560nm).

3.7.2 Total antioxidant status

The total antioxidant status (TAS) was determined using an automated direct ABTS radical cation technique (Erel, 2004). 5 µl of serum solution was mixed with 200 µl of Reagent 1 (acetate buffer 0.4 mol/l pH 5.8). Following R1, 20 µl of Reagent 2 (ABTS⁺ in acetate buffer 30 mmol/l pH 3.6) was added. Trolox was dissolved in phosphate buffer (30 mmol/l pH 7.4) as a standard. The assay's first absorbance (A1) was recorded at 660 nm before R1 and R2 (as a sample blank) were mixed, and the last absorbance (A2) was taken 5 minutes later (660 nm).

3.7.3 Thiobarbituric Acid Reactive Substance (TBARS) value

The approach of Witte *et al.* (1970) was used to estimate the TBARS value with minor changes.

For 2 minutes, 10g of sample was triturated in a 2 M orthophosphoric acid solution with 25 ml of pre-cooled 20% trichloroacetic acid (TCA). After rinsing with 25 mL cooled distilled water, the contents were quantitatively transferred into a beaker. The contents were filtered via ash-free filter paper after adequate mixing (Whatman filter paper No. 1 supplied by GE Healthcare U.K). In test tubes, 3 ml of TCA extract (filtrate) was combined with 3 ml of TBA reagent (0.005M) and left in the dark for 16 hours. 3 ml of 10% TCA and 3 ml of 0.005 M TBA reagent were combined to make a blank sample. Using a UV-VIS spectrophotometer, the absorbance (O.D.) was measured at a fixed wavelength of 532 nm (HITACHI, UV-Spectrophotometer U-1800). By multiplying the O.D. value with the k factor 5.2, the TBARS value was determined as mg malonaldehyde per kg of sample.

3.8 Slaughter and carcass characteristics

At the conclusion of the trial, a total of 42 healthy broiler chickens (2 birds per replication, or 6 birds from each treatment) were selected for the study of carcass characteristics. The birds were fasted overnight and water was taken away three to four hours before slaughter. The birds were weighed to determine their pre-slaughter live weight then slaughtered using the Halal procedure, with a 2-minute bleeding period permitted. After bleeding, the carcass was weighed to ascertain the amount of blood lost. The carcass was then scalded for 1.5 minutes at 600 °C. To calculate the feather loss, feathers were plucked and the de-feathered weight was recorded. The head and shanks were removed and carcasses were visually observed for grading as per the standard procedure (BIS Grading). The carcasses were eviscerated by removing the interior contents after vents were opened. For each carcass, the eviscerated weight and weight of giblets (heart, liver, and gizzard) were calculated and expressed as a percentage of PSLW. The carcass was then cut into standard portions such as breast, thighs, drumsticks, wings, back, and neck, with each part weighing separately. Blood loss, feather loss, dressing yield, cutup parts yield, giblet yield were determined using the following formula:

Where, PSLW, BL, FL EL is pre-slaughter live weight, blood loss, feather loss and evisceration loss respectively.

$$\text{Blood loss (\%)} = \frac{\text{Pre-slaughter live weight(PSLW)} - \text{weight after bleeding}}{\text{Pre-slaughter live weight}} \times 100$$

$$\text{Feather loss (\%)} = \frac{\text{PSLW} - \text{BL} - \text{Weight after de-feathering}}{\text{Pre-slaughter live weight}} \times 100$$

$$\text{Dressing yield (\%)} = \frac{\text{PSLW} - \text{BL} - \text{FL} - \text{EL} - \text{Weight of head and shanks}}{\text{Pre-slaughter live weight}} \times 100$$

$$\text{Giblet yield (\%)} = \frac{\text{Weight of giblets}}{\text{Pre-slaughter live weight}} \times 100$$

$$\text{Cut up part yield (\%)} = \frac{\text{Weight of specific cut}}{\text{Dressed weight}} \times 100$$

3.9 Sensory evaluation of meat

Meat was sensory evaluated in association with the Department of Livestock Products and Technology, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, using an 8-point hedonic scale for descriptive attributes of product, as described by Keeton (1983).

Preparation of meat samples

Each treatment group had eight breast meat samples of equal size removed out of the deep freezer and allowed to thaw at room temperature for about two hours. Fat was removed from the samples after they were rinsed in running tap water. Weighed samples were maintained in an autoclave. The autoclave was filled with a volume of 1.2 percent salt solution equal to the sample weight. In the autoclave, the meat samples were cooked for roughly 10 minutes at a pressure of 15 psi. The samples were removed from the autoclave and put in bone china plates to cool to room temperature. The samples were scored on an 8-point scale by a professional panel of 10 judges, who had not eaten or drunk anything for at least one hour previous to the assessment. The samples were judged on their overall appearance, taste, tenderness, juiciness, and acceptability. Judging was as follows:

Very good	Good	Fair	Poor	Very Poor
8-9	6-7	4-5	2-3	0-1

3.10 Proximate composition of meat

The per cent moisture, protein, ether extract, and ash content of product samples were assessed using the Association of Official Analytical Chemists' standard protocol (AOAC, 2000).

3.11 Economics

The economics were calculated at the end of the study using the cost of day old chicks, feed costs, miscellaneous cost (labour cost, electricity charge, vaccination, medication, etc.), body weight gain and the price of the birds sold in the market on a live weight basis.

3.12 Statistical analysis

One way ANOVA was used to analyse the data. The test statistics were then compared against p-values to determine their significance. Any P-value less than 0.05 ($P < 0.05$) was considered statistically significant, and the analysis was done using the statistical software SPSS version 20.00 Chicago, United States of America for Windows.

Chapter- 4

EXPERIMENTAL FINDINGS

4.1 Proximate analysis

4.1.1 *Artemisia absinthium*

The proximate composition of feed additive under study i.e. *Artemisia absinthium* is given in Table 4.1. The Moisture content of *Artemisia absinthium* leaves was 12.91 per cent and the per cent dry matter was 87.09. The per cent Crude Protein content was 23.56 whereas ether extractable Fat content was found to be 4.31 per cent and the Crude Fibre content was found to be 20.30 per cent. The per cent total Ash content of *Artemisia absinthium* leaves was 8.65 per cent and the Nitrogen Free Extract was 43.18 per cent.

Table 4.1: Proximate composition of *Artemisia absinthium*

Attributes	Mean
Dry Matter (%)	87.09±0.075
Moisture (%)	12.91±0.075
Crude Protein (%)	23.56±0.159
Ether Extract (%)	4.31±0.037
Crude Fibre (%)	20.30±0.58
Total Ash (%)	8.65±0.084
Nitrogen Free Extract (%)	43.18±0.79
Metabolizable Energy (Kcal/kg)*	2753.72

*Calculated as per Ponzenga (1985) $ME = 37 \times \%CP + 81 \times \%EE + 35.5 \times \%NFE$

4.2 Body weight (g)

The mean body weight of broiler chicken fed diets supplemented with different levels of *Artemisia absinthium* leaves with or without enzyme treatment during different ages is given in Table 4.2 and the graphical representation of weekly body weight is depicted in Fig 4.1. The average body weight (g) of the broiler chicken at the end of 1st week recorded was 160.16g±0.94, 161.71g±0.17, 160.80g±0.835, 161.77g±0.23, 160.05g±0.19, 162.74g±0.65 and 161.49g±0.29 respectively in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups. There was no Statistical significant differences observed among the different groups at the end of first week of age. The average body weight (g) of broiler chicken at the end of 2nd week recorded was 353.19g±4.97, 387.46g±0.41, 393.39g±1.77, 398.69g±0.50, 405.73g±1.75, 407.63g±1.81 and 411.78g±0.79 for T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. Significantly (P≤0.05) highest body weight was recorded in the T₇ group in which 2% enzyme treated *A.absinthium* was supplemented in the diet. The average body weight (g) of broiler chickens at 3rd week was 637.91g±4.49, 689.64g±3.95, 703.80g±4.46, 707.15g±7.59, 711.4g±4.63, 728.20g±5.14 and 730.35g±5.51 for T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively with significantly (P≤0.05) highest body weight recorded in T₇ group.. The mean body weight of the broiler chickens at the end of 4th week recorded was 996.80g±5.87, 1064.41g±5.09, 1102.13g±7.86, 1109.00g±4.36, 1112.49g±5.08, 1139.33g±8.64 and 1147.33g±5.16 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. Significantly (P≤0.05) highest body weight was recorded in T₆ and T₇ groups when compared to the other treatment groups and the control group. At the end of 5th week of age the mean body weight (g) of the broiler chickens was recorded as 1417.99g±2.098, 1514.38g±3.23, 1552.30g±6.25, 1590.05g±2.86, 1576.88g±3.77, 1659.13g±12.06 and 1690.91g±4.61 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively with significantly (P≤0.05) highest body weight was recorded in the T₇ group in which 2% enzyme treated *A.absinthium* was supplemented in the diet.

Table 4.2: Average weekly body weight (g) of broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Treatment groups	Age (Week)				
	1	2	3	4	5
T ₁	160.16 ±0.94	353.19 ^e ±4.97	637.91 ^d ±4.49	996.80 ^d ±5.87	1417.99 ^f ±2.098
T ₂	161.71 ±0.17	387.46 ^d ±0.41	689.64 ^c ±3.95	1064.41 ^c ±5.09	1514.38 ^e ±3.23
T ₃	160.80 ±0.83	393.39 ^{cd} ±1.77	703.80 ^{bc} ±4.46	1102.13 ^b ±7.86	1552.30 ^d ±6.25
T ₄	161.77 ±0.23	398.69 ^{bc} ±0.50	707.15 ^{abc} ±7.59	1109.00 ^b ±4.36	1590.05 ^c ±2.86
T ₅	160.05 ±0.19	405.73 ^{ab} ±1.75	711.4 ^{abc} ±4.63	1112.49 ^b ±5.08	1576.88 ^{cd} ±3.77
T ₆	162.74 ±0.65	407.63 ^{ab} ±1.81	728.20 ^{ab} ±5.14	1139.33 ^a ±8.64	1659.13 ^b ±12.062
T ₇	161.49 ±0.29	411.78 ^a ±0.79	730.35 ^a ±5.51	1147.33 ^a ±5.16	1690.91 ^a ±4.61
CD(P≤0.05)	1.740	6.98	16.125	16.588	18.00

Means within same column with different superscripts differ significantly (P≤0.05)

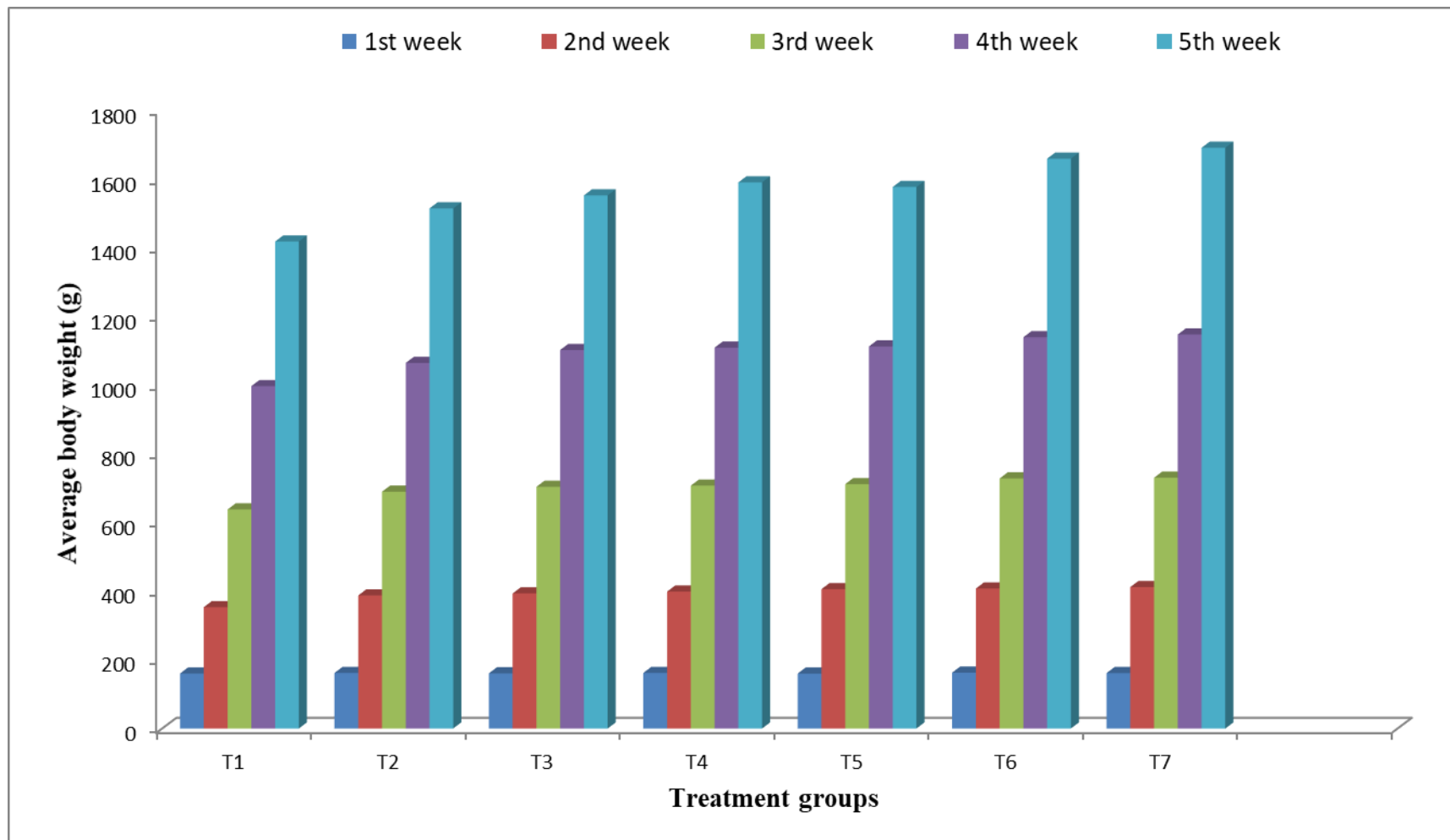


Fig. 4.1: Average body weight (g) of different treatment groups at weekly interval

During first week of age no significant differences were observed among the different groups. However, as the age of birds advanced significant ($P \leq 0.05$) differences were observed in average weekly body weight of birds fed *Artemisia absinthium* with or without enzyme treatment when compared to the control group. The group of birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves had significantly ($P \leq 0.05$) higher body weight when compared with the group of birds fed diets supplemented with different levels of raw *Artemisia absinthium* leaves. Among all the treatment groups, diets supplemented with 2% *Artemisia absinthium* leaves with enzyme showed ($P \leq 0.05$) significantly higher body weight when compared to birds fed diets supplemented with 1% and 1.5% levels.

4.3 Body weight gain (g)

The mean body weight gain (g) in broiler chickens fed diets supplemented with different levels of *Artemisia absinthium* leaves with or without enzyme treatment is given in Table 4.3 and the graphical representation of average body weight gain is depicted in Fig: 4.2.

The average body weight gain (g) of broiler birds during 1 -2 week of age was $193.02g \pm 4.42$, $225.74g \pm 0.28$, $232.58g \pm 2.46$, $236.91g \pm 0.65$, $245.68g \pm 1.85$, $244.89g \pm 2.46$ and $250.288g \pm 0.947$ in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups, respectively with significantly ($P \leq 0.05$) highest body weight recorded in T₇ group and lowest body weight gain in control group.

During 2-3 week of age the mean body weight gain (g) was found to be $284.72g \pm 7.13$, $302.18g \pm 4.06$, $310.41g \pm 2.71$, $308.46g \pm 7.62$, $305.74g \pm 4.62$, $320.56g \pm 5.05$ and $318.57g \pm 5.38$ in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups, respectively. Statistical analysis revealed that the body weight gain in T₆ and T₇ groups was significantly ($P \leq 0.05$) higher than all other treatment groups.

Table 4.3: Average weekly body weight gain (g) in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Treatment groups	Age (Week)				
	1-2	2-3	3-4	4-5	1-5
T ₁	193.02 ^e ±4.42	284.72 ^b ±7.13	358.89 ^c ±3.43	421.19 ^d ±5.28	1257.83 ^f ±1.94
T ₂	225.74 ^d ±0.28	302.18 ^{ab} ±4.06	374.76 ^{bc} ±8.68	449.97 ^c ±2.88	1352.67 ^e ±3.12
T ₃	232.58 ^{cd} ±2.46	310.41 ^{ab} ±2.71	398.33 ^{ab} ±5.33	450.16 ^c ±8.34	1391.50 ^d ±7.02
T ₄	236.91 ^{bc} ±0.65	308.46 ^{ab} ±7.62	401.85 ^a ±9.76	481.05 ^b ±6.70	1428.28 ^c ±2.72
T ₅	245.68 ^{ab} ±1.85	305.74 ^{ab} ±4.62	401.02 ^{ab} ±2.02	464.39 ^{bc} ±3.61	1416.83 ^{cd} ±3.60
T ₆	244.89 ^{ab} ±2.46	320.56 ^a ±5.05	411.12 ^a ±6.08	519.80 ^a ±3.71	1496.39 ^b ±12.65
T ₇	250.288 ^a ±0.947	318.57 ^a ±5.38	416.97 ^a ±9.75	543.58 ^a ±3.40	1529.41 ^a ±4.86
CD (P≤0.05)	7.107	16.863	17.255	15.613	18.844

Means within same column with different superscripts differ significantly (P≤0.05)

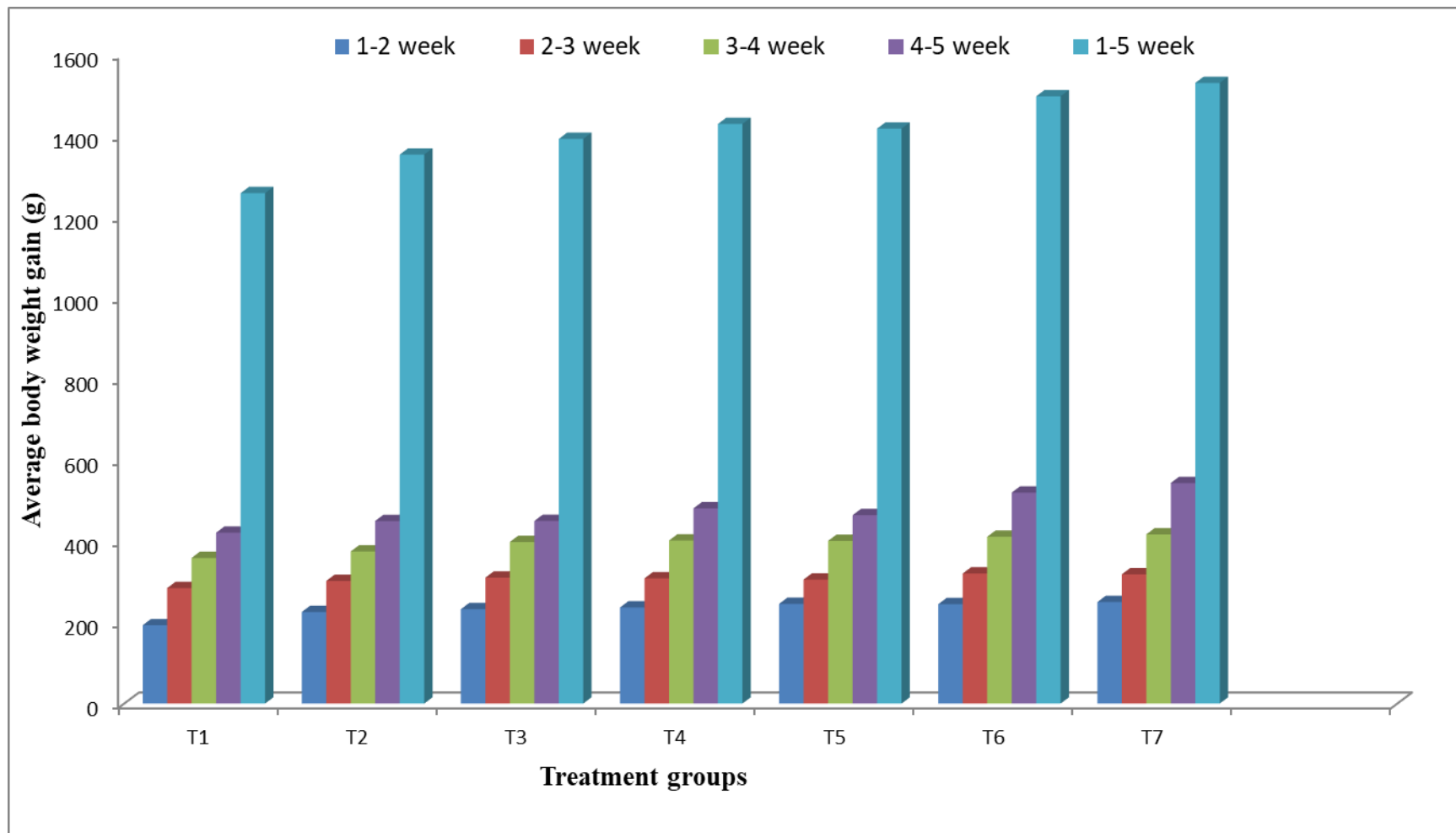


Fig. 4.2: Average body weight gain (g) of different treatment groups at weekly interval

During 3-4 week of age the mean body weight gain (g) was found to be 358.89g±3.43, 374.76g±8.68, 398.33g±5.33, 401.85g±9.76, 401.02g±2.02, 411.12g±6.08, 416.97g±9.75 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. Statistical analysis revealed that the body weight gain in T₆ and T₇ group was significantly ($P\leq 0.05$) higher than all other treatment groups. During 4-5 week of age the mean body weight gain (g) recorded was 421.19g±5.28, 449.97g±2.88, 450.16g±8.34, 481.05g±6.70, 464.39g±3.61, 519.80g±3.71 and 543.58g±3.40 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. Significantly ($P\leq 0.05$) highest body weight was recorded in T₆ and T₇ treatment groups.

During 1-5 weeks of age the mean body weight gain (g) was recorded as 1257.83g±1.94, 1352.67g±3.12, 1391.50g±7.02, 1428.28g±2.72, 1416.83g±3.60, 1496.39g±12.65 and 1529.41g±4.86 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. The body weight gain of T₇ treatment group was found to be significantly ($P\leq 0.05$) highest among all other treatment groups.

A significant ($P\leq 0.05$) increase in body weight gain was observed in all the treatment groups when compared to the control group but the highest body weight gain was found in birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves. However significantly ($P\leq 0.05$) highest body weight gain was found in birds fed diets supplemented with 2% enzyme treated *Artemisia absinthium* leaves i.e. T₇ group.

4.4 Feed consumption

The results of average feed consumption (g) of broiler chicken fed diets supplemented with different levels of *Artemisia absinthium* leaves with or without enzyme treatment has been summarized in Table 4.4 and depicted in Fig. 4.3.

During 1 to 2 weeks of age the average feed consumption (g) recorded was 251.64g±4.74, 278.21g±5.05, 279.57g±9.37, 283.75g±4.32, 282.58g±7.00, 290.36g±1.52, 294.18g±3.59 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups, respectively. Significantly ($P\leq 0.05$) higher feed consumption was observed in birds fed diets

supplemented with raw and enzyme treated *Artemisia absinthium* leaves when compared to the control group.

During 2 to 3 weeks of age the average feed consumption (g) of birds recorded was 455.18g±11.00, 458.15g±1.17, 463.11g±3.12, 459.30g±1.78, 461.16g±5.66, 469.29g±5.47 and 465.80g±4.59 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups, respectively. No significant difference was found in the feed consumption of birds in various treatment groups during this week.

The average feed consumption (g) of birds during 3 to 4 weeks of age recorded was 684.71g±9.38, 696.95g±3.33, 706.67g±6.84, 703.63g±9.67, 725.95g±10.35, 726.12g±7.75 and 733.27g±6.56 for T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. Significantly ($P \leq 0.05$) highest feed consumption was observed in birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves i.e. T₅, T₆ and T₇ groups.

The average feed consumption (g) of birds during 4 to 5 weeks of age recorded was 855.24g±8.10, 866.66g±3.46, 863.19g±4.01, 869.92g±17.30, 874.63g±12.84, 887.31g±2.61 and 891.72g±5.82 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. No significant difference was observed in the feed consumption of birds in various treatment groups.

The overall feed consumption (g) of birds during 1 to 5 weeks of age recorded was 2246.77g±13.52, 2299.97g±4.88, 2312.55g±14.39, 2316.62g±13.34, 2344.33g±15.87, 2372.72g±6.72 and 2384.98g±2.93 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. The group of birds fed different levels of enzyme treated *Artemisia absinthium* leaves had significantly ($P \leq 0.05$) higher feed consumption when compared to the birds fed diets supplemented with raw *Artemisia absinthium* leaves and the control group. Among the birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves highest feed consumption was recorded in birds fed diets supplemented with 2% *Artemisia absinthium* leaves i.e. T₇ group during the entire growth period from 1 to 5 weeks of age.

Table 4.4: Average and weekly feed consumption (g) in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Treatment groups	Age (Week)				
	1-2	2-3	3-4	4-5	1-5
T ₁	251.64 ^b ±4.741	455.18 ±11.00	684.71 ^b ±9.38	855.24 ±8.10	2246.77 ^c ±13.52
T ₂	278.21 ^{ab} ±5.05	458.15 ±1.17	696.95 ^{ab} ±3.33	866.66 ±3.46	2299.97 ^{bc} ±4.88
T ₃	279.57 ^a ±9.37	463.11 ±3.12	706.67 ^{ab} ±6.84	863.19 ±4.01	2312.55 ^{abc} ±14.39
T ₄	283.75 ^a ±4.32	459.30 ±1.78	703.63 ^{ab} ±9.67	869.92 ±17.30	2316.62 ^{abc} ±13.34
T ₅	282.58 ^a ±7.00	461.16 ±5.66	725.95 ^a ±10.35	874.63 ±12.84	2344.33 ^{ab} ±15.87
T ₆	290.36 ^a ±1.52	469.29 ±5.47	726.12 ^a ±7.75	887.31 ±2.61	2372.72 ^{ab} ±6.72
T ₇	294.18 ^a ±3.59	465.80 ±4.59	733.27 ^a ±6.56	891.72 ±5.82	2384.98 ^a ±2.93
CD(P≤0.05)	17.125	-	24.558	-	47.37

Means within same column with different superscripts differ significantly (P≤0.05)

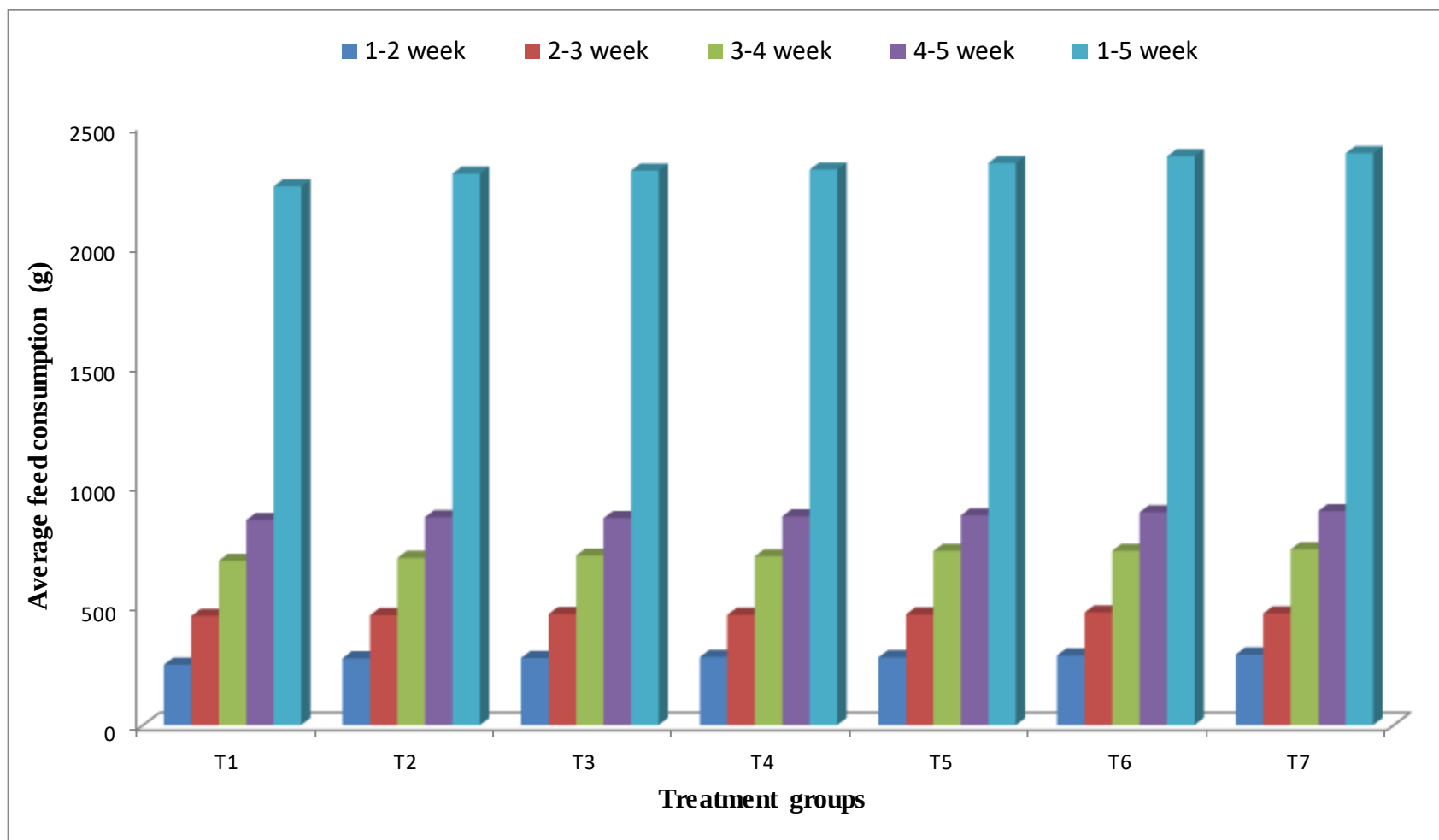


Fig. 4.3: Average feed consumption (g) of different treatment groups at weekly interval

4.5 Feed conversion ratio

The results of weekly feed conversion ratio in broiler chicken fed diets supplemented with different levels of *Artemisia absinthium* leaves with or without enzyme treatment has been summarized in Table 4.5 and depicted in Fig. 4.4.

Significant difference ($P \leq 0.05$) was observed in the FCR of birds fed diets supplemented with different levels of *Artemisia absinthium* leaves with or without enzyme treatment when compared with the control birds during 1-2 weeks of age. During 2-3 and 3-4 weeks of age no significant difference was observed in the FCR of birds in different treatment groups. During 4-5 weeks of age significant ($P \leq 0.05$) difference was observed in the FCR of birds in different treatment groups. Significantly ($P \leq 0.05$) better FCR was observed in birds fed diets supplemented with 2% enzyme treated *Artemisia absinthium* leaves i.e. T₇ group when compared to the control and other treatment groups.

The cumulative FCR (1-5 weeks of age) found was 1.77 ± 0.01 , 1.69 ± 0.009 , 1.66 ± 0.006 , 1.61 ± 0.01 , 1.65 ± 0.01 , 1.58 ± 0.01 and 1.55 ± 0.003 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. Overall feed conversion ratio of birds fed diets supplemented with 2% enzyme treated *Artemisia absinthium* leaves was significantly ($P \leq 0.05$) improved when compared to the birds fed diets supplemented with 1% and 1.5% enzyme treated *Artemisia absinthium* leaves or birds fed different levels of raw *Artemisia absinthium* leaves and the control diet.

Table 4.5: Average weekly feed conversion ratio in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Treatment groups	Age (Week)				
	1-2	2-3	3-4	4-5	1-5
T ₁	1.32 ^a ±0.03	1.57 ±0.08	1.90 ±0.04	2.02 ^a ±0.01	1.77 ^a ±0.01
T ₂	1.22 ^{ab} ±0.02	1.51 ±0.03	1.86 ±0.07	1.92 ^{ab} ±0.02	1.69 ^{ab} ±0.009
T ₃	1.19 ^b ±0.02	1.49 ±0.01	1.77 ±0.01	1.92 ^{ab} ±0.05	1.66 ^{bc} ±0.006
T ₄	1.19 ^b ±0.01	1.48 ±0.04	1.76 ±0.04	1.78 ^{cd} ±0.02	1.61 ^{bcd} ±0.01
T ₅	1.14 ^b ±0.04	1.50 ±0.02	1.81 ±0.02	1.86 ^{bc} ±0.02	1.65 ^{bc} ±0.01
T ₆	1.18 ^b ±0.01	1.46 ±0.04	1.76 ±0.02	1.70 ^{de} ±0.01	1.58 ^{cd} ±0.01
T ₇	1.17 ^b ±0.01	1.46 ±0.02	1.75 ±0.04	1.63 ^e ±0.01	1.55 ^d ±0.003
CD(P≤0.05)	0.078	-	-	0.077	0.037

Means within same column with different superscripts differ significantly (P≤0.05)

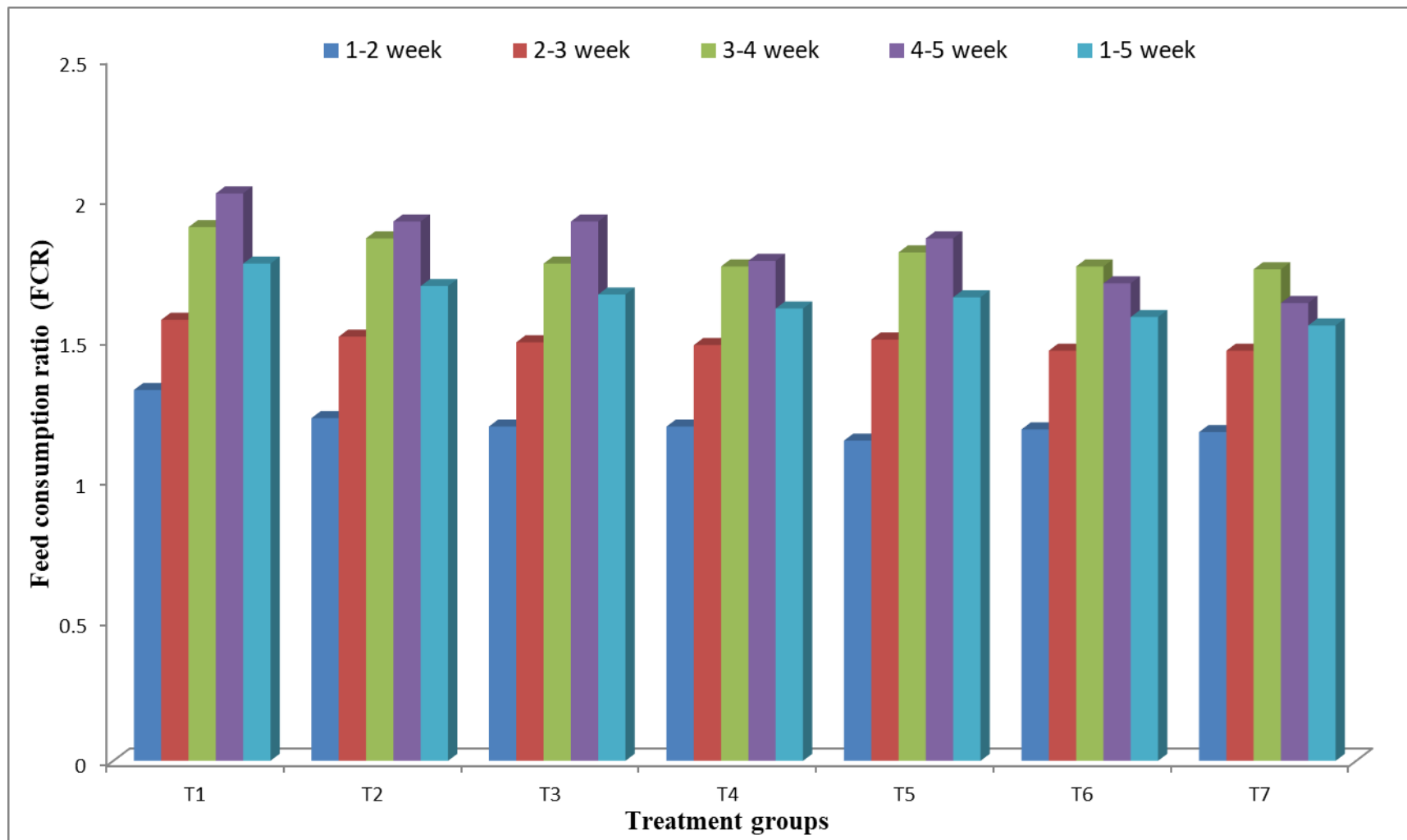


Fig. 4.4: Feed consumption ratio of different treatment groups at weekly interval

4.6 Mortality

The weekly and overall mortality per cent of broiler chickens fed different levels of *Artemisia absinthium* with or without enzyme supplementation is presented in Table 4.6 and shown graphically in Fig. 4.5. During the 1st week of age no mortality was recorded in any of the treatment groups. During the 2nd week of age the per cent mortality recorded was 3.3 per cent in T₄ group and during the 3rd week of age the per cent mortality recorded was 3.3 in T₆ and T₇ groups. During the 4th and 5th week of age no mortality was recorded in any of the treatment groups.

Table 4.6: Weekly and total mortality (%) in broilers chicken fed *Artemisia absinthium* with or without enzyme

Treatment group	Age in week					Overall
	1	2	3	4	5	1-5
T ₁ (Control)	0	0	0	0	0	0
T ₂ (1% <i>A. absinthium</i>)	0	0	0	0	0	0
T ₃ (1.5% <i>A. absinthium</i>)	0	0	0	0	0	0
T ₄ (2% <i>A. absinthium</i>)	0	2.7	0	0	0	2.7
T ₅ (1% <i>A. absinthium</i> +E)	0	0	0	0	0	0
T ₆ (1.5% <i>A. absinthium</i> + E)	0	0	2.7	0	0	2.7
T ₇ (2% <i>A. absinthium</i> +E)	0	0	2.7	0	0	2.7

4.7 Percent digestibility of nutrients (%)

The results of average nutrient digestibility in experimental birds fed diets supplemented with different levels of *Artemisia absinthium* with or without enzyme treatment is given in Table 4.7.

The dry matter digestibility was 69.43 ± 0.56 , 70.83 ± 0.58 , 72.15 ± 0.31 , 72.51 ± 0.29 , 72.57 ± 0.40 , 73.74 ± 0.52 and 74.61 ± 0.42 respectively for treatment groups T₁, T₂, T₃, T₄, T₅, T₆ and T₇. Significantly ($P \leq 0.05$) higher dry matter digestibility was observed in treatment groups fed diets supplemented with 2% enzyme treated *Artemisia absinthium* i.e. T₇ group as compared to control and treatment groups. The digestibility of crude protein was found to be 62.49 ± 0.17 , 63.47 ± 0.18 , 64.70 ± 0.11 , 65.24 ± 0.13 , 64.83 ± 0.03 , 65.68 ± 0.18 and 66.64 ± 0.16 for treatment groups T₁, T₂, T₃, T₄, T₅, T₆ and T₇ respectively. Significantly ($P \leq 0.05$) higher digestibility of crude protein (CP) was observed in treatment groups fed diets supplemented with 2% enzyme treated *Artemisia absinthium* i.e. T₇ group as compared to other treatment groups the control group.

The digestibility of Ether Extract was 64.12 ± 0.43 , 64.62 ± 0.44 , 65.62 ± 0.48 , 65.94 ± 0.49 , 66.02 ± 0.55 , 66.80 ± 0.44 and 66.98 ± 0.49 for treatment groups T₁, T₂, T₃, T₄, T₅, T₆ and T₇ respectively. The crude fibre (CF) digestibility was 37.01 ± 0.32 , 37.52 ± 0.31 , 38.14 ± 0.33 , 38.50 ± 0.35 , 38.98 ± 0.36 , 39.23 ± 0.38 and 39.78 ± 0.36 for treatment groups T₁, T₂, T₃, T₄, T₅, T₆ and T₇ respectively without any significant difference across different treatment groups.

Table 4.7: Average nutrient digestibility (%) in broiler fed diets supplemented with *Artemisia absinthium* with or without enzyme

Parameters	Treatment Group							CD (P≤0.05)
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	
Dry Matter	69.43 ^d ±0.56	70.83 ^{cd} ±0.58	72.15 ^{bc} ±0.31	72.51 ^{abc} ±0.29	72.57 ^{abc} ±0.40	73.74 ^{ab} ±0.52	74.61 ^a ±0.42	0.898
Crude Protein	62.49 ^e ±0.17	63.47 ^d ±0.18	64.70 ^c ±0.11	65.24 ^{bc} ±0.13	64.83 ^c ±0.03	65.68 ^b ±0.18	66.64 ^a ±0.16	0.453
Ether Extract	64.12 ±0.43	64.62 ±0.44	65.62 ±0.48	65.94 ±0.49	66.02 ±0.55	66.80 ±0.44	66.98 ±0.49	-
Crude Fibre	37.01 ±0.32	37.52 ±0.31	38.14 ±0.33	38.50 ±0.35	38.98 ±0.36	39.23 ±0.38	39.78 ±0.36	-

Means with different superscripts in same row differ significantly (P≤0.05)

4.8 Blood biochemicals

The results of various blood biochemical parameters estimated in broiler chicken fed diets supplemented with different levels of *Artemisia absinthium* with or without enzyme supplementation is presented in Table 4.8.

The serum Glucose levels (mg/dl) in different treatment groups was between 200.66 ± 10.68 and 245.33 ± 3.05 . The mean value of glucose was found to be significantly ($p < 0.05$) reduced in the group of birds fed diets supplemented with 1.5 and 2% *Artemisia absinthium* without enzyme and the groups fed 1, 1.5 and 2 % *Artemisia absinthium* with enzyme supplementation when compared to the control group and the group of birds fed diets supplemented 1% *Artemisia absinthium* without enzyme supplementation.

The serum cholesterol levels (mg/dl) in different treatment groups ranged between 161.00 ± 6.50 and 197.33 ± 5.45 . There was a significant reduction ($p < 0.05$) in the serum cholesterol levels in birds fed diets supplemented with different levels of enzyme treated *Artemisia absinthium* when compared to the birds fed diets supplemented with *Artemisia absinthium* without enzyme treatment and the control group. Among the group of birds fed diets supplemented with different levels of *Artemisia absinthium* without enzyme treatment, the serum cholesterol levels were significantly ($p < 0.05$) lower in birds fed diets supplemented with 2% *Artemisia absinthium* when compared to the birds fed diets supplemented with 1 and 1.5% levels of *Artemisia absinthium*.

The serum AST levels (u/l) varied between 252.30 ± 7.88 and 282.33 ± 6.64 among various dietary treatment groups fed diets supplemented with different levels of *Artemisia absinthium* with or without enzyme treatment with no statistical difference between the different treatment groups and the control group. The serum ALT levels (u/l) in various treatment groups varied between 7.33 ± 0.33 and 9.00 ± 0.57 with no statistically significant difference between the different treatment groups and the control group.

The lipid profile including serum triglycerides (mg/dl), high density lipoproteins (HDL) (mg/dl), and low density lipoproteins (LDL) (mg/dl) in various treatment groups varied significantly ($p<0.05$). The serum triglycerides ranged between 54.66 ± 2.33 and 70.66 ± 0.66 with significantly ($p<0.05$) highest levels in the control group and lowest in the group fed diets supplemented with 2% *Artemisia absinthium* with enzyme treatment. Overall significant ($p<0.05$) difference was observed in the triglyceride levels in the birds fed diets supplemented with *Artemisia absinthium* with enzyme treatment when compared to the birds fed diets supplemented with *Artemisia absinthium* without enzyme treatment and the control group. Similar trend was observed in the serum high density lipoproteins (HDL) and low density lipoproteins (LDL) levels.

The serum high density lipoproteins (HDL) (mg/dl) levels in various treatment groups ranged between 83.66 ± 2.18 and 99.66 ± 1.33 with significantly ($p<0.05$) highest levels found in T₇ group in which diet was supplemented with 2% *Artemisia absinthium* treated with enzyme supplementation and lowest levels in the control group. No significant difference was observed among the other treatment groups.

The serum low density lipoproteins (LDL) (mg/dl) levels in various treatment groups ranged between 67.00 ± 2.00 and 101.66 ± 2.40 . Significantly ($p<0.05$) higher levels were observed in the control group and the groups fed diets supplemented with *Artemisia absinthium* without enzyme treatment while as significantly lower levels were observed in birds fed diets supplemented with different levels of enzyme treated *Artemisia absinthium* with the lowest LDL found in group fed 2% levels.

The serum creatinine levels (mg/dl) ranged between 0.19 to 0.24 and were within the normal levels. No significant difference was observed in the serum creatinine level of birds fed diets supplemented with *Artemisia absinthium* with or without enzyme treatment and the control group.

Table 4.8: Blood biochemistry in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Parameters	Treatment Group							CD (P≤0.05)
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	
Glucose (mg/dl)	245.66 ^a ±3.05	239.33 ^a ±6.76	220.66 ^{ab} ±3.52	221.66 ^{ab} ±8.87	226.00 ^{ab} ±5.13	222.66 ^{ab} ±10.17	200.66 ^b ±10.68	22.866
Cholesterol (mg/dl)	197.33 ^a ±5.45	194.66 ^a ±1.33	196.66 ^a ±7.51	181.0 ^{ab} ±6.35	179.66 ^{ab} ±7.31	173.00 ^{ab} ±6.24	161.00 ^b ±6.50	18.776
AST(u/l)	258.33 ±12.67	257.34 ±5.17	281.33 ±16.18	253.66 ±11.68	252.30 ±7.88	260.66 ±8.11	282.33 ±6.64	-
ALT(u/l)	7.66 ±0.66	9.00 ±0.57	8.00 ±0.57	7.33 ±0.33	7.33 ±0.34	7.66 ±0.33	8.00 ±0.57	-
Triglyceride (mg/dl)	70.66 ^a ±0.66	68.66 ^a ±1.76	69.66 ^a ±1.20	68.00 ^a ±3.78	64.33 ^{ab} ±2.84	61.66 ^{ab} ±2.40	54.66 ^b ±2.33	7.198
HDL(mg/dl)	83.66 ^b ±2.18	89.66 ^{ab} ±4.84	86.00 ^{ab} ±4.16	85.66 ^{ab} ±1.45	93.66 ^{ab} ±3.84	94.00 ^{ab} ±3.18	99.66 ^a ±1.33	9.980
LDL(mg/dl)	101.66 ^a ±2.40	95.66 ^{ab} ±2.84	98.33 ^{ab} ±4.33	94.33 ^{ab} ±1.76	83.00 ^{bc} ±2.50	71.00 ^c ±3.40	67.00 ^c ±2.00	10.824
Creatinine (mg/dl)	0.20 ±0.04	0.21 ±0.01	0.24 ±0.01	0.19 ±0.01	0.21 ±0.01	0.22 ±0.01	0.19 ±0.01	-

Means with different superscripts in same row differ significantly (P≤0.05)

4.9 Slaughter traits and carcass characteristics

The results of slaughter and carcass characteristics in birds fed diets supplemented with different levels of *Artemisia absinthium* with or without enzyme treatment is presented in Table 4.9 and Table 4.10 respectively. There was a significant ($p<0.05$) increase in the dressing percentage of birds fed diets supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium*. Significantly ($p<0.05$) higher dressing percentage was observed in treatment group T₇. There was no significant difference between the different treatment groups and the control group with respect to blood loss, feather loss, visual carcass score.

The data on percent cutability characteristics of birds fed diets supplemented with different levels of *Artemisia absinthium* with or without enzyme treatment revealed no significant effect on various parameters like breast, drumsticks, thighs, wings, back and neck weights among various treatment groups and the control group.

4.10 Histomorphology

4.10.1 Liver

The histomorphology of liver in birds among all the treatment groups revealed normal structure, the lobulae being undelimited by conjunctive septa, being indentified on the basis of centrolobular venula and the Kiernan spaces near it. Hepatic cells had a polygonal shape, with 1 or 2 nuclei, with nucleolus. Liver of all treatment groups showed normal structure of central vein, hepatic sinusoids, endothelium, portal vein, sinusoids and kupffer cells (Plate 3-9).

4.10.2 Small intestine

The histomorphological structure of intestine in different treatment groups is presented in Plate no. 10-16. There was no variation in histomorphology of intestine of birds fed diets supplemented with *Artemisia absinthium* with or without enzyme supplementation from normal structure. Villi were lined by

Table 4.9: Slaughter traits in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Slaughter trait (%)	Treatment Group							CD (P≤0.05)
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	
Blood loss	3.69±0.11	3.86±0.15	4.18±0.37	3.737±0.40	3.91±0.23	4.12±0.07	3.69±0.26	-
Feather loss	7.29±0.22	7.42±0.55	8.29±0.59	9.00±0.40	8.16±0.49	8.38±0.69	8.73±0.43	-
Dressing	72.04 ^d ±0.89	72.20 ^d ±0.79	73.56 ^{cd} ±0.65	75.10 ^{bc} ±0.96	74.88 ^{bc} ±0.85	76.85 ^{ab} ±0.83	77.64 ^a ±0.78	1.045
Visual carcass score	A	A	A	A	A	A	A	

Means with different superscripts in same row differ significantly (P≤0.05)

Table 4.10: Carcass characteristics in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Cut up parts (%)	Treatment group						
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Breast	31.18±0.41	31.48±0.59	34.17±1.25	31.27±0.95	32.48±0.83	31.73±1.06	31.60±1.47
Drumsticks	13.56±0.42	13.44±0.21	13.03±0.29	13.54±0.18	13.17±0.53	14.07±0.58	12.77±0.76
Thighs	13.90±0.31	14.69±0.36	13.86±0.23	13.92±0.06	13.81±0.08	13.98±0.06	13.51±0.99
Wings	12.21±0.33	12.62±0.10	12.70±0.22	11.93±0.34	12.97±0.77	12.16±0.28	11.61±0.72
Back	15.70±0.34	15.98±0.37	14.56±1.20	16.00±0.88	15.96±0.49	15.41±1.50	14.15±0.50
Neck	4.89±0.55	6.48±1.09	6.38±1.03	5.66±1.10	7.06±1.90	4.19±0.14	6.73±1.62

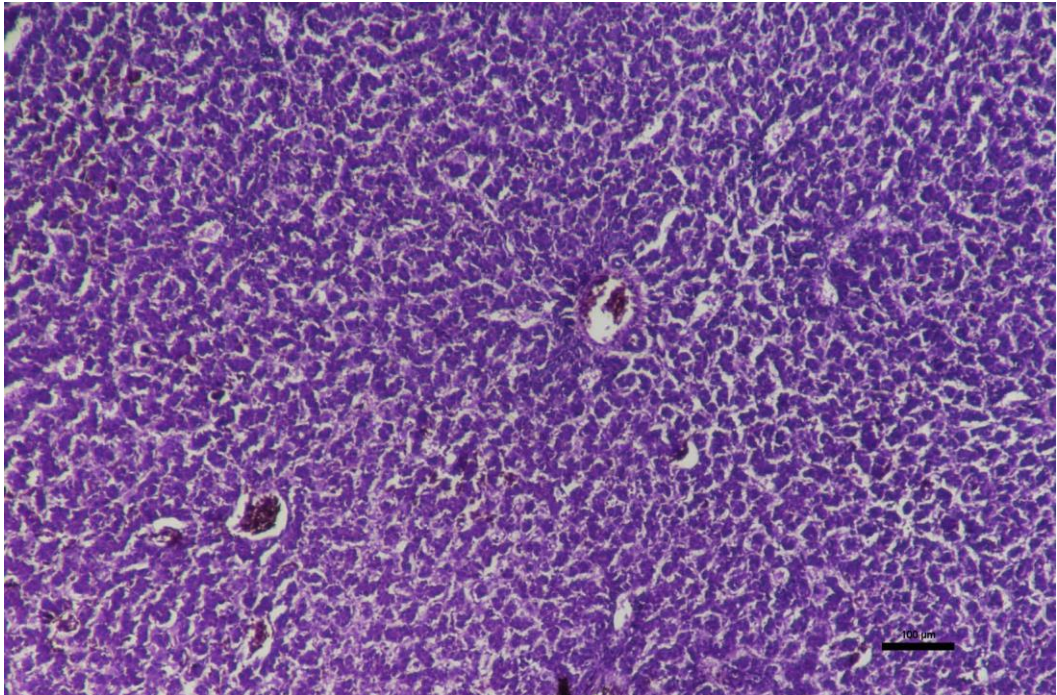


Plate-3: Histological section of Liver from control group (HE×100)

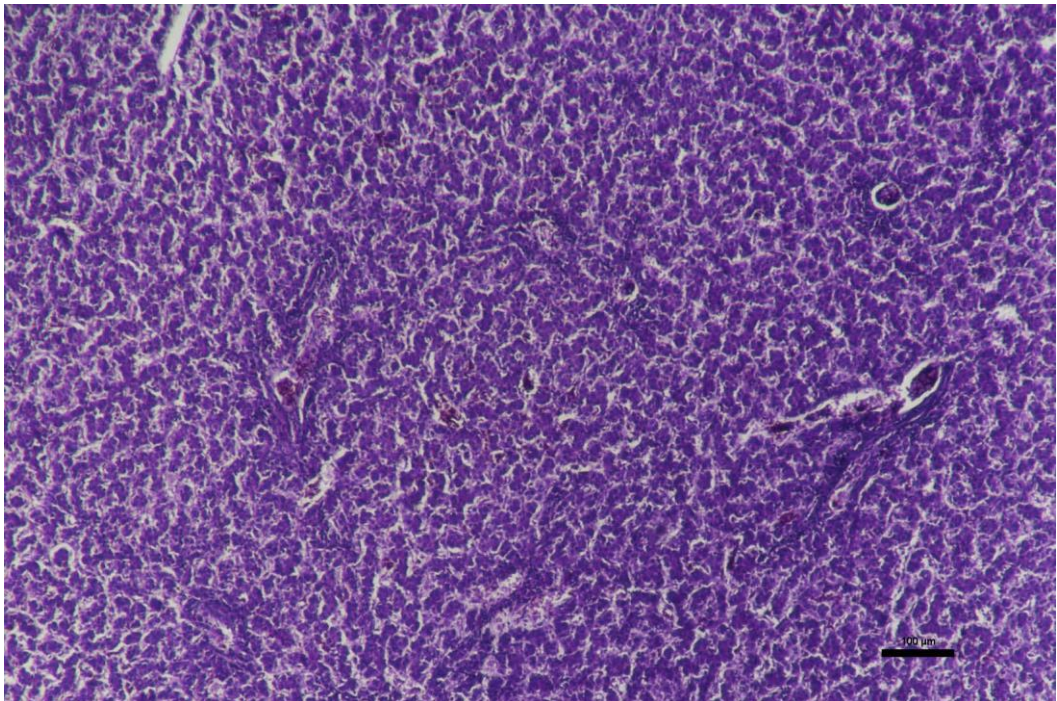


Plate-4: Histological section of Liver from group fed 1% *Artemisia absinthium* (HE×100)

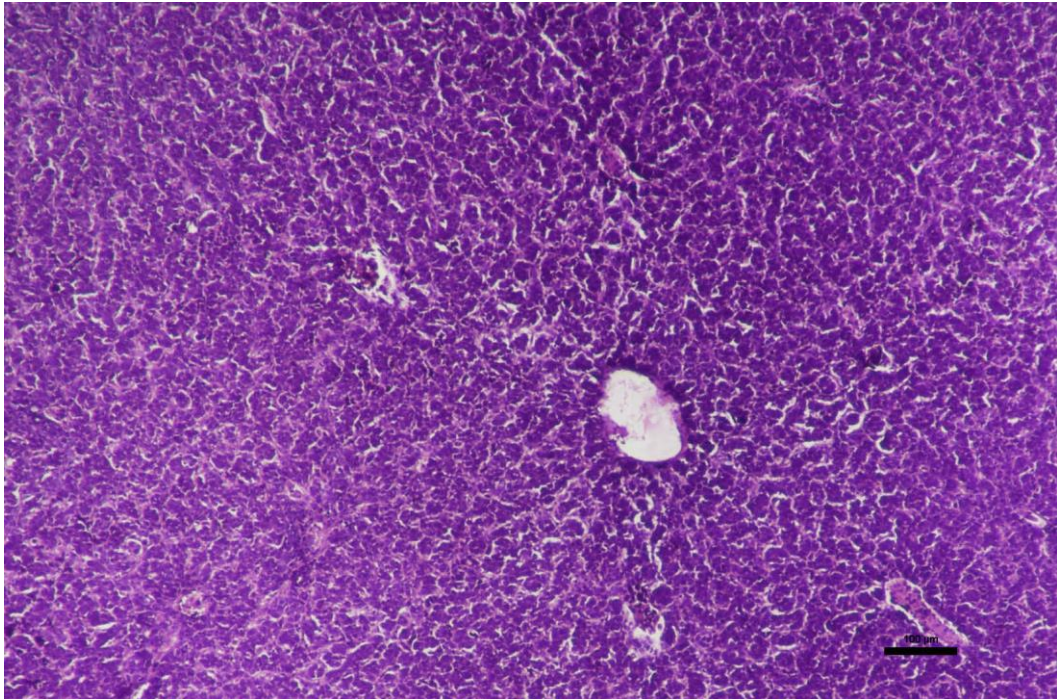


Plate-5: Histological section of Liver from group fed 1.5% *Artemisia absinthium* (HE×100)

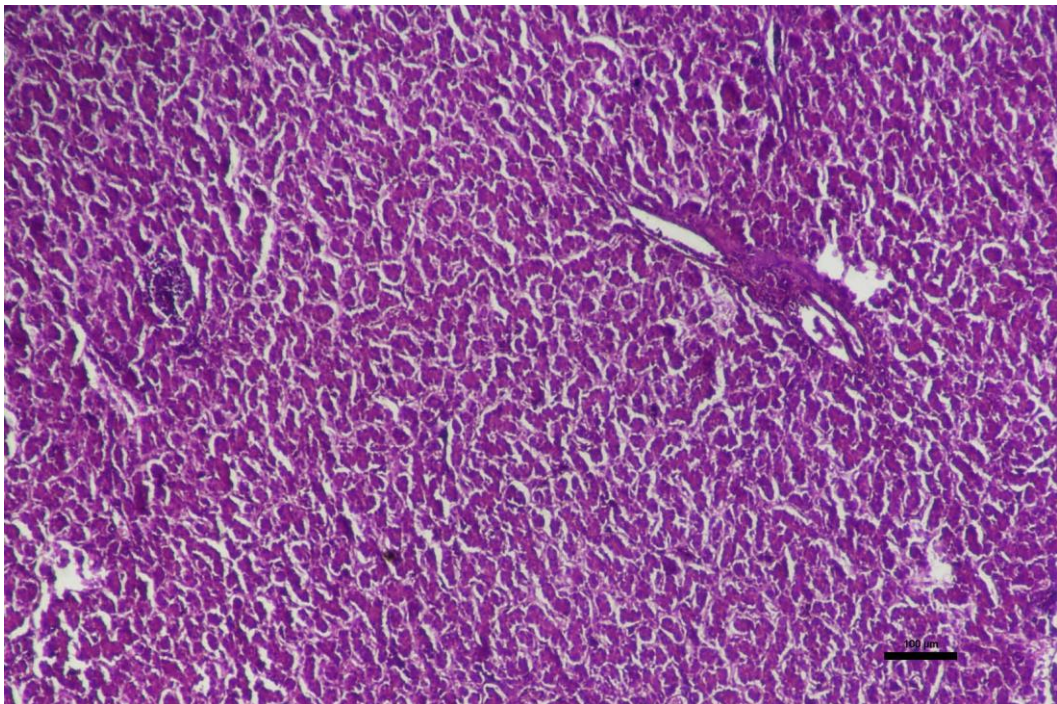


Plate-6: Histological section of Liver from group fed 2% *Artemisia absinthium* (HE×100)

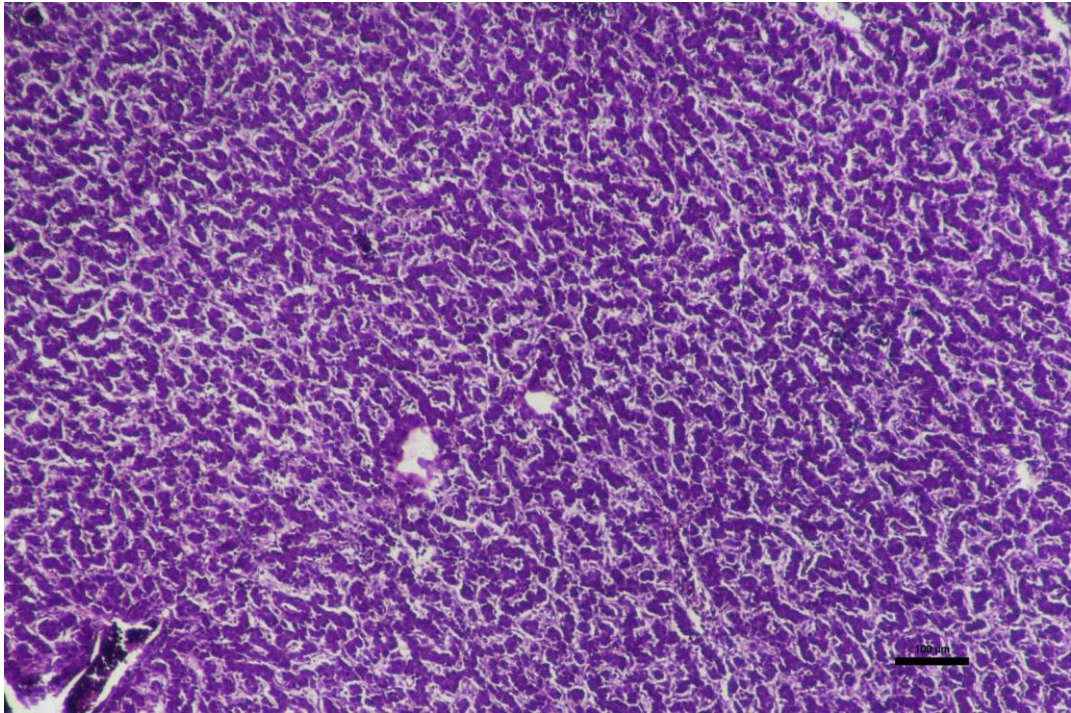


Plate-7: Histological section of Liver from group fed 1% *Artemisia absinthium* with enzyme (HE×100)

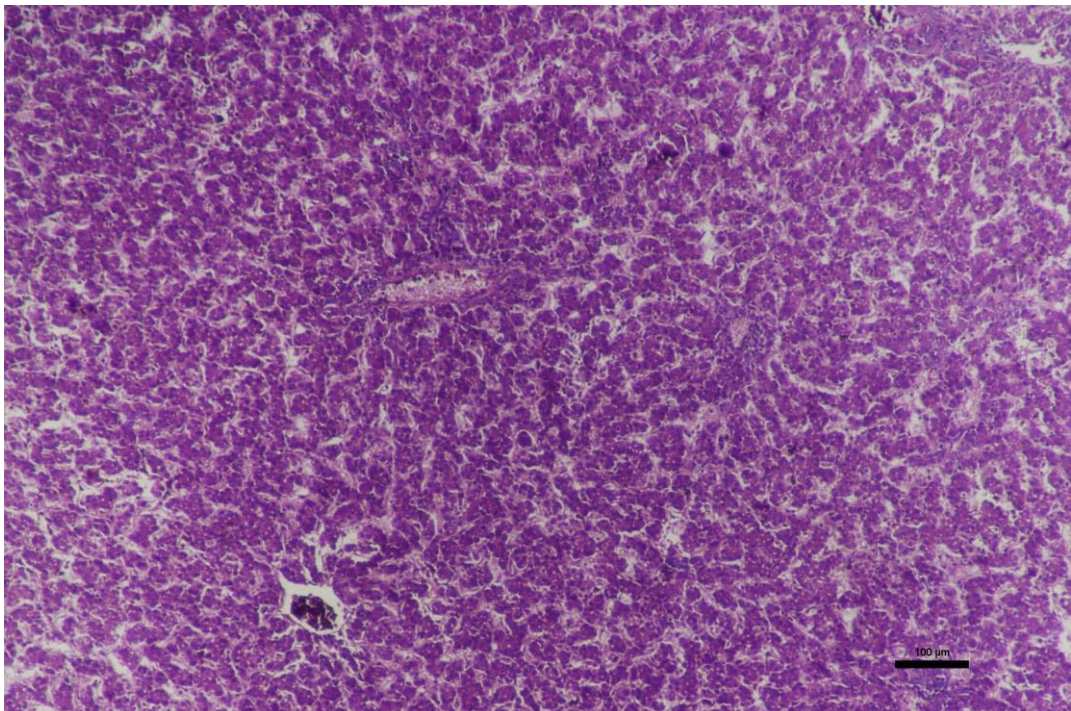


Plate-8: Histological section of Liver from group fed 1.5% *Artemisia absinthium* with enzyme (HE×100)

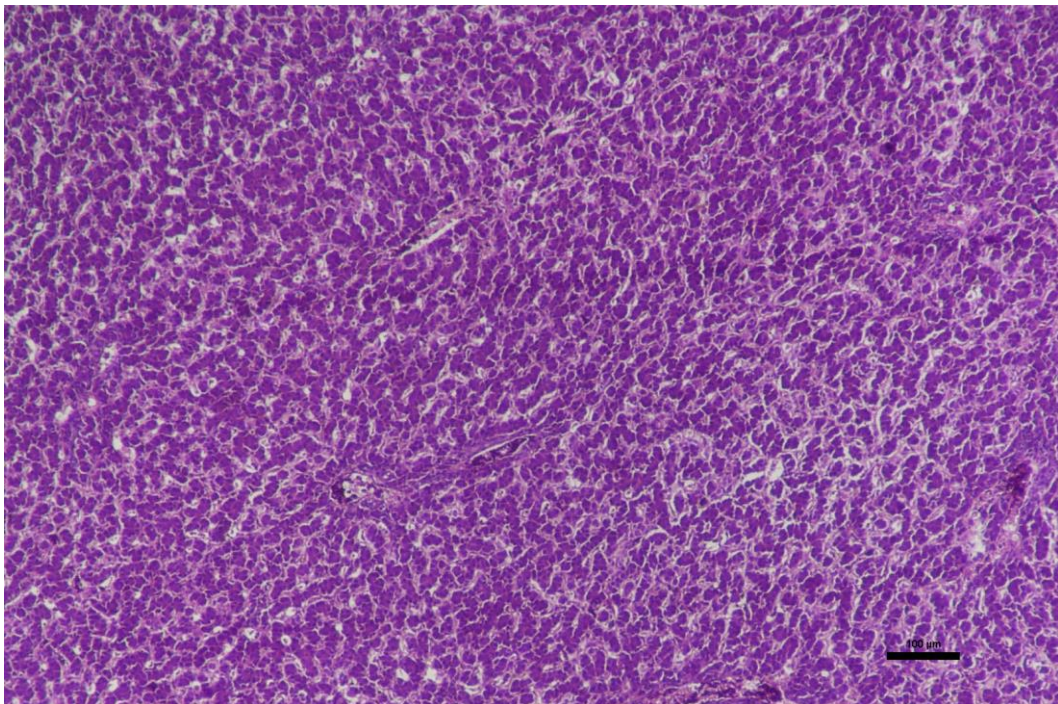


Plate-9: Histological section of Liver from group fed 2% *Artemisia absinthium* with enzyme (HE×100)

columnar epithelial cells, enterocytes. Enterocytes were having apical microvilli (brush border). The epithelium of villi was intact with no degeneration or denudation. There was no infiltration of inflammatory cells (Plate 9-16).

4.11 Cecal microbial population

The effect of diets supplemented with *Artemisia absinthium* with or without enzyme treatment on the cecal viable count and cecal coliform count of broilers is given in Table 4.11 (Plate17-18). Statistical analysis showed significant ($P \leq 0.05$) effect on the reduction of cecal viable count and caecal coliform count in the groups fed 1.5 and 2% raw and enzyme treated *Artemisia absinthium* as compared to the groups fed 1% raw and enzyme treated *Artemisia absinthium* and the control group. Among all the treatment groups, Significantly ($P \leq 0.05$) lower cecal viable count and cecal coliform counts were observed in treatment groups T₆ and T₇ i.e. the groups fed 1.5% and 2% enzyme treated *Artemisia absinthium*.

4.12 Immune competence

4.12.1 Anti SRBC antibody titre

The total anti SRBC titre of birds is presented in Table 4.12 (Plate-19). There was a significant ($p < 0.05$) difference between the various treatment groups and the control group. Significant ($p < 0.05$) difference was observed within the groups as well. The total anti SRBC titre of birds in different treatment groups was found to be 1.70 ± 0.26 , 1.93 ± 0.03 , 2.23 ± 0.20 , 2.33 ± 0.12 , 2.33 ± 0.13 , 2.56 ± 0.11 , 2.63 ± 0.08 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. Among the different treatment groups significantly ($p < 0.05$) higher anti SRBC antibody titre was observed in T₆ and T₇ groups i.e. the groups fed diets supplemented with 1.5% and 2% *Artemisia absinthium* treated with enzyme.

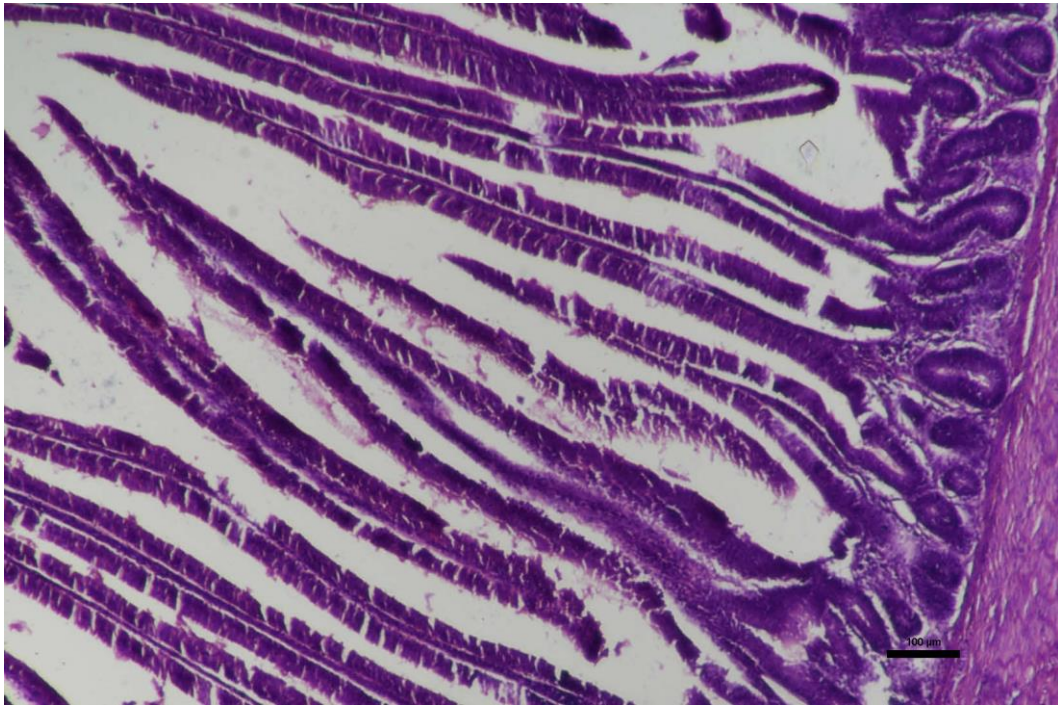


Plate 10: Histology of duodenum from control group showing normal villus architecture and histomorphology (HE×100)

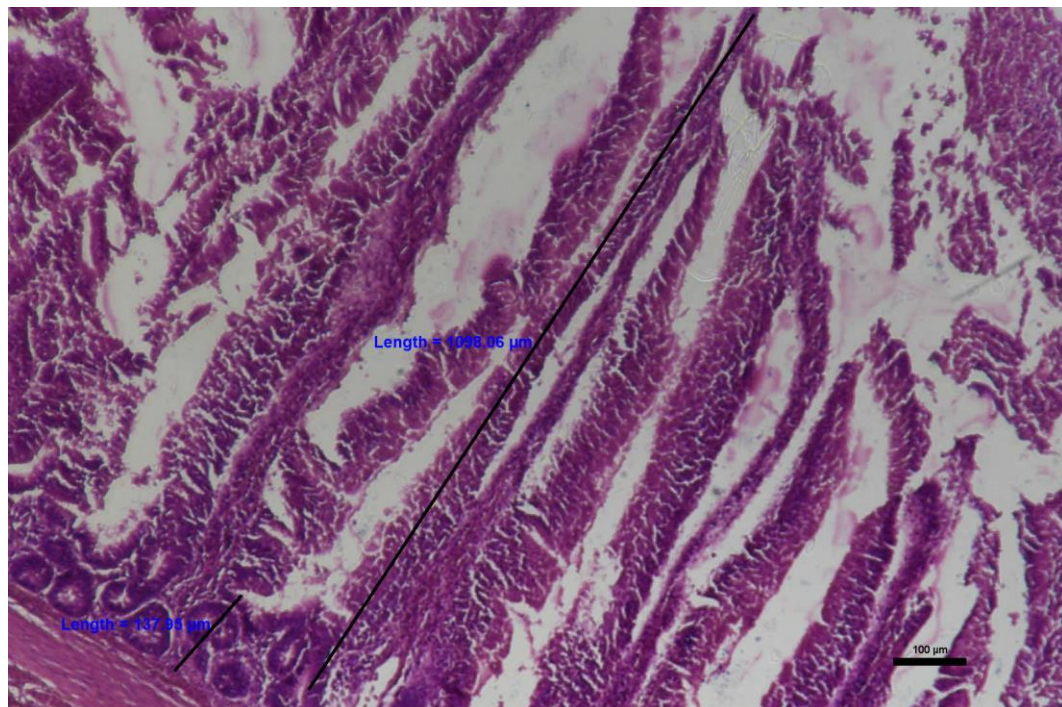


Plate 11: Histology of duodenum from group fed 1% *Artemisia absinthium* showing normal villus architecture and histomorphology (HE×100)

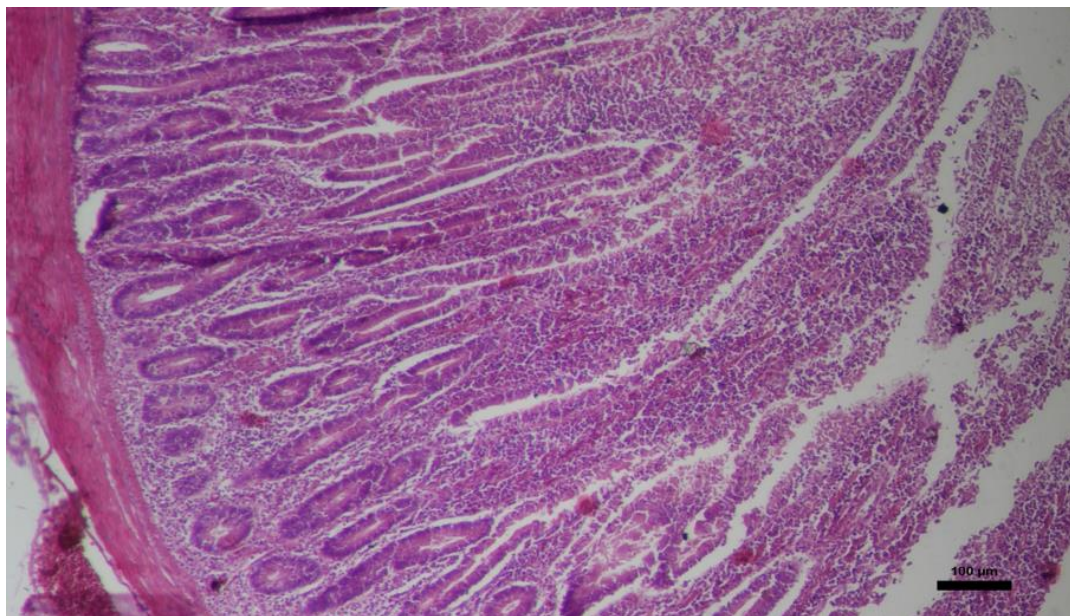


Plate 12: Histology of duodenum from group fed 1.5% *Artemisia absinthium* showing normal villus architecture and histomorphology (HE×100)

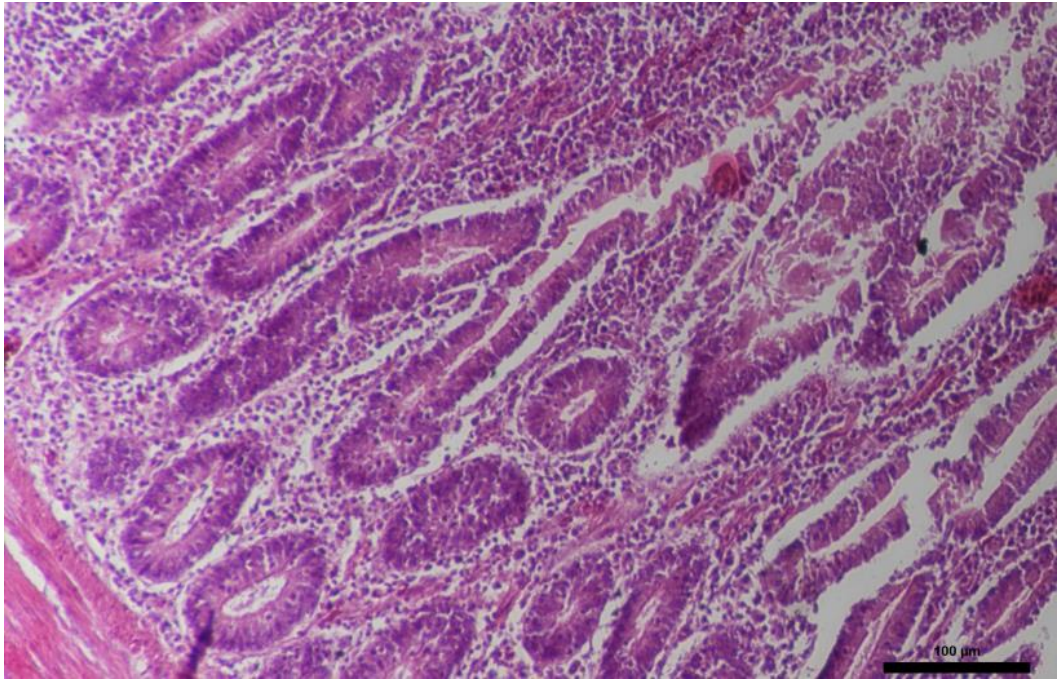


Plate 13: Histology of duodenum from group fed 2% *Artemisia absinthium* showing normal villus architecture and histomorphology (HE×100)

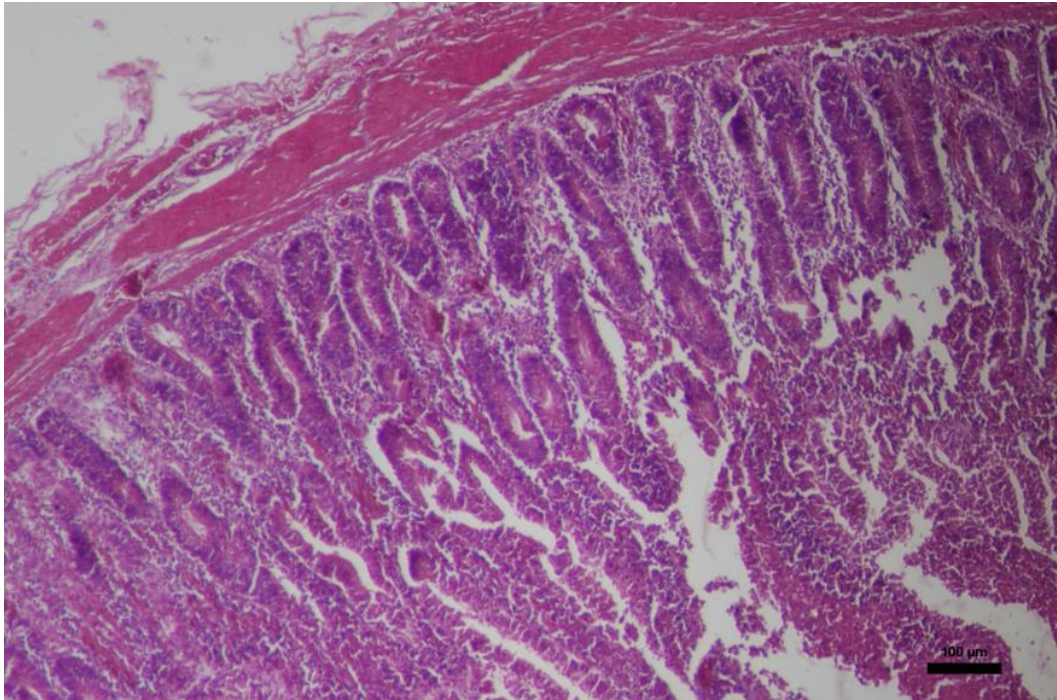


Plate 14: Histology of duodenum from group fed 1% *Artemisia absinthium* with enzyme showing normal villus architecture and histomorphology (HE×100)

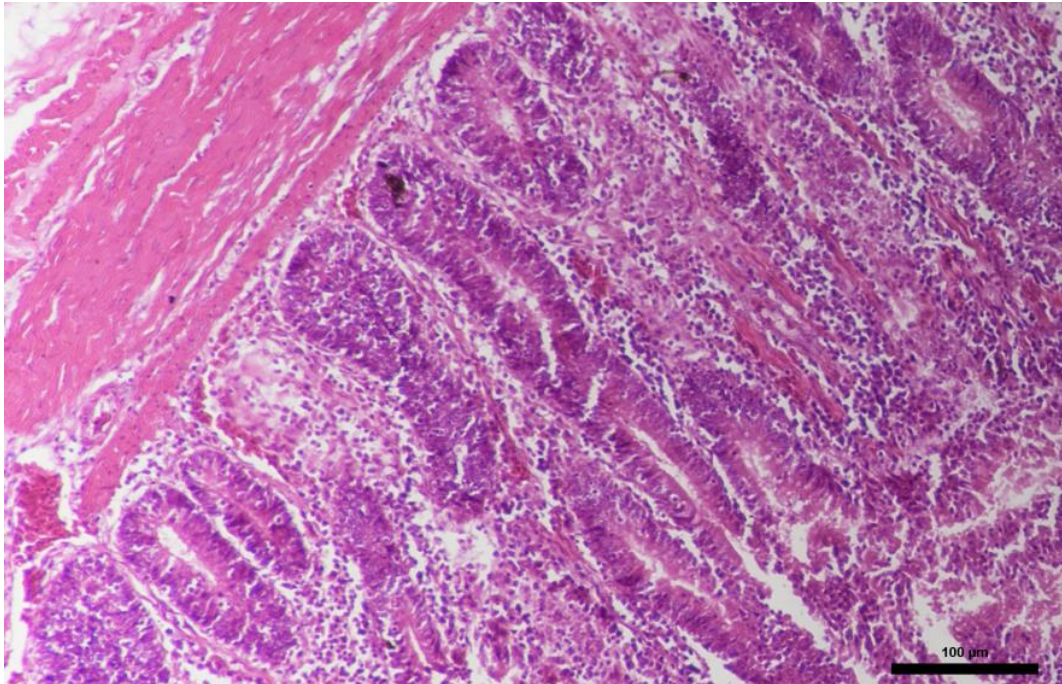


Plate 15: Histology of duodenum from group fed 1.5% *Artemisia absinthium* with enzyme showing normal villus architecture and histomorphology (HE×100)

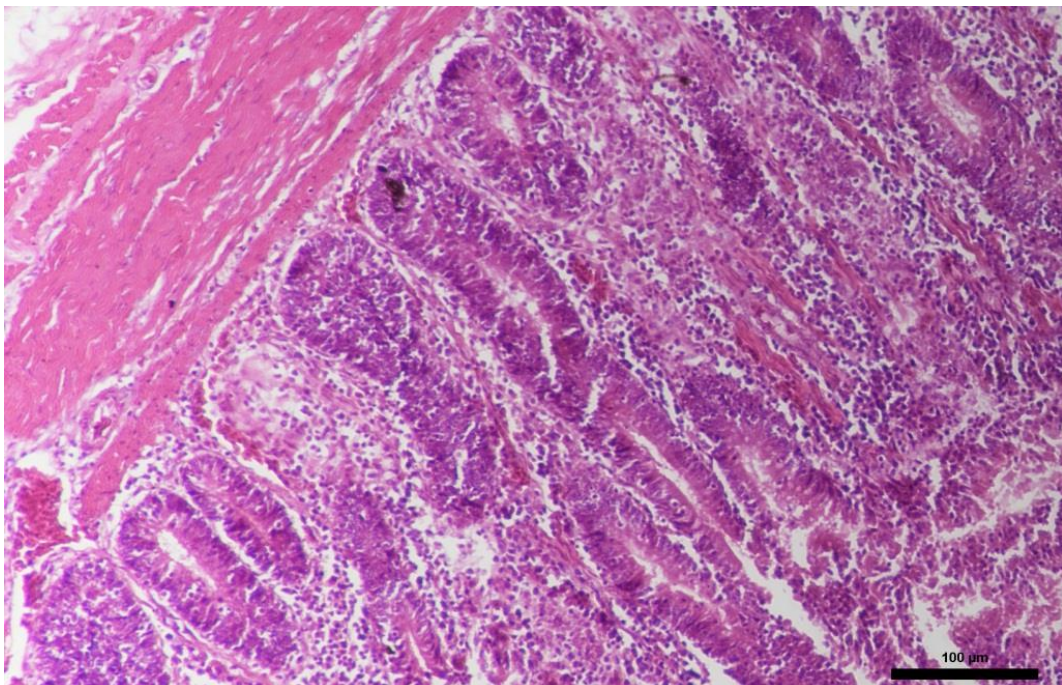


Plate 16: Histology of duodenum from group fed 2% *Artemisia absinthium* with enzyme showing normal villus architecture and histomorphology (HE×100)



Plate-17: Bacterial growth on Brain heart infusion agar for estimation of total viable count

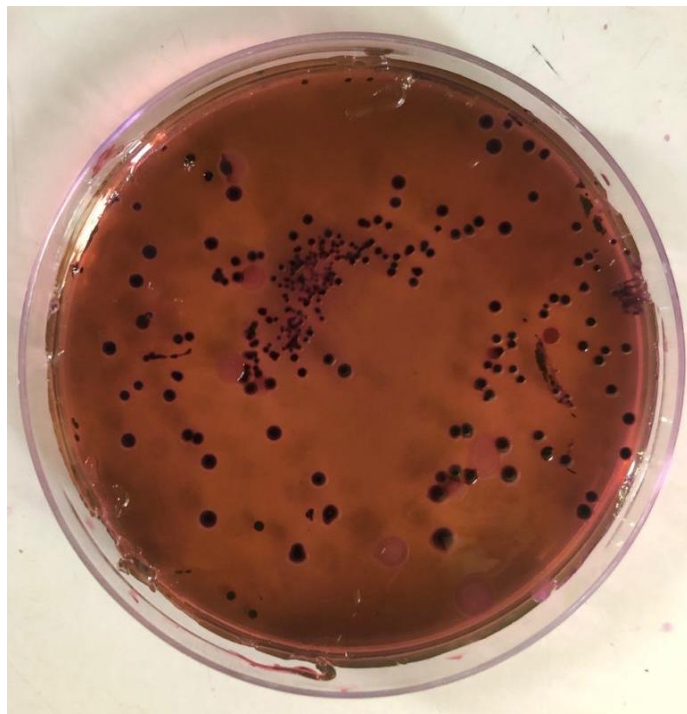


Plate-18: Bacterial growth on MacConkey agar for estimation of total coliform count

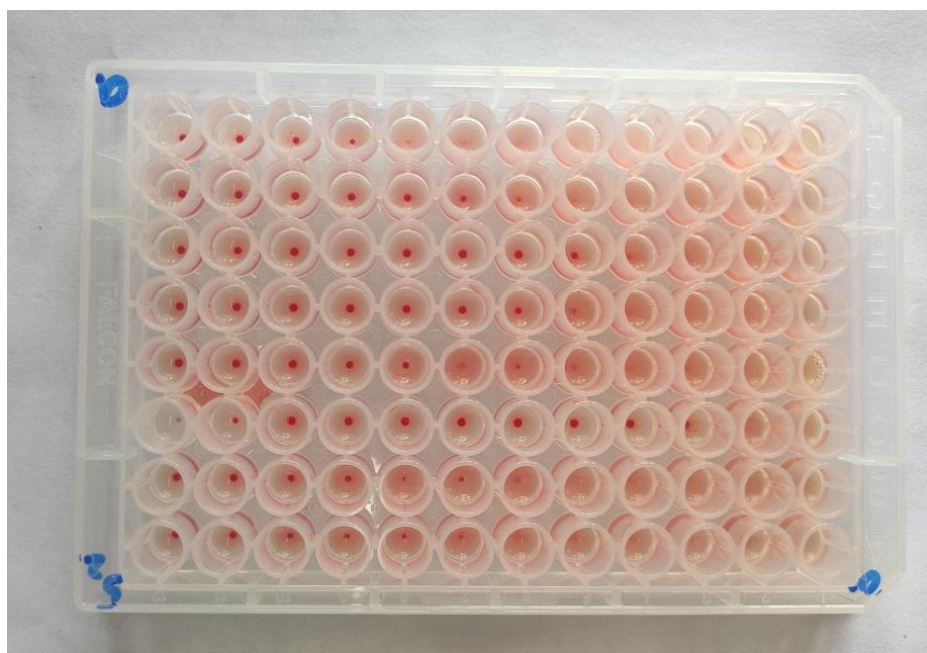


Plate-19: Anti-SRBC antibody titre on microtitreplate

Table 4.11: Cecal microbial population in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Parameter	Treatment Group							CD ($P \leq 0.05$)
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	
Cecal viable count (cfu/g)	8.62 ^a ±0.06	8.03 ^b ±0.05	7.65 ^c ±0.02	7.62 ^c ±0.02	7.95 ^b ±0.02	7.43 ^d ±0.03	7.41 ^d ±0.02	0.113
Cecal coliform count (cfu/g)	5.55 ^a ±0.03	5.18 ^b ±0.01	4.87 ^c ±0.02	4.85 ^c ±0.03	5.10 ^b ±0.01	4.61 ^d ±0.07	4.60 ^d ±0.02	0.126

Means with different superscripts in same row differ significantly ($P \leq 0.05$)

Table 4.12: Total anti SRBC antibody titre in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Parameter	Treatment Group							CD ($P \leq 0.05$)
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	
Total anti SRBC antibody titre (log ₁₀ HA)	1.70 ^b ±0.26	1.93 ^{ab} ±0.03	2.23 ^{ab} ±0.20	2.33 ^{ab} ±0.12	2.33 ^{ab} ±0.13	2.56 ^a ±0.11	2.63 ^a ±0.08	0.468

Means with different superscripts in same row differ significantly ($P \leq 0.05$)

4.12.2 Skin thickness

The skin thickness (mm) in birds fed diets supplemented with *Artemisia absinthium* with or without enzyme treatment is presented in Table 4.13 (Plate-20). It was found to be 1.01 ± 0.03 , 1.36 ± 0.03 , 1.36 ± 0.05 , 1.41 ± 0.22 , 1.61 ± 0.34 , 1.95 ± 0.01 and 1.98 ± 0.04 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ groups respectively. Significant difference was observed between the different treatment groups and the control group. Among the treatment groups significantly ($p < 0.05$) higher skin thickness was observed in T₆ and T₇ groups in which diets were supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium* when compared to other groups. Among all the groups, significantly lower skin thickness was observed in the control group.

4.12.3 Weight of immune organs

The effect of diets supplemented with *Artemisia absinthium* with or without enzyme treatment on the weight of immune organs of broiler chicken is presented in Table 4.14. No significant difference was observed in the per cent weight of bursa, thymus and spleen in the different treatment groups and the control group.

4.13 Antioxidant assay

4.13.1 Total antioxidant status (TAS) and total oxidant status (TOS)

The results of total antioxidant status (TAS) and total oxidant status (TOS) in various treatment groups have been summarized in Table 4.15. Significant ($p < 0.05$) difference was observed between the treatment groups and the control group. There was significantly ($p < 0.05$) lower total oxidant status in the treatment group T₅, T₆ and T₇ i.e. the groups fed diets supplemented with 1%, 1.5% and 2% enzyme treated *Artemisia absinthium* when compared to other groups. The plasma total antioxidant status was significantly ($p < 0.05$) higher in the T₇ group as compared to other treatment groups. Among all the groups, significantly lower total antioxidant status and higher total oxidant status was observed in the control group.

Table 4.13: Skin thickness (mm) in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Parameter	Treatment Group							CD (P≤0.05)
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	
Mean skin thickness (mm)	1.01 ^b ±0.03	1.36 ^{ab} ±0.03	1.36 ^{ab} ±0.05	1.41 ^{ab} ±0.22	1.61 ^{ab} ±0.34	1.95 ^a ±0.01	1.98 ^a ±0.04	0.487

Means with different superscripts in same row differ significantly (P≤0.05)

Table 4.14: Weight of immune organs in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Weight of immune organs (%)	Treatment group						
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Thymus	0.116±0.005	0.122±0.005	0.131±0.009	0.128±0.005	0.124±0.008	0.136±0.001	0.129±0.009
Bursa	0.138±0.007	0.138±0.01	0.140±0.001	0.138±0.004	0.163±0.014	0.138±0.006	0.146±0.008
Spleen	0.128±0.02	0.137±0.02	0.135±0.007	0.123±0.004	0.130±0.03	0.138±0.01	0.138±0.006

Table 4.15: Plasma total antioxidant status (TAS) and total oxidant status (TOS) in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Parameter	Treatment Group							CD (P≤0.05)
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	
TOS (μmol Trolox Eq/L)	7.74 ^a ±0.09	7.27 ^{ab} ±0.16	7.09 ^b ±0.12	7.05 ^b ±0.10	7.11 ^b ±0.08	6.86 ^b ±0.08	6.75 ^b ±0.15	0.365
TAS (μmol H ₂ O ₂ Eq/L)	1.24 ^c ±0.01	1.28 ^{bc} ±0.02	1.29 ^{abc} ±0.01	1.32 ^{abc} ±0.02	1.31 ^{abc} ±0.02	1.34 ^{ab} ±0.01	1.37 ^a ±0.01	0.055

Means with different superscripts in same row differ significantly (P≤0.05)



Plate 20: Increase in skin thickness of foot web by DNCB antigen

4.13.2 Thiobarbituric acid reactive substances (TBARS) assay

The effect of diets supplemented with *Artemisia absinthium* with or without enzyme treatment on the Thiobarbituric acid reactive substances (TBARS) assay in the broiler meat has been presented in Table 4.16. Statistically significant ($P<0.05$) difference was observed between the various treatment groups and the control group. The highest value of TBARS was observed in the control group. Among the various treatment groups, Significantly ($P<0.05$) lower TBARS value of meat was observed in the birds fed enzyme treated *Artemisia absinthium* as compared to the treatment groups fed raw *Artemisia absinthium*.

4.14 Sensory evaluation/organoleptic evaluation

The results of sensory evaluation of the meat from different treatments is presented in Table 4.17. There was no significant difference among the various sensory attributes i.e. appearance, flavor, juiciness, texture, mouth coating and the overall acceptability ranged from 6.86 to 7.12.

4.15 Proximate composition of meat

The effect of diets supplemented with *Artemisia absinthium* with or without enzyme treatment on the proximate composition of broiler meat is presented in Table 4.18. No significant effect was found on the moisture, crude protein, crude fat and ash content of broilers of different treatment groups.

4.16 Economics

The economics of rearing broilers fed diets supplemented with *Artemisia absinthium* with or without enzyme treatment is presented in table 4.19. Feed cost/kg live weight gain worked out to be highest (Rs.70.80) for the birds of T₁ group (control) followed by Rs. 68.44, Rs. 67.64, Rs. 66.01, Rs. 67.27, Rs. 64.81 and Rs. 59.94 for T₂, T₃, T₄, T₅ T₆ and T₇ respectively. The difference in feed cost compared to the control group worked out to be highest for T₆ and T₇ groups.

Table 4.16: TBARS assay of meat of broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Parameter	Treatment Group							CD ($P \leq 0.05$)
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	
TBARS (nmol MDA/mg of meat)	0.55 ^a ±0.003	0.50 ^{ab} ±0.003	0.49 ^{ab} ±0.002	0.477 ^{ab} ±0.004	0.46 ^b ±0.002	0.45 ^b ±0.003	0.44 ^b ±0.002	0.031

Means with different superscripts in same row differ significantly ($P \leq 0.05$)

Table-4.17: Sensory Evaluation/organoleptic parameters of meat in broilers fed diets supplemented with *Artemisia absinthium* with or without enzyme

Attributes	Treatment group						
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Appearance	6.98±0.12	7±0.11	7.19±0.11	7.02±0.11	7.09±0.13	7.10±0.13	7.01±0.12
Flavour	6.81±0.12	6.86±0.12	7±0.13	6.98±0.12	6.90±0.13	7.01±0.12	7±0.13
Juiciness	6.86±0.12	6.97±0.13	6.81±0.12	6.72±0.12	6.87±0.13	6.92±0.13	6.85±0.12
Texture	6.90±0.13	6.95±0.13	6.87±0.12	6.93±0.12	6.96±0.13	6.93±0.14	6.97±0.14
Mouthcoat	7.23±0.12	7.52±0.11	7.57±0.13	7.47±0.13	7.42±0.11	7.53±0.15	7.18±0.15
Overall acceptability	6.86±0.12	6.96±0.11	7±0.11	7.02±0.11	7.06±0.11	7.12±0.13	7.08±0.11

Table 4.18: Proximate composition of meat in broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Parameter	Treatment group						
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Moisture (%)	75.06± 0.08	75.50± 0.15	75.36± 0.08	75.13± 0.12	75.33± 0.24	75.30± 0.26	75.33± 0.17
Protein (%)	21.03± 0.16	21.15± 0.24	21.25± 0.23	21.42± 0.26	21.41± 0.12	21.27± 0.23	21.60± 0.23
Fat (%)	2.36± 0.17	2.16± 0.08	2.30± 0.29	2.07± 0.07	2.01± 0.05	2.07± 0.07	2.00± 0.01
Total ash (%)	1.76± 0.08	1.72± 0.08	1.47± 0.20	1.39± 0.20	1.60± 0.15	1.35± 0.10	1.29± 0.07

Table 4.19: Cost of production of broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme

Component	Treatment group						
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Basal diet (Rs/kg)	40	40	40	40	40	40	40
<i>Artemisia</i> (Rs)	0	0.5	0.75	1	0.5	0.75	1
Enzyme (Rs)					0.27	0.27	0.27
Total cost of feed (Rs)	40	40.5	40.75	41	40.77	41.02	41.27
FCR	1.77	1.69	1.66	1.61	1.65	1.58	1.55
Feed cost/kg live weight gain (Rs)	70.80	68.44	67.64	66.01	67.27	64.81	63.96
Difference in feed cost compared to control	-	2.44	3.24	4.87	3.61	6.07	6.92

Chapter 5

DISCUSSION

5.1 Proximate composition

The various proximate components and bioactive compounds contribute towards the nutritional value of herbs and medicinal plants. Results of proximate analysis in present study revealed that the moisture content of *Artemisia absinthium* leaves was 12.91 per cent and the per cent dry matter was 87.09. The per cent crude protein content was 23.56 whereas ether extractable fat content was found to be 4.31 per cent and the crude fibre content was 20.30 per cent. The per cent total ash content of *Artemisia absinthium* leaves was 8.65 per cent and the nitrogen free extract was 43.18 per cent. The proximate composition of different species of *Artemisia* has been reported by many workers. The proximate composition of *Artemisia* varies depending upon the species, geographical conditions, level of plant maturity and soil composition. Brisbie *et al.* (2008) found that *Artemisia annua* leaves contain about 19.66 per cent Crude protein, 14.4 per cent Crude fibre, 5.73 per cent Crude fat, 10.26 per cent Ash content, 49.94 per cent Digestible carbohydrates. Iqbal *et al.* (2012) reported that the chemical composition of *Artemisia annua* leaves comprised of 7.5 per cent Ash, 8.3 per cent Carbohydrate, 6.07 per cent Fat, 14.2 per cent Fibre, 11.4 per cent Moisture and 24.37 mg/100 g Protein on dry matter basis. Ashok and Upadhyaya (2013) reported that *Artemisia absinthium* leaves contain 17.2 per cent moisture, 2.50 per cent total ash, 11.66 per cent starch, 6.38 per cent sugar, 0.20 per cent tannin and 2.78 per cent phenol whereas *Artemisia annua* leaves comprises of 15.7 per cent moisture, 14.18 per cent total ash, 13.95 per cent starch, 9.69 per cent sugar, 0.18 per cent tannin and 1.83 per cent phenol. Ait-Kaki *et al.* (2018) *Artemisia herba-alba* contain 29.6 per cent crude fibre, 11 per cent crude protein which consists of 72% of amino acids and 16.5 per cent total nitrogen content. The mineral composition consists of Ca, P, K, Na and Mg as 10.6, 3.3, 35.5, 0.4 and 2.5g/kg respectively.

5.2 Body weight and weight gain

The results showed that the group of birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves had significantly ($P \leq 0.05$) higher body weight and body weight gain when compared with the group of birds fed diets supplemented with different levels of raw *Artemisia absinthium* leaves and the control group during the growth period from 1 to 5 weeks of age. In the current study the improvement in live body weight and weight gain of broilers fed diets supplemented with enzyme treated *Artemisia absinthium* might be due to its high crude protein content (23.5% in the present study), minerals, Vitamin A, C, E, antioxidants and flavonoids such as quercitin and coumarins (Brisbie *et al.*, 2008; Bilia *et al.*, 2006) which are very important for growth and development of poultry as well as for increasing the weight of animals (Brisibe *et al.*, 2008). The beneficial effects of dietary *Artemisia absinthium* supplementation on body weight and performance of birds might possibly be attributed to its high concentrations of essential oils, which are beneficial in maintaining a healthy microbiota balance (Greathead, 2003) and its antimicrobial activity with a positive effect on gut health (Khalaji *et al.*, 2011).

The results of present study are in agreement with Brisbie *et al.* (2008a) who reported that the broilers supplemented with 10% and 20% of dried *Artemisia annua* leaves in the commercial mash had significantly ($P < 0.05$) higher live weight gain. Similarly, Gholamrezaie *et al.* (2013) reported that 0.5, 1 and 1.5% of *Artemisia annua* leaf powder with 2000 and 4000 ppm methanolic extract improved weight gain of broilers, Dragan *et al.* (2014) and Kostadinovic *et al.* (2015) found that 1.5% of *Artemisia annua* and 100g, 150g and 200g/kg of *Artemisia absinthium* significantly ($P < 0.05$) increased the weight gain of broilers. Similarly Popovic *et al.* (2017) observed that the addition of 200 g/kg of *Artemisia absinthium* (wormwood) in rabbit diet led to the highest body weight with significant differences compared to the control treatment. The supplementation of *Artemisia absinthium* herb alone as feed additive in complete

diets in lambs significantly ($P<0.05$) enhanced the growth rates (Beigh *et al.*, 2018). Wang *et al.* (2019) also found that diets supplemented with *A. vulgaris* meal improved final body weight of rabbits. Dietary supplementation with 2 g/kg enzymatically treated *Artemisia annua* L. significantly ($P<0.05$) increased growth performance, in weaned pigs (Yiu Niu *et al.*, 2020). Dietary inclusion of *Artemisia annua* L. aqueous extract (AAE) significantly improved the average daily weight gain of broilers (Shiwei Guo *et al.*, 2020)

In contrast to the findings of the current study, dietary supplementation of 1 percent *Artemisia sieberi* (Khalaji *et al.*, 2011), 10 and 20 g/kg dried *Artemisia annua* leaves (Engberg *et al.*, 2012), 2 and 4 percent *Artemisia annua* (Cherian *et al.*, 2013), and 0.4 percent *Artemisia dracunculus* to broilers (Gharetappe *et al.*, 2015) did not effect the weight gain of broilers. According to Hosseinzadeh and Moghaddam (2014), dietary supplementation with 0.125 and 0.25 percent *Artemisia dracunculus* had no effect on weight gain, while a 0.5 percent dosage decreased the same in broilers. Incorporating 5 mg/kg of *Artemisia sieberi* granule extract in the diet of broilers lowered live weight at 42 days when compared to the control group (Kheirabadi *et al.*, 2014).

The enzyme treatment in present study increased the efficiency of the additive because the enzymatic treatment of phytogetic compounds with cellulases, pectinases, and hemicellulases, either alone or in combination, has been found to hydrolyze and disintegrate the plant's cell wall, allowing intracellular bioactive chemicals to be released more easily (Puri *et al.*, 2012). Similarly the plant cell wall integrity gets disrupted by enzymes, resulting in the release of nutrients enclosed by the cell wall (Ravindran, 2013).

5.3 Feed consumption

The group of birds fed different levels of enzyme treated *Artemisia absinthium* leaves had significantly ($P\leq0.05$) higher feed consumption when compared to the birds fed diets supplemented with raw *Artemisia absinthium*

leaves and the control group. Among the birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves highest feed consumption was recorded in birds fed diets supplemented with 2% *Artemisia absinthium* leaves i.e. T₇ group during the entire growth period from 1 to 5 weeks of age.

The improvement in feed consumption with the supplementation of enzyme treated *Artemisia absinthium* leaves in the current study could have been because of the presence of essential amino acids, minerals, vitamins A, C and E, antioxidants and flavonoids such as quercetin and coumarins in *Artemisia spp.* (Brisibe *et al.*, 2008; Bilia *et al.*, 2006) and due to its positive effects on digestive secretions and the activities of digestive enzymes as reported by Patel and Srinivasan (2004) that the phytogetic feed additives increase the digestive secretions and enzymatic activity thus having positive effects on the digestive tract. Appetizing and digestion-stimulating characteristics, as well as antibacterial actions, are found in herbs, spices, and diverse plant extracts (Hernandez *et al.*, 2004). Lee *et al.* (2004) also found that the intestinal activities of trypsin, lipase and amylase in broilers is enhanced by the phytogetic compounds.

Similar findings were reported by Song *et al.* (2018) who found that dietary supplementation of 1g/kg enzymatically treated *Artemisia annua* increased ($P<0.05$) feed intake in heat stressed broilers compared to the control group. Saracila *et al.* (2018) found that broilers fed diets treated with a mixture of *A. annua* oil and powder had a significantly ($p<0.05$) higher average daily feed intake than those fed the conventional diet or those treated with *A. annua* oil only. Brisbie *et al.* (2008) found that *Artemisia annua* L. leaves as a botanical coccidiostat in coccidia-infested broilers increased the feed consumption as compared to the control group. Kostadinovic *et al.* (2015) found that the broilers fed diets supplemented with 100g, 150g and 200g/kg of *Artemisia absinthium* had increased feed consumption compared to control group. Diets supplemented with *Artemisia apiacea* Hance at 25, 50, and 75 g/kg significantly ($P<0.05$) improved feed intake in rabbits compared to control group (Ding *et al.*, 2019). Wang *et al.*

(2019) also found that diets supplemented with *A. vulgaris* meal improved feed intake in rabbits.

Contrary to the present findings, Khalaji *et al.* (2011) found that the addition of 1% *Artemisia sieberi* leaves significantly ($P \leq 0.01$) decreased feed intake in broilers up to 21 days of age. Engberg *et al.* (2012) found that the supplementation of dried *A. annua* leaves (0, 5, 10 and 20 g/kg) and n-hexane extract from fresh *A. annua* leaves resulted in dose dependent decrease feed consumption in broilers. Supplementation of *Artemisia vulgaris* L. powder and *Pinus densiflora* powder at 1 and 2% in broilers diets had no effect on feed consumption (Kim *et al.*, 2012a). Supplementation of 2000, 4000 ppm methanolic extract and 0.5%, 1% and 1.5% leaf powder of *Artemisia annua* significantly ($P < 0.05$) reduced daily feed intake in broiler chicken (Gholamrezaie *et al.*, 2013). Supplementation of broiler diets with 100, 200 and 300 ppm of Wormwood (*Artemisia absinthium*) and Cumin (*Cuminum cyminum*) essential oil had no significant effects on feed consumption of broiler chicken (Habibi *et al.*, 2016). Wan *et al.* (2016) reported no significant effect of the supplementation of broiler diets with enzymatically treated *Artemisia annua* L. on the average daily feed intake of broilers. Dietary inclusion of *Artemisia annua* L. aqueous extract (AAE) significantly decreased the feed intake of broilers (Shiwei Guo *et al.*, 2020).

5.4 Feed conversion ratio

The feed conversion ratio was significantly ($P \leq 0.05$) improved in the birds fed diets supplemented with 1.5 % and 2% enzyme treated *Artemisia absinthium* leaves when compared to the birds fed diets supplemented with 1% enzyme treated *Artemisia absinthium* leaves or birds fed different levels of raw *Artemisia absinthium* leaves and the control diet. This improvement in FCR might be due to its positive effects on digestive secretions and the activities of digestive enzymes as reported by Patel and Srinivasan (2004) that the phytogetic feed additives increase digestive secretions and enzymatic activity thus exerting positive effects within the digestive tract and also due to the presence of flavonoids which

decrease the population of pathogenic bacteria in digestive tract thus improving the overall performance of broilers and a better FCR (Srividya *et al.*, 2010).

The findings of this study are consistent with those of Brisibe *et al.* (2008), who found that dietary supplementation of *Artemisia* species increased feed efficiency in broilers and laying hen productivity.. Similarly, Dragan *et al.* (2010) also found that *Artemisia annua* oil extract significantly improved feed conversion in broilers when compared to the control group. Engberg *et al.* (2012) found that the supplementation of n-hexane extract from fresh *A. annua* leaves (0, 125, 250 and 500 mg/kg) and dried *A. annua* leaves (0, 5, 10 and 20 g/kg) and resulted in an improved feed conversion ratio. Kim *et al.* (2012b) also observed that supplementation of 2.5 and 5.0 g/kg of *Lactobacillus*-fermented *Artemisia princeps* (LFA) in diets significantly ($P < 0.05$) improved feed efficiency in broilers. Kostadinovic *et al.* (2015) found that the inclusion of 100g, 150g and 200g/kg of *Artemisia absinthium* in broiler diets significantly ($P < 0.05$) improved feed efficiency in treatment groups compared to control group. Addition of 0.5% *Artemisia herba* powder significantly ($P < 0.05$) increased feed conversion ratio in laying hens (Multag *et al.*, 2018). Dietary inclusion of *Artemisia annua* L. aqueous extract (AAE) significantly improved the feed conversion ratio of broilers (Shiwei Guo *et al.*, 2020)

On the contrary, adding 1% *Artemisia sieberi* leaves had no effect on body weight or feed conversion ratio, although it did reduce feed consumption significantly up to 21 days of age ($P \leq 0.01$) in broilers (Khalaji *et al.*, 2011). Cherian *et al.* (2013) and Wan *et al.* (2016) found no significant difference in feed efficiency of broilers fed diets supplemented with 0, 2 and 4% *Artemisia annua* leaf powder compared to control group. Popovic *et al.* (2018) found no significant difference in the feed efficiency of broilers fed diets supplemented with medicinal plants mixtures (*Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum*).

5.5 Mortality

In the present study, no significant effect on the mortality of birds was observed. The possible reason for this could be the presence of various bioactive compounds in *Artemisia absinthium* having diverse pharmacological effects such as anti-inflammatory (Lee *et al.*, 2004), antimicrobial (Caner *et al.*, 2008), antioxidant (Mahmoudi *et al.*, 2009). Similarly Brzoska *et al.* (2010) reported that herb mixture supplementation had no significant effect on chicken mortality. Abo Omar *et al.* (2016) reported that the supplementation of natural herb extract in broilers reduced mortality by improving the general performance of birds and building up the immunity.

5.6 Digestibility of nutrients

Significantly ($P \leq 0.05$) higher digestibility of dry matter and crude protein was recorded in treatment groups fed diets supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium* i.e. T₆ and T₇ groups as compared to the control and other treatment groups. The improvement in digestibility was due to the appetite and digestion-stimulating properties and antimicrobial effects present in the herbs, spices, and various plant extracts. Also, essential oils have previously been shown to have beneficial effects on the digestive system (Hernández *et al.*, 2004), including stimulating effects on the output of digestive enzymes from the pancreas, gut mucosa, and enhanced bile flow (Platel and Srinivasan, 2004; Jamroz *et al.*, 2005; Jang *et al.*, 2007). These changes might contribute to better nutrient absorption in the small intestine. As a result, the improved digestibility of dry matter and crude protein in the present study might be attributed to the activation of endogenous digestive enzymes in birds by the supplementation of *Artemisia absinthium*. Also the antimicrobial property of *Artemisia absinthium* resulted in controlling the gut microflora and the enzyme treatment further enhanced this effect resulting in increased digestibility of crude protein and dry matter.

Results of present study are in agreement with Hernandez *et al.* (2004) who reported an increase in digestibility coefficient of dry matter, ether extract, crude protein and organic material with the dietary supplementation of Labiatae plants (*Salvia officinalis*, *Rosmarinus officinalis* and *Dymus vulgaris*). In another study by Cross *et al.* (2007), herbs or their essential oils were shown to have a beneficial impact on apparent metabolisable energy and total tract apparent digestibility coefficients of dry and organic matter. Emami *et al.* (2012) also reported that feeding broilers a diet supplemented with 200 mg/kg peppermint essential oil significantly ($P<0.01$) increased the digestibility of crude protein. Murugesan *et al.* (2015) also found that supplementation of phytogenic feed additives significantly ($P<0.05$) increased the apparent total tract digestibility of crude protein and ether extract when compared to the control group. Hassan *et al.* (2015) reported an increase in digestibility coefficient of dry matter, ether extract, crude protein and organic material with the dietary supplementation of artichoke extracts in broilers.

However, Lee *et al.* (2003) in an experiment on female cobb broilers fed with corn-soybean meal diet supplemented with thyme, cinnamaldehyde or a commercial essential oil preparation reported no differences in ileal digestibilities of crude protein and starch as well as for the total tract fat digestibility between the non-supplemented control and the phytoadditive treatments at 21 and 40 days. Similarly, Jamroz *et al.* (2005) found no effect of phytogenic feed additive on apparent ileal digestibility of nutrients in broilers. Khaligh Gharetappe *et al.* (2015) found that the supplementation of *Artemisia dracuncululus* and *Mentha piperita* leaves in broilers had no significant effect on the digestibility of nutrients. Wang *et al.* (2021) also found that the addition of phytogenic feed additive in broilers did not cause significant differences in the digestibility of dry matter, crude protein, and ileal digestible energy among the treatments at 21 and 42 days of age.

5.7 Blood biochemistry

The average value of glucose was observed to be significantly ($p < 0.05$) reduced in the group of birds fed diets supplemented with 2% *Artemisia absinthium* with enzyme supplementation when compared to other treatment groups and the control group. The reduction in glucose levels by supplementing *Artemisia* spp. might be due to the increased insulin secretion from pancreatic beta cells (LalSamyal *et al.*, 2011) and increased uptake of glucose in tissues (Oblski *et al.*, 2011). *Artemisia* species are rich in flavonoids which contain β -flavonoid rings (Xu, 2010). Flavonoids decrease glucose absorption by establishing hydrogen bonds with the active sites of carbohydrate digestion and metabolism enzymes. Flavonoids have been shown to reduce the activities of α -glucosidase (Rosak and Mertes, 2009) and α -amylase (Tadera *et al.*, 2006), slowing glucose absorption. The results are consistent with the findings of Parsaei *et al.* (2014) who reported that incorporating olive leaf (*Artemisia herba-alba*) in the chick diet significantly ($P \leq 0.05$) reduced the blood glucose. According to Qamar *et al.* (2015), adding herbal medications to broiler drinking water significantly ($P \leq 0.05$) reduced blood glucose levels. Saleh *et al.* (2018) also found that the supplementation of broiler diets with phytogenics (herb mixture) significantly ($P < 0.05$) reduced the plasma glucose levels. Ait kaki *et al.* (2018) also found that broilers fed diet supplemented with 2% *Artemisia herba-alba* (olive leaf) powder had significantly ($P < 0.05$) lower blood glucose levels compared to control group. Supplementation of broiler diets with phytogenics (herb mixture) significantly ($P < 0.05$) reduced the plasma glucose in treatment groups compared to the control group (Saleh *et al.*, 2018)

However, Hosseinzadeh and Farhoomand (2014) found no significant difference in the levels of glucose of broilers fed different levels (0.125%, 0.25% and 0.5%) of *Artemisia dracunculus* powder. Belaid Gater *et al.* (2021) also found no effect on blood glucose levels of broilers fed *Artemisia Herba-Alba* and Fenugreek powder.

The lipid profile including serum cholesterol, triglycerides, high density lipoproteins (HDL) and low density lipoproteins (LDL) in various treatment groups varied significantly ($P < 0.05$). There was a significant ($P < 0.05$) reduction in the serum cholesterol levels in birds fed diets supplemented with 2% enzyme treated *Artemisia absinthium* when compared to the birds of other treatment groups and the control group. Significant ($P < 0.05$) difference was observed in the triglyceride levels in the birds fed diets supplemented with *Artemisia absinthium* with enzyme treatment when compared to the birds fed diets supplemented with *Artemisia absinthium* without enzyme treatment and the control group. Similar trend was observed in the serum high density lipoproteins (HDL) and low density lipoproteins (LDL) levels. According to Lutgen (2013), various *Artemisia* species can improve lipid metabolism and lower blood cholesterol levels. Lowering de novo cholesterol production has been shown to enhance the expression of LDL cholesterol receptors on hepatocytes, leading to increased LDL absorption by liver hepatocytes and, as a result, reduced blood LDL levels (Ghasemi *et al.*, 2014). The fibre content of *Artemisia* spp. may induce the binding of cholesterol with bile acids, and the suppression of micelle formation combined with the effect of fermentation on short-chain fatty acid synthesis may explain the drop in cholesterol levels (Baghban-Kanani *et al.*, 2018). Studies have also shown that the medicinal plants reduce the activity of HMG-CoA in hepatic reductase. For every 5% inhibition of the activity of HMG-CoA reductase, the key enzyme in cholesterol synthesis, cholesterol production is reduced by 2% resulting in lower cholesterol levels, LDL and VLDL concentrations in blood. (Ghazvinian *et al.*, 2018). *Artemisia* is rich in flavonoids and these flavonoids have been shown to affect fat metabolism by altering fatty acid profiles and the omega 3 to omega 6 ratio, lowering cholesterol and triglyceride levels (Kamboh and Zhu, 2013). Souri *et al.* (2015) reported that the bioactive compounds in medicinal plants lower plasma triglyceride concentration by decreased lipid intake, or high lipid catabolism or decrease in the activity of acetyl coenzyme A carboxylase resulting in decreased esterification reactions that ultimately decrease triacylglycerol

synthesis. Flavonoids have antioxidant properties and can alter lipid metabolism by preventing LDL production. (Habibian *et al.*, 2019). All these combined effects could be the cause of their positive effects on the serum biochemistry of broilers.

The results are consistent with the findings of Kim *et al.* (2012b) who found that inclusion of *Artemisia vulgaris* L. and *Pinus densiflora* powder in broiler diets significantly ($P<0.05$) decreased the levels of total serum cholesterol and LDL-cholesterol and increased the levels of HDL-cholesterol. Ait kaki *et al.* (2018) also found that broilers fed diet supplemented with 2% *Artemisia herba-alba* (olive leaf) powder had significantly lower blood cholesterol levels compared to control group. Parsaei *et al.* (2014) reported that supplementation of olive leaf in the chick diet significantly ($P<0.05$) reduced the blood cholesterol. Popovic *et al.* (2018) found that medicinal plants mixtures (*Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum*) statistically ($P<0.05$) increased the HDL-cholesterol and decreased LDL cholesterol concentration in treatment group compared to control group. Supplementation of broiler diets with phytogenics (herb mixture) significantly ($P<0.05$) reduced the plasma total cholesterol and triglyceride while increasing the plasma high-density lipoprotein compared to the control group (Saleh *et al.*, 2018). Baghban Kanani *et al.* (2019) also found that supplementation of *Artemisia annua* leaves significantly reduced the plasma LDL and cholesterol but did not affect the triglycerides and HDL levels in laying hens

However, Khalaji *et al.* (2011) found that supplementation of *Artemisia sieberi* leaves in the diet of broilers did not significantly alter plasma cholesterol, triglyceride, HDL, LDL, VLDL, LDL: HDL ratio. Hosseinzadeh and Farhoomand (2014) also found no significant difference in the levels of glucose, cholesterol, triglycerides, VLDL, LDL, and HDL between various treatments fed different levels (0.125%, 0.25% and 0.5%) of *Artemisia dracunculus* powder. Lee *et al.* (2019) found no significant differences in triglyceride, total cholesterol, HDL-cholesterol and VLDL+LDL-cholesterol among the different diet groups fed 0.5%

Lactobacillus plantarum-fermented *A. annua* dried leaves and non fermented *A. annua* dried leaves. Belaid Gater *et al.* (2021) also found no effect on lipid profile (blood cholesterol, LDL, HDL and triglycerides) levels of broilers fed *Artemisia Herba-Alba* and Fenugreek powder. Abdul Hafeez *et al.* (2020) found no significant difference on total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride levels of birds fed diet supplemented with phytonics (ajwan, black cumin and coriander powder).

The serum aspartate amino transferase (AST) and alanine amino transferase (ALT) levels were found to be within the normal range and no significant difference was observed among the various treatment groups and the control group. Khattak *et al.* (2013) also found no effect of feeding natural blend of essential oils from basil, caraway, laurel, lemon, oregano, sage, tea, and thyme on the ALT and AST levels of broilers. Baghban kanani *et al.* (2018) depicted no significant differences in ALT and AST concentrations among dietary treatments fed *Artemisia annua* leaves, probiotic, and organic acid compared to those of the control. Saleh *et al.* (2018) also found no significant difference in the plasma AST and ALT levels of birds fed diets supplemented with 0.5, 1 and 1.5% phytogetic mixture. The serum ALT and AST levels of birds in serum among broilers fed diets containing 0.00, 150, and 300 ppm levels of phytogetic feed additive were among the normal range and did not differ significantly (El-Ashram and Abdelhafez, 2020). Shahin *et al.* (2020) also found no significant differences in the serum AST and ALT levels of birds fed different levels (0.05, 0.1 and 0.20%) of phytogetic feed additives. Amber *et al.* (2021) found no difference in the ALT and AST levels of birds receiving 1 and 1.5% Aloe vera gel in drinking water. However, Reis *et al.* (2018) recorded reduced serum levels of ALT and AST in birds fed with either concentration (0.05 and 1%) of phytogetic feed additives on day 14 of age.

The serum creatinine levels also showed no significant difference between the treatment groups and the control group suggesting that *Artemisia absinthium*

exhibited non-toxic effect on the kidneys. Kalia *et al.* (2018) found no significant differences in the creatinine levels of birds fed diets supplemented with *H. rhamnoides* aqueous extract at level of 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken. Shahin *et al.* (2020) found no significant differences in the serum creatinine levels of birds fed different levels (0.05, 0.1 and 0.20%) of phytogetic feed additives. Similarly, the serum creatinine levels of broilers that received drinking water with or without Aloe vera gel showed no significant differences with the control group ($P \geq 0.05$) (Amber *et al.*, 2021).

5.8 Slaughter traits and carcass characteristics

There was a significant ($p < 0.05$) increase in the dressing percentage of birds fed diets supplemented with enzyme treated *Artemisia absinthium*. Significantly ($p < 0.05$) higher dressing percentage was observed in treatment groups T₅, T₆ and T₇ when compared with the other treatment groups and the control group. Results are in agreement with Issa and Abo Omar (2012), Abo Omar *et al.* (2016) and Mudalal *et al.* (2020) who reported increased ($P < 0.05$) dressing percentages in birds supplemented with herb extracts compared to control birds. Al Kassie *et al.* (2014) reported that the inclusion of mixture of hot red pepper and black pepper at a level of 0.75 and 1% in the diets significantly ($p < 0.05$) improved the dressing percentage of broilers. The improvement in these groups might have been due to the better growth rate and efficient utilization of nutrients as the groups fed diet supplemented with enzyme treated *Artemisia absinthium* had better final body weight compared to other groups. This is possibly due to the high crude protein content of *Artemisia absinthium* (23.5% in the present study) presence of minerals, Vitamin A, C, E, antioxidants and flavonoids such as quercitin and coumarins (Brisbie *et al.*, 2008; Bilia *et al.*, 2006) which are very important for growth and development of poultry as well as increasing the weight of animals (Brisibe *et al.*, 2008).

No significant difference between the different treatment groups and the control group with respect to blood loss, feather loss, visual carcass score was

observed. The data on percent cutability characteristics of birds fed diets supplemented with different levels of *Artemisia absinthium* with or without enzyme treatment also revealed no significant effect on various parameters like breast, drumsticks, thighs, wings, back and neck weights among various treatment groups and the control group.

The results are in agreement with Khalaji *et al.* (2011) who found that dietary supplementation of *Artemisia sieberi* leaves in broilers did not affect the relative weight and length of different parts of the carcass. Hosseinzadeh and Farhoomand (2014) also found no statistically significant effect of feeding different levels (0.125%, 0.25% and 0.5%) of *Artemisia dracunculus* powder on the relative weight of heart, liver, spleen, bursa, cook carcasses and abdominal fat of broilers. Habibi *et al.* (2016) found that the different levels of cumin and wormwood essential oil in diets of broilers had no significant effect on carcass yield and relative weights of liver, intestine, heart, gizzard, thigh, breast and abdominal fat at 42 days of age. Saracila *et al.* (2018) also observed that supplementation with *Artemisa annua* oil and powder did not significantly affected the carcass and organ weights of heat stressed broilers. Cetin *et al.* (2019) found no significant difference in the carcass characteristics, relative weights of heart, gizzard and abdominal fat of broilers fed diets supplemented with dried *Artemisia absinthium*.

5.9 Caecal microbial population

Statistical analysis showed significant ($P \leq 0.05$) reduction in caecal viable count and caecal coliform count in the groups fed 1.5 and 2% raw and enzyme treated *Artemisia absinthium* as compared to the groups fed 1% raw and enzyme treated *Artemisia absinthium* and the control group. Among all the treatment groups, Significantly ($P \leq 0.05$) lower caecal viable count and caecal coliform counts were observed in treatment groups T₆ and T₇ i.e. the groups fed 1.5% and 2% enzyme treated *Artemisia absinthium*. Lopes-Lutz *et al.* (2008) reported that oils of different species of *Artemisia* had varying degrees of growth inhibition

against microorganisms such as *Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. Fiamegos *et al.* (2011) reported that the essential oils occurring in flowers and aerial parts from *A. absinthium* have antimicrobial properties. This beneficial action could be due to the higher concentration of terpenoids, including monoterpenes in the *Artemisia* essential oil. These compounds are well-known to cause membrane disruption in microorganisms by the action of lipophilic compounds in the oils, thereby impairing the antibacterial activity (Sahoo *et al.*, 2012). Coumarins, flavonoids, and other terpenoids constituents present in *Artemisia absinthium* are reported to have significant pharmacological activities such as antitumor and antibacterial activities (Erel *et al.*, 2012; Singh *et al.*, 2012).

The results are in agreement with the findings of Khalaji *et al.* (2011) who observed that the supplementation of broiler diets with *Artemisia sieberi* leaves significantly ($P < 0.05$) reduced coliform and *E. coli* populations in cecum of birds. Kim *et al.* (2012b) reported that dietary supplementation of *Lactobacillus*-fermented *Artemisia princeps* (LFA) significantly increased the concentrations of *Lactobacillus* spp. and reduced the concentrations of *Salmonella* spp. in the ileal content of birds. Kim *et al.* (2015) studied the antimicrobial effects of Artemisinin extracts from *Artemisia annua* L. extracts and found that water, methanol, ethanol and acetone extracts of *Artemisia annua* L. had antimicrobial activity against the periodontopathic microorganisms like *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* subsp. *animalis*, *Fusobacterium nucleatum* subsp. *polymorphum*, and *Prevotella intermedia*. Supplementation of *Artemisia annua* powder in heat stressed broilers significantly ($P < 0.05$) reduced the number of intestinal *Enterobacteriaceae* and increased the count of lactobacilli (Panaite *et al.*, 2018, Saracila *et al.*, 2018).

However, Ahsan *et al.* (2018) found no difference in the total aerobic bacteria, *Escherichia coli*, coliforms and *Lactobacilli* count among the different treatments groups fed different levels of dietary phyto-genic feed additive. Also it

was observed that the dietary supplementation with phytogenic blend (PB) of *Aerva lanata*, *Piper betle*, *Cynodon dactylon*, and *Piper nigrum* did not significantly affect the total aerobic count, *Clostridium* count, *Lactobacillus* count, *Bifidobacterium* count and Coliform count in broilers (Oso *et al.*, 2019). Saeed El-Ashram and Gamal A. Abdelhafez (2020) found that supplementation of broiler diets with essential oils of thyme and star anise did not affect the intestinal bacterial count and *Lactobacillus* population but significantly increased the *Enterococcus* counts in treatment groups compared to the control group.

5.10 Histomorphology

5.10.1 Liver

The histomorphology of liver in birds among all the treatment groups had normal structure suggesting that *Artemisia absinthium* was not hepatotoxic and did not alter the structure of liver of treatment groups. The results are in harmony with Gilani and Janbaz (1995), Amat *et al.* (2010) who reported hepatoprotective effect of *Artemisia absinthium*. Mohammadian *et al.* (2015) also reported the hepatoprotective effect of hydroalcoholic extract of *Artemisia absinthium* L. in rats and preserving the structural integrity of the hepatocellular membrane. Tunc *et al.* (2019) reported that supplementation of *Artemisia dracuncululus* in broilers did not change the structure of the liver. Park *et al.* (2020) also reported the hepatoprotective effect of *Artemisia annua* on liver injury in mice after lipopolysaccharide/D-galactosamine treatment.

5.10.2 Small intestine

The histomorphology of the intestine of birds of all the treatment groups showed normal histomorphology. There was no variation in histomorphology of intestine of birds fed diets supplemented with *Artemisia absinthium* with or without enzyme supplementation from normal structure. In the present study, no significant effect was observed on the villus height and crypt depth of the birds of various treatment groups. Similar findings were reported by Peric *et al.* (2010)

who found that the phytogenic additive had no effect ($P>0.05$) on gut morphology (villus height or villus surface area) of broilers. Mounia *et al.* (2018) found that supplementation of phytogenic products did not affect the gut morpho-histology (villus height of duodenum, jejunum and ileum) of broilers but only the depths of the duodenal crypt were found to be significantly ($P<0.05$) deeper in birds supplemented with phytogenic products and shallower for broilers in the control group. Many studies suggest that phytogenic compounds have little effect on gut morphology and histology, making it impossible to correlate better intestinal morphology to improved animal performance (Mounia *et al.*, 2018). Tunc *et al.* (2019) also reported that supplementation of *Artemisia dracunculus* in broilers did not alter the morphology of intestinal tissues, but the longest jejunum villus was observed in groups fed 0.2% *Artemisia dracunculus*.

However, herbal extracts (carvacrol, cinnamaldehyde and capsaicin) supplementation in broilers significantly ($P<0.005$) improved the intestinal villus height but did not affect the crypt depth and villus/crypt ratio (Petrolli *et al.*, 2012). In broilers, Khalaji *et al.* (2011) and Amad *et al.* (2013) found that a blend of essential oils, a blend of thymol, anethole, and cinnamaldehyde, as well as a blend of phytochemicals produced the best results in intestinal micrometry, such as improved villus/crypt ratios. Supplementation of phytogenic feed components in broilers significantly ($P<0.05$) elevated the villus height within the duodenum, jejunum and ileum, decreased the crypt depth in jejunum and diminished the mucosal thickness within the duodenum and ileum in comparison to the control group (Murugesan *et al.*, 2015). Song *et al.* (2018) found that enzymatically treated *Artemisia annua* (EA) supplementation in broilers challenged with heat stress significantly increased ($P<0.05$) the villus height and ratio of villus height to crypt depth and decreased the crypt depth. Oso *et al.* (2019) observed that the broilers fed diets supplemented with 1 and 2 % of phytogenic mixture of *Piper nigrum*, *Piper betle*, *Cynodon dactylon* and *Aerva*

lanata had longer villi height in duodenum and jejunum, reduced crypt depth and higher villus/crypt ratio as compared to the control.

5.11 Immune competence

Significant ($P < 0.05$) differences were observed between the treatment groups and the control group with respect to the immune response. The immune response of birds was measured in terms of anti SRBC antibody titre, mean skin thickness and the relative weight of immune organs. Significantly ($P < 0.05$) higher anti SRBC antibody titre and skin thickness was recorded in the birds fed 1.5 and 2% enzyme treated *Artemisia absinthium*. The relative weight of immune organs i.e., spleen bursa and thymus and had no significant difference amongst the treatment groups.

Increase in the immune response by use of natural products has been reported by many research workers (Kong *et al.*, 2004; Taheri *et al.*, 2005). Several herbs and essential oils have been reported to have immunomodulatory effects such as immunoglobulin secretion, cytokine modulation, expression of lymphocyte, phagocytosis and histamine release (Mahima *et al.*, 2012). Rubio *et al.* (2013) also proposed that phytochemical feed additives may work by increasing the expression of antioxidant enzymes, which may help to reduce inflammation. The flavonoids present in *Artemisia* increase the action of vitamin C, serve as antioxidants, and may thereby improve immunological function. (Manach *et al.*, 1996). *Artemisia* spp. have been found to have antioxidant and anti-inflammatory effects, which decrease prostaglandin formation, resulting in improved humoral response. (Bhakuni *et al.*, 2001; Brisibe *et al.*, 2009).

The results of present study are in agreement with Li *et al.* (2012) who found that Artemisinin possessed immunomodulatory function. Gholamrezaie Sani *et al.* (2013) found that *A. annua* extract and leaf powder increased the serum IgG contents and weight of thymus and bursa of fabricius in broilers. *A. annua* has been reported to exert anti-inflammatory action by reducing the pro-inflammatory

cytokines (Ryu *et al.*, 2013). Studies on the immunomodulatory potential of herbs like *W. somnifera*, *T. cordifolia*, and *A. indica* against chicken infectious anaemia revealed that these herbs have a high capacity for stimulating both cellular and humoral immune responses in chicks against the causative virus, as well as ameliorating viral pathogenesis (Latheef *et al.*, 2013). Addition of 200 ppm *Artemisia absinthium* essential oil to broiler diets significantly ($P<0.05$) increased haemagglutination titre against sheep red blood cells (SRBC) and weight of bursa of fabricius in broilers after day 7 and 14 of injection. (Habibi *et al.*, 2016). Song *et al.*, 2018 reported that supplementation of broiler diets with enzymatically treated *Artemisia annua* (EA) significantly ($P<0.05$) increased the concentrations of Intestinal secretory immunoglobulin A (SIgA) and immunoglobulin G (IgG) concentrations in heat stressed broilers compared with the control. Saleh *et al.* (2018) found that phytogenic supplementation significantly ($P<0.05$) improved the plasma levels of total protein and antibodies titers for Newcastle disease virus in broilers compared with the control treatment. Aroche *et al.* (2018) found that 0.5% of mixed powder of medicinal plant leaves significantly ($P<0.05$) increased the IgG concentration in broilers. Wang *et al.* (2019) found that *A. vulgaris* meal supplementation on the immune function in rabbits and found significantly ($P<0.05$) increased blood levels of IgA and IgM, lymphocytes of bursale, thymus, CD4 and CD8 hence improving the immunity in rabbits.

However, Malayoglu *et al.* (2010) found that dietary supplementation of enzyme and oregano essential oil, alone and in combination had no significant effect on immune response of broilers. Khalaji *et al.* (2011) also found that supplementation of *Artemisia sieberi* leaves in broilers did not affect primary or secondary antibody response against the sheep red blood cells.

5.12 Oxidative stability

The anti-oxidative activities of *Artemisia absinthium* on broilers was assessed by determining the total oxidative status (TOS) and total anti-oxidative status (TAS) of birds. Significant ($P<0.05$) improvement in the oxidative status of

birds fed diets supplemented with raw and enzyme treated *Artemisia absinthium* when compared to control group was observed. Birds fed 1.5% and 2% enzyme treated *Artemisia absinthium* leaf powder had significantly ($P<0.05$) reduced levels of TOS and increased levels of TAS compared to other treatment groups. The oxidative stability of broiler meat was measured by determining the value of TBARS. Statistically significant ($P<0.05$) difference was observed between the various treatment groups and the control group. Significantly ($P<0.05$) lower values of TBARS were observed in birds fed diets supplemented with raw and enzyme treated *Artemisia absinthium* when compared to control. Significantly ($P<0.05$) lower values of TBARS was observed in groups fed 1.5% and 2% enzyme treated *Artemisia absinthium* leaf powder compared to other treatment groups.

The antioxidant effect of medicinal plants is mainly attributed to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes. These compounds can be used as electron or hydrogen suppliers to reduce free radical formation and scavenging free radicals which is responsible for their antioxidant capacity (Pietta, 2000). Phenolic compounds prevent radical formation, scavenge free-radicals, chelate metal ions and improve the antioxidant endogenous system and therefore act as powerful antioxidants (Qingming *et al.*, 2010). The antioxidant activity of *Artemisia spp.* could be attributed to its flavonoidal content. They are rich sources of sesquiterpene lactones, antioxidant phenolics, flavonoids and other biologically active compounds. Free-radical scavenging action is likely mediated by flavonoids such as rutin, quercetin, and other flavonoid glycosides, as well as phenolic acids such as coumaric, syringic, salicylic, chlorogenic, and vanillic acids (Rice Evans *et al.*, 1996). These flavonoids act as scavengers of numerous oxidizing species and additionally they act as quenchers of singlet oxygen (Das and Ratty, 1986). Various flavonoids and phenolic chemicals found in *A. absinthium* may be implicated in the mechanism of free radical scavenging action (Javed *et al.*, 2019).

Results of present study are in agreement with the findings of Jasna *et al.* (2004) who reported the free radical scavenging activity of *Artemisia absinthium* Linn. extract by measuring their ability to scavenge stable 2,2-diphenyl-1-picrylhydrazyl free radical and reactive hydroxyl radical. Cherian *et al.* (2013) found that birds fed diets supplemented with *Artemisia annua* leaf powder had significantly ($P<0.05$) lower malondialdehyde content in the breast and thigh muscles compared with the control birds. Kostadinovic *et al.* (2015) reported that supplementation of broiler diets with 200g/kg *A. absinthium* significantly ($P<0.05$) higher oxidative stability of broiler meat and reduced activity of malondialdehyde in blood, plasma and liver and increased the blood levels of glutathione peroxidase as compared to control group. Chu *et al.* (2015) reported that supplementation of broiler diets with 1,000 mg/kg *Artemisia argyi* aqueous extract (AAE) significantly ($P<0.05$) broilers improved the serum superoxide dismutase (SOD) activity and decreased the serum malondialdehyde (MDA) content. Wan *et al.* (2016a) found that the diets supplemented with various levels of enzymatically treated *Artemisia annua* L. significantly ($P<0.05$) decreased the malondialdehyde concentrations and increased free radical scavenging activities of breast and thigh muscles in broilers. Popovic *et al.* (2017) reported that feeding 100g/kg dried *Artemisia absinthium* plant to rabbits significantly ($P<0.05$) reduced the levels of malondialdehyde in liver homogenate while increasing the levels of glutathione peroxidase, superoxide-dismutase and glutathione reductase. Song *et al.* (2018) reported that the broiler diets supplemented with enzymatically treated *Artemisia annua* (EA) significantly ($P<0.05$) increased the jejunal total superoxide dismutase activity and glutathione peroxidase content and decreased the jejunal malondialdehyde concentration in the heat stressed broilers compared with the control group.. Wan *et al.* (2017) found that enzymatically treated *Artemisia annua* significantly ($P<0.05$) reduced the reactive oxygen metabolites and thiobarbituric acid reactive substances, increased the antioxidant enzyme activities in heat stressed broilers. Baghban Kanani *et al.* (2019) found significantly ($P<0.05$) higher glutathione peroxidase level and lower and

malondialdehyde levels in hens fed 7.5% *A. annua* leaves compared to other treatments (i.e. 2.5% and 5% *A. annua* leaves) and control. *Artemisia dracunculus* (Tarragon) supplementation significantly ($P<0.05$) reduced the malondialdehyde levels of the serum, liver and yolk, decreased the plasma corticosterone (CORT) and serum total oxidant (TOS) levels induced by high stocking density in laying hens (Kaya *et al.*, 2020).

5.13 Sensory and organoleptic evaluation

There was no significant difference among the various sensory attributes i.e. appearance, flavor, juiciness, texture, mouth coating and the overall acceptability which ranged from 6.86 to 7.12 on an 8-point hedonic scale for descriptive attributes of product, as described by Keeton (1983). Similar results were also recorded earlier by Puvaca *et al.* (2015) who found that the inclusion of spices such as garlic, black pepper, and strong red pepper in the diet had no negative impact on meat quality, with good sensory scores for scent, juiciness, and overall impression. However, there were favourable impacts on various sensory qualities in chicken when phytochemical feed additives were used. Supplementing poultry diets with polyherbal products improved overall meat quality features such as fillet and tender yield, sensory characteristics, organoleptic cooking parameters, overall palatability, and customer acceptability of meat (Waskar *et al.*, 2011). However, Dzinic *et al.* (2013) tested the sensory quality of breast chicken meat after adding 2% garlic powder to the feed and found that the garlic flavour and odour were transmitted to the meat. The sensory parameter values in poultry supplemented with phytochemicals were equivalent to those fed a negative control diet (Goliomytis *et al.*, 2014).

5.14 Proximate composition

In this study no significant effect of the supplementation of diets with raw and enzyme treated *Artemisia absinthium* on the proximate composition of broiler meat was observed. Similar observations were also recorded by Gardzielewska *et*

al. (2003) who reported that the addition of echinacea (*Echinacea purpurea*), garlic (*Allium sativum*), and ginger (*Zingiber officinale*) to broiler chicken feed had no effect on meat moisture content. Contrary to this findings, Onibi *et al.* (2009) found that spice herbs in broiler diet had a significant ($P<0.05$) impact on fat content, with the thigh muscle having the highest fat concentration (82.9 g/kg), followed by the drumstick muscle (66.9 g/kg), and the breast muscle having the lowest (49.1 g/kg). Similarly, Marcincak *et al.* (2011) also found alterations in the composition of the thigh muscle of broilers fed diets containing clove powder, *Agrimonia eupatoria*, and *Melissa officinalis* extracts (a greater proportion of crude protein and a lower proportion of fat). Puvaca *et al.* (2014) found that the addition of garlic powder to chicken diet at a concentration of 2.0 g/100 g resulted in improved protein content (22.9 g/100 g) in breast meat compared with unsupplemented diet (21.8 g/100 g). Kim *et al.* (2012a) found that inclusion of *Artemisia princeps* (1% or 2%) and *Pinus densiflora* powder (1% or 2%) in the diets significantly ($P<0.05$) decreased crude fat contents of chicken thigh meat compared with the control groups. Kostadinovic *et al.* (2015) also found that birds fed with 200 g/kg of *A. absinthium* had higher protein content and significantly ($P<0.05$) lower fat content as compared to the control.

5.15 Economics

The feed cost/kg live weight gain worked out to be highest (Rs.70.80) for the birds of T₁ group (control) followed by Rs 68.44, Rs. 67.64, Rs. 66.01, Rs. 67.27, Rs. 64.81 and Rs.59.94 for T₂, T₃, T₄, T₅, T₆ and T₇ respectively. The difference in feed cost compared to the control group worked out to be highest for T₆ and T₇ groups. This improvement in economics could be attributed to better FCR and weight gain in birds fed diets supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium*. These results are in agreement with Tanzim *et al.* (2017) who found that adding phyto-genic feed additive (PFA) to broiler diets improved feed utilisation, increased weight growth, and improved feed conversion ratio, resulting in higher return and improved benefit/cost ratio. Herbal powder

supplementation in broiler diets resulted in the lowest feed cost/kg body weight growth (Puvaca *et al.*, 2016). It has been observed that the broiler diets including commercial herbal preparations were more cost-effective. This improvement might be attributable to a higher FCR or a reduction in the amount of feed required to produce one unit of meat (Omar *et al.*, 2019; Singh *et al.*, 2018). Abo Omar *et al.* (2016) also found better economic efficiency in birds fed with a commercial herbal preparation. When compared to other groups, the birds fed diet containing 0.20 percent phytogetic feed additive had significantly ($P<0.05$) highest total return, gross margin, benefit-cost ratio, and economic efficiency percent (Shahin *et al.*, 2020).

Chapter 6

SUMMARY AND CONCLUSION

An experiment was conducted to evaluate the effect of supplementing *Artemisia absinthium* in broiler diets alone or in combination with enzyme on the performance of broiler chicken reared for five weeks involving two hundred and fifty two day old commercial broiler chicks were purchased from a reputed hatchery and reared for the first week under standard brooding conditions. On the eighth day, the chicks were randomly divided into seven treatment groups, each with three replicates of twelve birds. The treatment groups were as follows:

- Treatment-1: Commercial diet without any additive (Control group)
- Treatment-2: Commercial diet supplemented with 1% *Artemisia absinthium* leaf powder
- Treatment-3: Commercial diet supplemented with 1.5% *Artemisia absinthium* leaf powder
- Treatment-4: Commercial diet supplemented with 2% *Artemisia absinthium* leaf powder
- Treatment-5: Commercial diet supplemented with 1% *Artemisia absinthium* leaf powder and enzyme
- Treatment-6: Commercial diet supplemented with 1.5% *Artemisia absinthium* leaf powder and enzyme
- Treatment-7: Commercial diet supplemented with 2% *Artemisia absinthium* leaf powder and enzyme

The cocktail enzyme was used at the rate of 25g/100 kg of feed.

The birds were raised in cages upto five weeks of age under standard managerial conditions with *ad lib* feeding and watering. All birds were vaccinated and assessed twice daily for mortality, if any. The body weight, feed

consumption and FCR were recorded at weekly interval. At the end of experiment blood biochemistry, carcass characteristics, immune competence, histopathology of liver and small intestine, cecal microbial population, antioxidant status, sensory evaluation and proximate composition of meat and economics were calculated. The percent digestibility of nutrients at six weeks of age was estimated involving twenty one birds.

The results revealed a significant ($P \leq 0.05$) improvement in live body weight and body weight gain in the treatment groups fed diets supplemented with raw or enzyme treated *Artemisia absinthium* leaves. However, the group of birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves had significantly ($P \leq 0.05$) higher body weight when compared with the group of birds fed diets supplemented with different levels of raw *Artemisia absinthium* leaves during the growth period from 1 to 5 weeks of age.

The cumulative feed consumption was significantly ($P \leq 0.05$) higher in group of birds fed different levels of enzyme treated *Artemisia absinthium* when compared to the birds fed diets supplemented with raw *Artemisia absinthium* leaves and the control group. Among the birds fed diets supplemented with enzyme treated *Artemisia absinthium* leaves highest feed consumption was recorded in birds fed diets supplemented with 2% *Artemisia absinthium* leaves i.e. T₇ group during the entire growth period from 1 to 5 weeks of age. Similarly, the overall feed conversion ratio (FCR) of birds fed diets supplemented with 1.5 % and 2% enzyme treated *Artemisia absinthium* leaves was significantly ($P \leq 0.05$) improved when compared to the birds fed diets supplemented with 1% enzyme treated *Artemisia absinthium* leaves or birds fed different levels of raw *Artemisia absinthium* leaves and the control diet.

Significantly ($P \leq 0.05$) higher digestibility of dry matter and crude protein (CP) was observed in treatment groups fed diets supplemented with 2% enzyme treated *Artemisia absinthium* i.e. T₇ group as compared to other treatment groups and the control group.

The blood biochemicals of birds revealed significant ($p < 0.05$) differences among the various treatment groups. The mean value of glucose (mg/dl) was found to be significantly ($p < 0.05$) reduced in the group of birds fed diets supplemented with 2% *Artemisia absinthium* with enzyme supplementation when compared to other treatment groups and the control group. The serum AST levels (u/l) and serum ALT levels (u/l) were found to be within the normal range and did not differ significantly between the treatment groups. The serum creatinine levels (mg/dl) were found to be within the normal range and did not differ significantly between the treatment groups. The lipid profile including total cholesterol (mg/dl) serum triglycerides (mg/dl), high density lipoproteins (HDL) (mg/dl), and low density lipoproteins (LDL) (mg/dl) in various treatment groups varied significantly ($p < 0.05$). There was a significant ($p < 0.05$) reduction in the serum cholesterol levels in birds fed diets supplemented with 2% *Artemisia absinthium* with enzyme supplementation when compared to other treatment groups and the control group. Significantly ($p < 0.05$) highest levels of triglyceride were found in the control group and lowest in the group fed diets supplemented with 2% *Artemisia absinthium* with enzyme treatment. Similar trend was observed in the serum high density lipoproteins (HDL) and low density lipoproteins (LDL) levels.

There was no significant effect in general on the slaughter traits and carcass characteristics of birds fed supplemented with *Artemisia absinthium* with or without enzyme supplementation. Although the the dressing percentage of birds fed diets supplemented with enzyme treated *Artemisia absinthium* was significantly ($p < 0.05$) improved. There was no variation in histomorphology of liver and intestine of birds fed diets supplemented with *Artemisia absinthium* with or without enzyme supplementation from normal structure.

There was significant ($P \leq 0.05$) reduction in cecal viable count and caecal coliform count in the groups fed 1.5 and 2% raw and enzyme treated *Artemisia absinthium* when compared to the group of birds fed 1% raw and enzyme treated *Artemisia absinthium* and the control group. Among all the treatment groups,

Significantly ($P \leq 0.05$) lower cecal viable count and cecal coliform counts was observed in treatment groups T₆ and T₇ i.e. the groups fed 1.5% and 2% enzyme treated *Artemisia absinthium*.

There was a significantly ($p < 0.05$) higher total antioxidant status and lower total oxidant status and TBARS value in the treatment group T₆ and T₇ i.e. the groups fed diets supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium* when compared to other groups. Similarly the immune response measured as anti SRBC antibody titre, mean skin thickness and weight of immune organs was significantly ($p < 0.05$) higher in the treatment group T₆ and T₇ i.e. the groups fed diets supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium* when compared to other groups. However, no significant difference was observed in the weight of immune organs between various treatment groups.

No significant difference was observed in the sensory evaluation and proximate composition of meat among various treatment groups.

There was reduction in the feed cost/kg live weight gain among different treatment groups. Feed cost/kg live weight gain worked out to be highest (Rs.70.80) for the birds of T₁ group (control) followed by Rs 68.44, Rs. 67.64, Rs. 66.01, Rs. 67.27, Rs. 64.81 and Rs.59.94 for T₂, T₃, T₄, T₅ T₆ and T₇ respectively. The difference in feed cost compared to the control group worked out to be highest for T₆ and T₇ groups.

CONCLUSION

- Supplementation of broiler diets with *Artemisia absinthium* with or without enzyme resulted in improvement in body weight, body weight gain and FCR..
- Supplementation of broiler diets with 1.5% and 2% enzyme treated *Artemisia absinthium* resulted in significantly ($P \leq 0.05$) higher digestibility of dry matter and crude protein.

- Supplementation of broiler diets with 1.5% and 2% enzyme treated *Artemisia absinthium* resulted in significantly ($P \leq 0.05$) lower serum glucose, cholesterol and LDL levels and significantly ($P \leq 0.05$) higher serum HDL levels.
- Significantly ($P \leq 0.05$) lower cecal viable count and cecal coliform counts were observed in treatment groups fed diets supplemented with 1.5% and 2% enzyme treated *Artemisia absinthium*
- Supplementation of broiler diets with *Artemisia absinthium* with or without enzyme did not have negative effects on the histomorphology of liver and intestines.
- Supplementation of broiler diets with 1.5% and 2% enzyme treated *Artemisia absinthium* resulted in significantly ($P \leq 0.05$) higher immune response by increasing the anti SRBC antibody titre and mean skin thickness with DNCB injection.
- Supplementation of broiler diets with 1.5% and 2% enzyme treated *Artemisia absinthium* resulted in significantly ($p < 0.05$) higher serum total antioxidant status, lower total oxidant status and lower TBARS values of broiler meat
- There was a reduction in feed cost/kg live weight gain of birds fed diets supplemented with enzyme treated *Artemisia absinthium* compared to birds fed raw *Artemisia absinthium* and the control.

LITERATURE CITED

- Abdel-Latif, M. A., El-Far, A. H., Elbestawy, A. R., Ghanem, R., Mousa, S. A and Abd El-Hamid, H. S. 2017. Exogenous dietary lysozyme improves the growth performance and gut microbiota in broiler chickens targeting the antioxidant and non-specific immunity mRNA. *PLoS One*. **12**:10.
- Abdul-Hafeez, Ali Shah, S. A., Khan, R.U., Quadrat Ullah. and Naz, S. 2020. Effect of diet supplemented with phytogenics and protease enzyme on performance, serum biochemistry and muscle histomorphology in broilers. *Journal of Applied Animal Research* **48**(1):326-330
- Abo Omar, J., Hejazi, A. and. Badran, R. 2016. Performance of Broilers Supplemented with Natural Herb Extract. *Open Journal of Animal Sciences* **6**: 68-74.
- Aftab, U. and Bedford, M. R. 2018. The use of NSP enzymes in poultry nutrition: myths and realities *World's Poultry Science Journal* **74**(2):1-10.
- Ahsan U, Kuter E Raza I, Köksal, B. H., Cengiz, O., Yildiz, M., Kizanlik, P. K., Kaya, M., Tatli,O. and Sevim, O. 2018. Dietary Supplementation of Different Levels of Phytogenic Feed Additive in Broiler Diets: The Dynamics of Growth Performance, Caecal Microbiota, and Intestinal Morphometry. *Brazilian Journal of Poultry Science* **20**(4):737-746.
- Ahsan, U., Raza, I., Koksall, B. H., Cengiz, O., Yildiz, M., Kizanlik, P. K., Kaya, M., Tatli, O. and Sevim, O. 2018. Dietary Supplementation of Different Levels of Phytogenic Feed Additive in Broiler Diets: The Dynamics of Growth Performance, Caecal Microbiota, and Intestinal Morphometry. *Brazilian Journal of Poultry Science* **20** (4): 737-746.

- Ait Kaki, A., Diaw, M. T., Geda, F. and Moula, N. 2018. Effects of *Artemisia herba-alba* or olive leaf (*Olea europaea*) powder supplementation on growth performance, carcass yield, and blood biochemical parameters in broilers. *Veterinary World* **11**(11):1624-1629.
- Ait-Kaki, A., Diaw, M. T., Geda, F. and Moula, N. 2018. Effects of *Artemisia herba-alba* or olive leaf (*Olea europaea*) powder supplementation on growth performance, carcass yield, and blood biochemical parameters in broilers. *Veterinary World* EISSN: 2231-0916.
- Al-Kassie Galib, A. M., Al-Nasrawi, M. A. M. and Saba, J. A. 2011. Use of black pepper (*Piper nigrum*) as feed additive in broilers diet. *Research Opinions in Animal and Veterinary Sciences* **1**(3):169-173.
- Amad, A. A., Wendler, K. R. and Zentek, J. 2013. Effects of a phytogenic feed additive on growth performance, selected blood criteria and jejunal morphology in broiler chickens. *Emirates Journal of Food and Agriculture* **25**(7):549-554.
- Amat, N., Upur, H. and Blazekovic, B. 2010. In vivo hepatoprotective activity of the aqueous extract of *Artemisia absinthium* L. against chemically and immunologically induced liver injuries in mice. *Journal of Ethnopharmacology* **131**(2):478-84.
- Amber, K., Nofel, R., Ghanem, R., Sayed, S., Farag, S. A., Shukry, M. and Dawood, M. A. O. 2021. Enhancing the Growth Rate, Biochemical Blood Indices, and Antioxidative Capacity of Broilers by Including Aloe vera Gel in Drinking Water. *Frontiers in Veterinary Science* **7**:632666.
- American Public Health Association (APHA). 1992. Compendium of methods for the microbiological examination of foods. Washington, D. C.

- AOAC. 2000. Official methods of analysis. 16th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA, p. 1094.
- Aroche, R., Martinez, Y., Ruan, Z., Guan, G., Waititu, S., Nyachoti, C.M., Mas, D. and Lan, S. 2018. Dietary Inclusion of a Mixed Powder of Medicinal Plant Leaves Enhances the Feed Efficiency and Immune Function in Broiler Chickens. *Journal of Chemistry* ID 4073068.
- Asad, M. H. H. B., Murtaza, G., Siraj, S., Khan, S. A., Azhar, S., Hussain, M. S., Ismail, T., Hussain, M. S. and Hussain, I. 2011. Enlisting the scientifically unnoticed medicinal plants of Pakistan as a source of novel therapeutic agents showing anti-venom activity. *African Journal of Pharmacology* 5(20): 2292- 2305.
- Ashok, P. K. and Upadhyaya, K. 2013. Evaluation of Analgesic and Anti-inflammatory Activities of Aerial Parts of *Artemisia vulgaris* L. in Experimental Animal Models. *Journal of Biologically Active Products from Nature* 3(1):101-105.
- Attia, Y.A., Abd EL-Razak, E., Tag El-Din., Zeweil,H.S., Hussein,A.S., El-Shahat, M. Q. and Mohamed Arafat, A. 2008. The effect of supplementation of enzyme on laying and reproductive performance in Japanese quail hens fed nigella seed meal. *Journal of Poultry Science* 45:110–115.
- Baghban-Kanani, P., Hosseintabar-Ghasemaba, B., Azimi-Youvalari, S., Seidavi, A., Ragni, M., Laudadio,V. and Tufarelli,V.2019. Effects of Using *Artemisia annua* Leaves, Probiotic Blend, and Organic Acids on Performance, Egg Quality, Blood Biochemistry, and Antioxidant Status of Laying Hens. *Journal of Poultry Science* 56(2):120-127.

- Basmaciog, L. M. L., Baysal, H., S. Misirliog Lu, Z., Polat, M., Yilmaz, H. and Turan, N. 2010. Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat—soybean meal diets. *British Poultry Science* **51**:67-80.
- Beigh, Y. A., Ganai, A. M., Ahmad, H. A., Masood, D., Ishfaq, A. and Farukh, M. 2018. Effect of supplementation of wormwood (*Artemisia absinthium* L.) herb and exogenous fibrolytic enzymes on growth of lambs. *Indian Journal of Small Ruminants* **24**(2):259-263.
- Beigh, Y. A., Ganai, A. M., Sheikh, G. G., Ahmad, H. A., Bilal, S., Amin, U. and Mir, M. S. 2017. Effect of feeding complete diet supplemented with feed additives alone and in combination on nutritional and hepatorenal function test profile in crossbred lambs. *Haryana Veterinary* **56**(1): 58-62.
- Belaid-Gater, N., Gaoua, S. and Kadi, S. A. 2021. Effect of sagebrush (*Artemisia Herba Alba* Asso) and fenugreek (*Trigonella Foenum-Graecum* L.) on growth performances, some biochemical traits and carcass qualities for broiler chickens. *Veterinaria* **70** (2):245-57.
- Bhat, A. R., Ishfaq, A., Ganai, A. M. Beigh, Y. A., Sheikh, G. G. and Masood, D. 2017. Effect of *Artemisia absinthium* (Titween) on nutrient intake, digestibility, nutrient balance and blood biochemical of sheep. *Indian Journal of Animal Research* ISSN:0367-6722.
- Bilia, A. R., Lazari, D., Messori, L. and Taglioli, V. 2006. Simultaneous analysis of Artemisinin and Flavonoids of several extracts of *Artemisia annua* L. obtained from a commercial sample and a selected cultivation. *Phytomedicine* **13**: 487.

- Bora K.S. and Sharma, A. 2010. Phytochemical and pharmacological potential of *Artemisia absinthium* Linn. and *Artemisia asiatica* Nakai : A Review. *Journal of Pharmacy Research* **3**(2):325-328.
- Braca, A., Fico, G., Morelli, I., Simone, F., Tome, F. and Tommasi, N. 2003. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *Journal of Ethnopharmacology* **86**(1):63-67.
- Brisibe, E. A., Umoren, U. E., Owai, P. U. and Brisibe, F. 2008. Dietary inclusion of dried *Artemisia annua* leaves for management of coccidiosis and growth enhancement in chickens. *African Journal of Biotechnology* **7**(22): 4083-4092.
- Brisibe, E. A., Umoren, U.E., Brisibe, F., Magalhaes, P.M., Ferreira, J.F.S., Luthria, D. and Prior, R.L. 2009. Nutritional characterization and antioxidant capacity of different tissues of *Artemisia annua* L. *Food Chemistry* **115**:1240-1246.
- Brzoska, F., Sliwinski, B. and Rutkowska, O. M. 2010. Effect of herb mixture on productivity, mortality, carcass quality and blood parameters of broiler chickens. *Annals of Animal Science* **10**(2): 157-165.
- Caner, A., Doskaya, M., Degirmenci, A., Can, H., Baykan, S., Uner, A., Basdemir, G., Zeybek, U. and Guruz, Y. 2008. Comparison of the effects of *Artemisia vulgaris* and *Artemisia absinthium* growing in western Anatolia against trichinellosis (*Trichinella spiralis*) in rats. *Experimental Parasitology* **119**(1):173-179.
- Çetin, M., Yurtseven, S., Kocyigit, A., Temamogullari, F., Altas, M. G., Ozyildiz, Z., Yilmaz, R. and Taskin, A. 2019. The Effects of Dried Wormwood (*Artemisia absinthium*) on Performance, Carcass Characteristics and

- Biochemical Parameters of Broiler Chicks. *KSU Journal of Agriculture and Nature* **22**(2): 301-314.
- Cherian, G., Orr, A., Burke, I. C. and Pan, W. 2013. Feeding *Artemisia annua* alters digesta pH and muscle lipid oxidation products in broiler chickens. *Poultry Science* **92**(4):1085-1090.
- Chopra. 1956. Glossary of Indian Medicinal Plants, NISCIAR, CSIR, New Delhi, P.25.
- Chu, G. M. and Song, Y. M. 2012. Effect of dietary addition of wormwood (*Artemisia montana Pampan*) on performance of fattening pigs and selected hematological and immunological indices. *Livestock Science* **147**: 188–191.
- Chu, W.B., Shi, B.L., Yan, S.M., Zhang, P.F., Zhao, F. and Sun, D.S. 2015. Effect of *Artemisiae argyi* extract on immune and antioxidative function in broilers. *Chinese Journal of Animal Science* **51** (19): 67-70.
- Cowan and Steele. 1993. Manual of identification of medicinal bacteria Ed. Cowan. Cambridge University Press, pp 317.
- Cowieson, A. J., Bedford, M. R. and Ravindran, V. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. *British Poultry Science* **51**:246-257.
- Cross, D., McDevitt, R. M., Hillman, K. and Acamovic, T. 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *British Poultry Science* **48**(4):496-506.
- Dalkilic, B. and Guler, T. 2009. The effects of clove extract supplementation on performance and digestibility of nutrients in broilers. *Ataturk University Journal of Veterinary Sciences* **23**: 161-166.

- Das, N. P and Ratty, A. K .1986. Effects of flavonoids on induced non-enzymic lipidperoxidation, in Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships (Cody V, Middleton E and Har-borne JB eds) pp 243–248. Alan R. Liss, Inc., New York.
- De Almeida, G. F, Horsted, K., Thamsborg, S. M., Kyvsgaard, N. C, Ferreira, J. F. and Hermansen, J. E. 2012. Use of *Artemisia annua* as a natural coccidiostat in free-range broilers and its effects on infection dynamics and performance. *Veterinary Parasitology* **186** (3-4):178-187.
- Ding, K., Wang, J., Liu, N. and Zhang, F. 2019. Effect of *Artemisia apiacea* Hance on growth performance, cecal opportunistic bacteria, and microbicidal peptides in rabbits. *Brazilian Journal of Animal Science*. ISSN 1806-9290.
- Dragan, L., Gyorke, A., Ferreira, J. F. S., Pop, I. A., Dunca, I. and Dragan M, Mircian, V., Dan, I. and Cozma,V. 2014. Effects of *Artemisia annua* and *Foeniculum vulgare* on chickens highly infected with *Eimeria tenella* (Phylum Apicomplexa). *Acta Veterinaria Scandinavica* **56**(1):22.
- Dragan, L., Titilincu, A., Dan, I., Dunca, I., Dragan, M. and Mircean,V. 2010. Effects of *Artemisia annua* and *Pimpinella anisum* on *Eimeria tenella* (Phylum Apicomplexa) low infection in chickens. *Scientia Parasitologica* **11**(2):77-82.
- Dzinic, N., Okanovic, D., Jokanovic, M., Tomovic, V. and Palic, D. 2013. The influence of garlic powder in broiler feed on carcass and breast meat quality. *Quality of Life* **4**: 55-61.

- EC (European Commission). Regulation 1831 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. 2003 *Official Journal of European Union Legislation* **268**:29.
- El-Ashram, S. and Abdelhafez, G.A. 2020. Effects of phytogetic supplementation on productive performance of broiler chickens. *Journal of Applied Poultry Research* **29**:852–862.
- Emami K. N., Samie A., Rahmani, H.R. and Ruiz-Feria, C. A.2012. The effect of peppermint essential Oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. *Animal Feed Science Technology* **175**:57–64.
- Engberg, R.M., Grevsen, K., Ivarsen, E., Frette, X., Christensen, L. P., Hojberg, O., Jensen, B. B. and Canibe, N. 2012. The effect of *Artemisia annua* on broiler performance, on intestinal microbiota and on the course of a *Clostridium perfringens* infection applying a necrotic enteritis disease model. *Avian Pathology* **41**(4): 369-376.
- Erel, O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry* **37**(4):277-85.
- Erel, S. B., Reznicek, G., Şenol, S. G., Yavasogulu, N. U. K., Konyalioglu, S. and Zeybek, A. U. 2012. Antimicrobial and antioxidant properties of *Artemisia*
- Fiamegos, Y. C., Kastitis, P. L., Exarchou, V., Han, H., Bonvin, A. M. J. J., Vervoort, J., Lewis, K., Hamblin, M. R. and Tegos, G. P. 2011. Antimicrobial and Efflux Pump Inhibitory Activity of Caffeoylquinic Acids from *Artemisia absinthium* against Gram-Positive Pathogenic Bacteria. *PLoS ONE* **6**(4): e18127.

- Gardzielewska, J., Pudyszak, K., Majewska, T., Jakubowska, M. and Pomianowski, J. 2003. Effect of plant-supplemented feeding on fresh and frozen storage quality of broiler chicken meat. *Electronic Journal of Polish Agricultural Universities. Series Animal Husbandry* **6**:2.
- Gharetappe, F. K., Hassanabadi, A., Semnaninezhad, H. and Nassiry, M. R. 2015. The effect of dietary tarragon (*Artemisia dracunculus*) and peppermint (*Mentha piperita*) leaves on growth performance and antibody response of broiler chickens. *Iranian Journal of Applied Animal Science* **5**(2):403-409.
- Ghasemi, H. A., Kasani, N. and Taherpour, K. 2014. Effects of black cumin seed (*Nigella sativa* L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers. *Livestock Science* **164**: 128-134.
- Ghazvinian, K., Seidavi A., Laudadio, V., Ragni, M and Tufarelli, V. 2018. Effects of various levels of organic acids and of virginiamycin on performance, blood parameters, immunoglobulins and microbial population of broiler chicks. *South African Journal of Animal Science* **48**(5) : 961-967.
- Gholamrezaie, S. L. Mohammadi, M., Jalali, S. J., Abolghasemi, S. A. and Roostaie Ali Mehr, M. 2013. Extract and leaf powder effect of *Artemisia annua* on performance, cellular and humoral immunity in broilers. *Iranian Journal of Veterinary Research* **14**(1): 15-20.
- Gilani, A. U. and Janbaz, K. H. 1995. Preventive and curative effects of *Artemisia absinthium* on acetaminophen and CCl₄ induced hepatotoxicity. *General Pharmacology* **26**(2):309-15.

- Goli, S. and Shahryar, H. A. 2015. Effect of enzymes supplementation (Rovabio and Kemin) on some blood biochemical parameters, performance and carcass characterizes in broiler chickens. *Iranian. Journal of Applied Animal Science* **19**:127–131.
- Goliomytis M, Tsourekis D, Simitzis PE, Charismiadou MA, Hager-Theodorides AL, Deligeorgis SG. 2014. The effects of quercetin dietary supplementation on broiler growth performance, meat quality, and oxidative stability. *Poultry Science* **93** (8):1957-62.
- Gouveia, S.C. and Castilho, P.C. 2013. *Artemisia annua* L. Essential oil and acetone extract composition and antioxidant capacity. *Industrial Crops and Products* **45**: 170-181.
- Greathead, H. 2003. Plants and plant extracts for improving animal productivity. *Proceedings of the Nutrition Society* **62** (2): 279-290.
- Guo, S., Ma, J., Xing, Y., Xu, Y., Jin, X., Yan, S. and Shi, B. 2020. *Artemisia annua* L. aqueous extract as an alternative to antibiotics improving growth performance and antioxidant function in broilers. *Italian Journal of Animal Science* **19**(1): 399-409.
- Habibi, H., Firouzi, S., Nili, H., Razavi, M., Asadi, S. L. and Daneshi, S. 2016a. Anticoccidial effects of herbal extracts on *Eimeria tenella* infection in broiler chickens: *in vitro* and *in vivo* study. *Journal of Parasitic Diseases* **40**: 401-407.
- Habibi, R., Jalilvand, G., Samadi, S. and Azizpour, A. 2016b. Effect of different levels of essential oils of wormwood (*Artemisia absinthium*) and cumin (*Cuminumcyminum*) on growth performance carcass characteristics and immune system in broiler chicks. *Iranian Journal of Applied Animal Science* **6**(2): 395-400.

- Habibian, M., Sadeghi, G. and Karimi, A. 2019. Comparative effects of powder, aqueous and methanolic extracts of purslane (*Portulaca oleracea* L.) on growth performance, antioxidant status, abdominal fat deposition and plasma lipids in broiler chickens. *Animal Production Science* **59** (1): 89-100.
- Hafeez, A., Iqbal, S., Sikandar, A., Din, S., Khan, I., Ashraf, S., Khan, R.U., Tufarelli, V. and Laudadio, V. 2021. Feeding of Phytobiotics and Exogenous Protease in Broilers: Comparative Effect on Nutrient Digestibility, Bone Strength and Gut Morphology. *Agriculture* **11**: 228.
- Hajati, H., Reazei, M. and Sayyazadeh, H. 2009. The Effects of Enzyme Supplementation on Performance, Carcass Characteristics and Some Blood Parameters of Broilers Fed on Corn-Soybean Meal-Wheat Diets. *International Journal of Poultry Science* **8**(12):199-205.
- Hassan, H. M. A., Youssef, A.W., Ali, H. M. and Mohamed, M. A.2015. Adding phytogenic material and/or organic acids to broiler diets: effect on performance, nutrient digestibility and net profit. *Asian Journal of Poultry Science* **9** (2): 97-105.
- Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Megias, M. D.2004. Influence of two plant extracts on broilers performance, digestibility and digestive organ size. *Poultry Science* **83** (2): 169-174.
- Hosseindoust, A., Lee, S., Gook Nho W., Song, Y. H., Shin, J.S., Ingale, S. L. A dose-response study to evaluate the effects of pH-stable β -mannanase derived from *Trichoderma citrinoviride* on growth performance, nutrient retention, and intestine morphology in broiler chickens. *Italian Journal of Animal Science* **18**:147–154.

- Hosseinzadeh, Z. and Farhoomand, P. 2014. The effects of *Artemisia dracunculus* powders different levels on blood parameters and internal organs weight broiler chickens. *International journal of Advanced Biological and Biomedical Research* **2**(3): 661-668.
- Hosseinzadeh, Z. and Farhoomand, P. 2014. The effects of *Artemisia Dracunculus* powders different levels on blood parameters and internal organs weight broiler chickens. *International Journal of Advanced Biological and Biomedical Research* **2**(3): 661-668.
- Hosseinzadeh, Z. and Moghaddam, G. 2014. Effects of tarragon powders' different levels (*Artemisia dracunculus*) on general performance and anetometric properties of digestive system of male broiler chickens. *International Journal of Advanced Biological and Biomedical Research* **2**(5):1599-1605.
- Hwang, K. E., Choi, Y. S., Choi, S. M., Kim, H. W., Choi, J. H., Lee., M. A. and Kim, C. J. 2013. Antioxidant action of ganghwayakssuk (*Artemisia princeps* Pamp.) in combination with ascorbic acid to increase the shelf life in raw and deep fried chicken nuggets. *Meat Science* **95**(3):593-602.
- Iqbal, S., Younas, U., Chan, K. W., Zia-Ul-Haq, M. and Ismail, M.2012. Chemical Composition of *Artemisia annua* L. Leaves and Antioxidant Potential of Extracts as a Function of Extraction Solvents. *Molecules* **17**: 6020-6032.
- Issa, K. and Abo Omar, J. 2012. Effect of garlic powder on performance and lipid profile of broilers. *Open Journal of Animal Science* **2**:62-68.
- Jamroz, D., Wiliczekiewicz, A., Wertelecki, T., Orda, J. and Skorupinska, J. 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. *British Poultry Science* **46** (4):485-493.

- Jang, I. S., Ko, Y. H., Kang, S. Y. and Lee, C. Y. 2007. Effect of commercial essential oils on growth performance, digestive enzyme activity, and intestinal microflora population in broiler chickens. *Animal Feed Science and Technology* **134** (3):304-315.
- Jasna, M. C., Djilas, S.M., Cetkovic, G.S. and Saponjac, V.T. 2005. Free-radical scavenging activity of wormwood (*Artemisia absinthium* L.) extracts. *Journal of the Science of Food and Agriculture* **85**(2):265 – 272.
- Jasna, M., Canadanovic, B., Sonja, M.D., Gordana, S.C., Vesna, T.T. 2004. Free-radical scavenging activity of wormwood (*Artemisia absinthium*) extracts. *Journal of the Science of Food and Agriculture* **85**:265–272.
- Javed A., Showkat, R.M. and Saima, A. 2019. A pharmacognostic review on *Artemisia absinthium* *International Research Journal of Pharmacy* **10**(1):25-31.
- Javed, A., Showkat, R.M. and Saima, A. 2019. A Pharmacognostic Review on *Artemisia absinthium*. *International Research Journal of Pharmacy* **10**(1):25-31.
- Kalia, S., Bharti, V.K., Giri, A., Kumar, B., Arora, A. and Balaje, S.S. 2018. *Hippophae rhamnoides* as novel phytogenic feed additive for broiler chickens at high altitude cold desert. *Scientific Reports* **8**:5954.
- Kamboh, A. A. and Zhu, W. Y. 2013. Effect of increasing levels of bioflavonoids in broiler feed on plasma anti-oxidative potential, lipid metabolites, and fatty acid composition of meat. *Poultry Science* **92** (2):454-61.
- Karaskova, K., Suchy, P. and Strakova, E. 2015. Current use of phytogenic feed additives in animal nutrition: A review. *Czech Journal of Animal Science* **60**(12):521-530.

- Kaya, H., Karaalp, M., Kaynar, O., Tekce, E., Aksakal, A. and Bayram, B. 2020. Tarragon (*Artemisia Dracunculus* L.) Could Alleviate Negative Effects of Stocking Density in Laying Hens. *Brazilian Journal of Poultry Science* **23**(1):1-12.
- Keeton, J. T. 1984. Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. *Journal of Food Science* **48**:878-881.
- Keeton, J.T. 1983. Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. *Journal of Food Science* **48**: 878-881.
- Kennedy A. and Deans S. 2001. *Artemisia absinthium*. Artemisia. Medicinal and Aromatic Plants - Industrial Profiles: CRC Press: 79-89.
- Khalaji, S., Zaghari, M., Hatami, K.H., Hedari-Dastjerdi, S., Lotfi, L. and Nazarian, H. 2011. Black cumin seeds, *Artemisia* leaves (*Artemisia sieberi*), and *Camellia* L. plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. *Poultry Science* **90**(11): 2500-10.
- Khaligh Gharetappe, F., Hassanabadi, A., Semnaninezhad, H. and Nassiry, M.R. 2015. The Effect of Dietary Tarragon (*Artemisia dracunculus*) and Peppermint (*Mentha piperita*) Leaves on Growth Performance and Antibody Response of Broiler Chickens. *Iranian Journal of Applied Animal Science* **5**(2):403-409.
- Khan, S. H., Sardar, R. and Siddique, B. 2006: Influence of enzymes on performance of broilers fed sunflower-corn based diets. *Pakistan Veterinary Journal* **26**:109–114.
- Khattak, F., Ronchi, A., Castelli, P. and Sparks, N. 2013. Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. *Poultry Science* **93**(1):132-137.

- Kheirabadia, K.P., Katadj, J.K., Bahadoran, S., Teixeira da Silva, J.A., Samani, A.D. and Bashi, M.C. 2014. Comparison of the anticoccidial effect of granulated extract of *Artemisia sieberi* with monensin in experimental coccidiosis in broiler chickens. *Experimental Parasitology* **141**:129-133.
- Kim, C. H., Kim, G. B., Chang, M. B., Bae, G. S., Paik, I. K. and Kil, D. Y. 2012b. Effect of dietary supplementation of *Lactobacillus*-fermented *Artemisia princeps* on growth performance, meat lipid peroxidation, and intestinal microflora in Hy-line Brown male chickens. *Poultry Science* **91** (11):2845-51.
- Kim, W., Choi, W.J., Lee, S., Kim, W.J., Lee, D.C., Sohn, U.D., Shin, H.S. and Kim, W. 2015. Anti-inflammatory, Antioxidant and Antimicrobial Effects of Artemisinin Extracts from *Artemisia annua* L. *Korean Journal of Physiology and Pharmacology* **19**: 21-27.
- Kim, Y. J., Kim, C. M., Choi, J. H. and Choi, I. H. 2012. Effect of dietary mugwort (*Artemisia vulgaris* L.) and pine needle powder (*Pinus densiflora*) on growth performance, serum cholesterol levels, and meat quality in broilers. *African Journal of Biotechnology* **11**(55): 11866-11873.
- Kordali, S., Aslan, I., Calmasur, O. and Cakir, A. 2006. Toxicity of essential oils isolated from three *Artemisia* species and some of their major components to granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Industrial Crops and Products* **23**(2):162-170.
- Kostadinovic, L. M., Cabarkapa, I. S., Levic, J. D., Kormanjos, S. M., Teodosin, S. J. and Sredanovic, S. A. 2014. Effect of *Artemisia absinthium* essential oil on antioxidative systems of broiler's liver. *Food and Feed Research* **41**(1): 11-17.

- Kostadinovic, L., Levic, J., Galonja-Coghill, T. and Ruzicic, L. 2012. Anticoccidian effects of the *Artemisia absinthium* L. extracts in broiler chickens. *Archiva Zootechnica* **15**(2): 69-77.
- Kostadinovic, L., Levic, J., Popovic, S., Cabarkapa, I., Puvaca, N., Duragic, O. and Kormanjos, S. 2015. Dietary inclusion of *Artemisia absinthium* for management of growth performance, antioxidative status and quality of chicken meat. *European Poultry Science* **79** ISSN:1612-9199.
- Koul, B., Taak, P., Kumar, A., Khatri, T. and Sanyal, I. 2017. The *Artemisia* genus: a review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation. *Journal of Glycomics Lipidomics* **7**:1.
- Lal Samyal, M., Hitesh, K. and Bharat, P. 2011. Evaluation anti-diabetic and anti hyperlipidemic effects of extract of *Artemisia dracunculus* L. in streptozotocin- induced -diabetic rats. *Phamacology Online* **2**: 1230-1237.
- Latheef, S. K., Dhama, K., Wani, M. Y., Samad, H. A., Barathidasan, R., Tiwari R., Singh, S.D. and Rai, R. B. 2013. Ameliorative effects of four herbs (*Withania somnifera*, *Tinospora cordifolia*, *Azadirachta indica* and E care SE herbal) on the pathogenesis of chicken infectious anaemia virus. *International Journal of Current Research* **5**(8) 2327-2331.
- Lee, A. R., Niu, K. M., Lee, W. D., Kothari, D. and Kim, S. K. 2019. Comparison of the Dietary Supplementation of *Lactobacillus plantarum*, and Fermented and Non- Fermented *Artemisia Annua* on the Performance, Egg Quality, Serum Cholesterol, and Egg yolk - Oxidative Stability During Storage in Laying Hens. *Brazilian Journal of Poultry Science* **21**(4):001-008.

- Lee, A. R., Niu, K.M., Lee, W.D., Kothari, D. and Kim, S.K. 2019. Comparison of the Dietary Supplementation of *Lactobacillus plantarum*, and Fermented and Non-Fermented *Artemisia Annua* on the Performance, Egg Quality, Serum Cholesterol, and Egg yolk-Oxidative Stability During Storage in Laying Hens. *Brazilian Journal of Poultry Science* **21**(4):1-8.
- Lee, H. G., Kim, H. and Oh, W. K. 2004. Tetramethoxy hydroxyflavone p-7F downregulates inflammatory mediators via the inhibition of nuclear factor kappa B. *Annals of the New York Academy of Sciences* **1030**:555-68.
- Lee, K. W., Everts, H., Kappert, H. J., Frehner, M., Losa, R. and Beynen, A. C. 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *British Journal of Poultry Science* **44**(3) : 450-457.
- Lee, K., Everts, H. and Beynen, A. 2004. Essential oils in broiler nutrition. *International Journal of Poultry Science* **3**(12):738-752.
- Li, T., Chen, H., Wei, N., Mei, X., Zhang, S., Liu, D. L., Gao, Y., Bai, S. F., Liu, X.G. and Zhou, Y. X. 2012. Anti-inflammatory and immunomodulatory mechanisms of artemisinin on contact hypersensitivity. *International Immunopharmacology* **12**(1):144-150.
- Lopes-Lutz, D., Alviano, D. S., Alviano, C. S. and Kolodziejczyk, P. P. 2008. Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. *Phytochemistry* **69**(8):1732-1738.
- Lutgen P. 2013. Artemisia ketone, phytosterols and lipid metabolism. *The World's Scientific and Social Network for Malaria Professionals* **18**:35.
- Mahima, R. A., Deb, R., Latheef, S. K., Abdul, S. H., Tiwari R., Verma, A. K., Kumar, A. and Dhama, K. 2012. Immunomodulatory and therapeutic

potentials of herbal, traditional/indigenous and ethnoveterinary medicines.
Pakistan Journal of Biological Sciences **15**(16): 754-774.

Mahmoudi, M., Ebrahimzadeh, M. A, Ansaroudi F, Nabavi, S.F. and Nabavi, S. M. 2009. Antidepressant and antioxidant activities of *Artemisia absinthium* L. at flowering stage. *African Journal of Biotechnology* **8**(24) : 7170-7175.

Malayoglu, H.B., Baysal, S., Misirlioglu, Z., Polat, M., Yilmaz, H. and Turan, N. 2010. Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat–soybean meal diets. *British Poultry Science* **51**(1):67-80.

Manach F., Regerat, F. and Texier O. 1996. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. *Nutrition Research* **16**(3):517-44.

Marcincak, S., Popelka, P., Zdolec, N., Martonova, M., Simkova, J. and Marcincakova, D. 2011. Effect of supplementation of phytogenic feed additives on performance parameters and meat quality of broiler chickens. *Slovenian Veterinary Research* **48**(1) :27-34.

Martinez-Diaz, R. A., Ibanez-Escribano, A., Burillo, J., Heras Lde, L., Prado, G. D., Agullo-Ortuno, M. T. Julio, L. F, Gonzalez-Coloma, A. 2015. Trypanocidal, trichomonacidal and cytotoxic components of cultivated *Artemisia absinthium* Linnaeus (Asteraceae) essential oil. *Memorias do Instituto Oswaldo Cruz* **110**(5):693-699

Mohammadian, A., Moradkhani, S., Ataei, S., Shayesteh, T.H., Sedaghat, M., Kheiripour, N, and Ranjbar, A. 2015. Antioxidative and hepatoprotective effects of hydroalcoholic extract of *Artemisia absinthium* L. in rat. *Journal of HerbMed Pharmacology* **5**(1): 29-32.

- Mohebodini, H., Jazi, V., Ashayerizadeh, A., Toghyani, M and Tellez-Isaias, G. 2021. Productive parameters, cecal microflora, nutrient digestibility, antioxidant status, and thigh muscle fatty acid profile in broiler chickens fed with *Eucalyptus globulus* essential oil. *Poultry Science* **100**:100922.
- Mohiti-Asli, M. and Ghanaatparast-Rashti, M. 2018. Comparing the effects of a combined phytogenic feed additive with an individual essential oil of oregano on intestinal morphology and microflora in broilers. *Journal of Applied Animal Research* **46**(1):184-189.
- Monika and Kaur, G. 2016. Phytochemical Screening and Antimicrobial Activity of *Artemisia absinthium*. *International Journal of Research and Review E-ISSN: 2349-9788*.
- Mounia, M., Nadir, A. and Omar, B. 2018. Effects of phytogenic products on gut morpho-histology of broiler chickens. *International Journal of Veterinary Science and Research* pp. 9-11.
- Mudalal, S., Zaazaa, A. and Abo Omar, J. 2020. Effects of Medicinal Plants Extract with Antibiotic Free Diets on Broilers Growth Performance and Incidence of Muscles Abnormalities. *Brazilian Journal of Poultry Science* **23**(1): 001-008.
- Murugesan, G. R., Syed, B., Haldar, S. and Pender, C. 2015. Phytogenic feed additives as an to antibiotic growth promoters in broiler chickens. *Frontiers in Veterinary Science* **3**(2):21.
- Mutlag, N. A., Al-Norri, M. A. and Farhan, S. M. 2018. The effect of adding turmeric and *Artemisia herba* powder to ration on productive performance of white laying hens. *Iraqi Journal of Veterinary Sciences* **32** (2): 237-242.
- Oblskiy, D., Pischel, I., Feistel, B., Glotov, N. and Heinrich, M. 2011. *Artemisia dracunculus* L. (tarragon): A Critical Review of Its Traditional Use,

Chemical Composition, Pharmacology and Safety. *Journal of Agricultural and Food Chemistry* **59**(21):11367-84.

Omar, A.E., Hanan, S., Al-Khalaifah, Wafaa, A. M., Mohamed, Heba, S. A., Gharib, Ali Osman, Naif, A. Al-Gabri. and Shimaa A. A. 2020. Effects of Phenolic-Rich Onion (*Allium cepa* L.) Extract on the Growth Performance, Behavior, Intestinal Histology, Amino Acid Digestibility, Antioxidant Activity, and the Immune Status of Broiler Chickens. *Frontiers in Veterinary Science* **7**: 582-612.

Omar, M. A., Abdel-Hamid, T. M., Sara Essam,S. and Omar, A. 2019. Growth and economic performance of using dried tomato pomace for mallard ducks. *Slovenian Veterinary Research* **56** (22): 699-706.

Onibi, G. E., Adebisi, O. E., Fajemisin, A. N. and Adetunji, A.V. 2009. Response of broiler chickens in terms of performance and meat quality to garlic (*Allium sativum*) supplementation. *African Journal of Agricultural Research* **4**(5): 511-517.

Oso, A. O., Suganthi, R. U., Reddy, G. B., Manjunatha Malik, P.K., Thirumalaisamy, G., Awachat, V.B., Selvaraju, S., Arangasamy, A. and Bhatta, R. 2019. Effect of dietary supplementation with phytogenic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. *Poultry Science* **98**(10): 4755-4766.

Panaite, T.D., Criste, R.D., Saracila, M., Tabuc, C., Turcu, R.P. and Olteanu,M. 2018. The use of ascorbic acid and *Artemisia annua* powder in diets for broilers reared under heat stress. *Romanian Biotechnological Letters* **23**(5):976-985.

Park, C. H., Choi, E., Yang, H., Hyun Ho, S., Park, S. J., Park, K. and Kim, S. 2020. Efficacy of *Artemisia annua* L. extract for recovery of acute liver failure. *Food Science Nutrition* **8**(7): 3738-3749.

- Parsaei, S., Amini, Z. and Houshmand, M. 2014. Effects of olive leaf on blood metabolites and humoral immunity response of broiler chicks. *International Journal of Advanced Biological and Biomedical Research* **2**(3): 741-751.
- Patel, K. and Srinivasan, K. 2004. Digestive stimulant action of spices: amyth or reality? *Indian Journal of Medical Research* **119**(5):167-79.
- Peric, L., Milosevic, N., Zikic, D., Sinisa Bjedov, S., Cvetkovic, D., Markov, S., Mohnl, M. and Steiner, T. 2010. Effects of probiotic and phytogetic products on performance, gut morphology and cecal microflora of broiler chickens. *Archiv Tierzucht* **53**(3):350-359.
- Petrolli, T.G., Albino, L.F.T., Rostagno, H.S., Gomes, P.C., Tavernari, F.C. and Balbino, E.M. 2012. Herbal extracts in diets for broilers. *Brazilian Journal of Poultry Science* **41** (7):1683-1690.
- Pietta, P.G. 2000. Flavonoids as Antioxidants. *Journal of Natural Products* **63**(7):1035-42.
- Popovic, S. J., Kostadinovic, L. M., Puvaca, N. M., Kokic, B. M., Cabarkapa, I. S. and Duragic, O. M. 2017. Potential of wormwood (*Artemisia absinthium*) as a feed supplement in rabbit diet: effect on controlling rabbit coccidiosis, antioxidative systems and growth performance. *Veterinarski Arhiv* **87**(6): 769-782.
- Popovic, S., Kostadinovic, L., Duragic, O., Acimovic, M., Cabarkapa, I., Puvaca, N. and Dragana, L.P. 2018. Influence of medicinal plants mixtures (*Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum*) in broilers nutrition on biochemical blood status. *Journal of Agronomy, Technology and Engineering Management* **1**(1): 91-98.

- Prakasita, V.C., Asmara, W., Sitarina Widayarini, S. and Wahyuni, A. E. T. 2019. Combinations of herbs and probiotics as an alternative growth promoter: An in vitro study. *Veterinary World* EISSN: 2231-0916.
- Puri, M., Sharma, D. and Barrow, C. J. 2012. Enzyme-assisted extraction of bioactives from plants. *Trends Biotechnology* **30**(1):37-44.
- Puvaca, N., Kostadinovic, L., Ljubojevic, D., Lukac, D., Levic, J., Popovic, S., Novakov, N., Bojana Vidovic, B. and Duragic, O. 2015. Effect of garlic, black pepper and hot red pepper on productive performances and blood lipid profile of broiler chickens. *European Poultry Science* **79** ISSN 1612-919.
- Puvaca, N., Simin, M.T., Kostadinovic, L., Lukac, D., Ljubojevic, D., Popovic, S. and Tasic, T. 2016. Economic efficiency costs of broiler chicken production fed dietary garlic, black pepper and hot red pepper supplements. <http://www.custose.agronegocioonline.com> **11**: 4.
- Puvaca, N., Stanacev, V., Beukovic, M., Ljubojevic, D., Kostadinovic, Lj. and Dzinic, N. 2014. Effect of phytogetic feed additive (*Allium sativum* L.) in broiler chicken nutrition on breast meat quality and tissues cholesterol content. *Proceedings of International Scientific Conference*, Vukovar, Croatia **7**:75-80
- Puvaca, N., Stanacev, V., Glamocic, D., Levic, J., Peric, L., Stanacev, V. and D. Milic, D.. 2013. Beneficial effects of phytoadditives in broiler nutrition. *World's Poultry Science Journal* **69**(1):27-35.
- Qingming, Y., Xianhui, P., Weibao, K. and Hong, Y. 2010. Antioxidant activities of malt extract from barley (*Hordeum vulgare* L.) toward various oxidative stress in vitro and in vivo. *Food Chemistry* **118**(1):84-89.

- Ravindran, V. 2013. Feed enzymes: the science, practice, and metabolic realities. *Journal of Applied Poultry Research* **22**:628–636.
- Rice-Evans, C. A., Miller, N.J. and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* **20** (7):933-956.
- Rosak, C. and Mertes, G. 2009. Effects of acarbose on proinsulin and insulin secretion and their potential significance for the intermediary metabolism and cardiovascular system. *Current Diabetes Reviews* **5**(3):157-64.
- Rubio, L., Motilva, M. J. and Romero, M. P. 2013. Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles. *Critical Reviews in Food Science and Nutrition* **53**: 943-53.
- Ryu, J. C., Park, S. M., Hwangbo, M., Byun, S. H., Ku, S. K., Kim, Y. W., Kim, S. H, Jee, S.Y. and Cho, I. L. J. 2013. Methanol extract of *Artemisia apiacea* hance attenuates the expression of inflammatory mediators via NF-j B Inactivation. *Evidence Based Complementary and Alternate Medicine* 494681.
- Saeed El-Ashram. and Gamal A. Abdelhafez. 2020. Effects of phytogenic supplementation on productive performance of broiler chickens. *Journal of Applied Poultry Research* **29**:852–862.
- Sahoo, G., Mulla, N. S. S., Ansari, Z. A. and Mohandass, C. 2012. Antibacterial activity of mangrove leaf extracts against human pathogens. *Indian Journal of Pharmaceutical Sciences* **74**(4):348-35.
- Saleh, A., Ebeid, T. A. and Abudabos. A. M. 2018. Effect of dietary phytogenics (herbal mixture) supplementation on growth performance, nutrient

utilization, antioxidative properties, and immune response in broilers. *Environmental Science and Pollution Research* **25**(15): 14606-14613.

Saracila M., Criste, R. D., Panaite, T. D., Vlaicu, P. A., Tabuc, C., Turcu, R. P., Olteanu, M. 2018. *Artemisia Annua* as Phytogenic Feed Additive in the Diet of Broilers (14-35 Days) Reared under Heat Stress (32 °C). *Brazilian Journal of Poultry Science* **20** (4): 825-832.

Saracila, M., Criste, R. D., Panaite, T. D., Vlaicu, P. A., Tabuc, C., Turcu, R. P., Olteanu, M. 2018. *Artemisia Annua* as Phytogenic Feed Additive in the Diet of Broilers (14-35 Days) Reared under Heat Stress (32°C). *Brazilian Journal of Poultry Science* **20**(4): 825-832.

Sarhadi, I., Alizadeh, E., Ahmadifar, E., Adineh, H. and Dawood, M.A.O. 2020. Skin mucosal, serum immunity and antioxidant capacity of common carp (*Cyprinus carpio*) fed *Artemisia* (*Artemisia annua*). *Annals of Animal Science* ISSN: 2300-8733.

Sarhadi, I., Alizadeh, E., Ahmadifar, E., Adineh, H. and Dawood, M.A.O. 2020. Skin mucosal, serum immunity and antioxidant capacity of common carp (*Cyprinus carpio*) fed *Artemisia* (*Artemisia annua*). *Annals of Animal Science* ISSN: 2300-8733.

Shah, A. H., Khan, S.A.A., Qudrat Ullah R.U., and Shabana, N. 2020. Effect of diet supplemented with phytogenics and protease enzyme on performance, serum biochemistry and muscle histomorphology in broilers. *Journal of Applied Animal Research* **48**(1): 326-330.

Shahin, S. E., Ibrahim, D. and Badawi, M. 2020. Effects of phytogenic supplementation on productive and economic performance in broilers. *Journal of Animal Health and Production* **9**(1): 42-49.

- Shirmohammad, F. and Mehri, M. 2011. Effects of dietary supplementation of multi-enzyme complex on the energy utilization in rooster and performance of broiler chicks. *African Journal of Biotechnology* **10**(38):7541-7547.
- Shiwei Guo, Jiaxin, M., Yuanyuan, X., Yuanqing , X., Xiao, J., Sumei, Y. and Binlin, Shi.2020. *Artemisia annua* L. aqueous extract as an alternative to antibiotics improving growth performance and antioxidant function in broilers. *Italian Journal of Animal Science* **19**(1): 399-409.
- Siegel, P. B and Gross, W. B. 1980. Production and non-persistence of antibodies in chicken to sheep erythrocytes. 1 Directional Selection. *Poultry Science* **59**(2):205-210.
- Singh, R., Verma, P. K. and Singh, G. 2012. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. *Journal of Intercultural Ethnopharmacology* **1**(2):101-104.
- Singh, V. B., Singh, V. K., Dwivedi, D., Tiwari, D., Singh, S. P. and Singh, V.V. 2018. Effect of a phytogenic feed additive supplemented diet on growth performance, hemato-biochemical profile and carcass characteristics of broiler chickens. *Animal Nutrition and Feed Technology* **18**(3): 321-331.
- Snedecor, G. W. and Cochran, W. G. 1994. Statistical methods. 6th Edition. Ames, IA. Iowa State University Press.
- Soltan, M.A., Shewita, R. and El-Katcha, M. I. 2008. Effect of dietary anise seed supplementation on growth performance, immune response, carcass traits and some blood parameters of broiler chickens. *International Journal of Poultry Science* **7**(11): 1078-1088.
- Song, Z. H., Cheng, K., Zheng, X. C., Ahmad, H., Zhang, L. L. and Wang, T. 2018. Effects of dietary supplementation with enzymatically treated

- Artemisia annua* on growth performance, intestinal morphology, digestive enzyme activities, immunity, and antioxidant capacity of heat-stressed broilers. *Poultry Science* **97**(2):430-437.
- Souri, H., Khatibjoo, A., Taherpoor, K., Hassan-Abadi, A., Fattahnia, F. and Askari, M. 2015. Effect of *Thymus Vulgaris* and *Satureja khuzestanica* Ethanolic extract on broiler chickens performance and immune response. *Iranian Journal of Applied Animal Research* **5** : 437-446.
- Srividya, A. R., Dhanabal, S. P., Misra, V. K. and Suja, G. 2010. Antioxidant and antimicrobial activity of *Alpinia officinarum*. *Indian Journal of Pharm Sci.* **72**:145-8.
- Tadera, K., Minami, Y., Takamatsu, K. and Matsuoka, T. 2006. Inhibition of α -glucosidase and α -amylase by flavonoids. *J. Nutr. Sci. Vitaminol.* **52**:149-153.
- Tunc, M.A., Yildirim, S. and Yoruk, M.A. 2019. Effects of tarragon (*Artemisia dracunculus*) powder on broiler performance parameters and histopathology of internal organs. *Austral Journal of Veterinary Sciences* **51**(3):113-118.
- Wan, X., Zhang, J., He, J., Bai, K., Zhang, L. and Wang, T. 2017. Dietary enzymatically treated *Artemisia annua* L. supplementation alleviates liver oxidative injury of broilers reared under high ambient temperature. *International Journal of Biometeorology* **61**:1629-1636.
- Wan, X.L., Niu, Y., Zheng, X.C., Huang, Q., Su, W.P., Zhang, J.F., Zhang, L.L. and Wang, T. 2016. Antioxidant capacities of *Artemisia annua* L. leaves and enzymatically treated *Artemisia annua* L. *in vitro* and in broilers. *Animal Feed Science and Technology* **221**(1): 27-34.

- Wang, J., Lin, L., Li, B., Zhang, F. and Liu, N. 2019. Dietary *Artemisia vulgaris* meal improved growth performance, gut microbes, and immunity of growing Rex rabbits. *Czech Journal of Animal Sciences* **64**:174-179.
- Wang, J., Su, S., Pender, C., Murugesan, R., Syed, B. and Kim, W. K. 2021. Effect of a Phytogenic Feed Additive on Growth Performance, Nutrient Digestion, and Immune Response in Broiler-Fed Diets with Two Different Levels of Crude Protein. *Animals* **11**:775.
- Waskar, V.S., Ravikanth, K. and Maini, S. 2011. Effect of polyherbal feed supplement and antimycotic product on meat quality attributes of chicken. *Journal of Indian Veterinary Association* **9**:39-45.
- Wati, T., Ghosh, T. K., Syed, B. and Haldar, S. 2015. Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens *Animal Nutrition* **3**: 213-219.
- Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. 2008. Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science* **86**: 140-148.
- Witte, V.C., Krause, G. F. and Bailey, M. E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science* **35**:582-585.
- Wright, C.W. 2002. *Artemisia. Medicinal and Aromatic Plants—Industrial Profiles*. Taylor & Francis, London.
- Xu, H. 2010. Inhibition kinetics of flavonoids on yeast α -glucosidase merged with docking simulations. *Protein Pept. Lett.* **17**: 1270-1279.

- Yang, S., Zhang, J., Jiang, Y., Qing Xu, Y., Jin, X., Yan, S.M. and Shi, B. L. 2021. Effects of *Artemisia argyi* flavonoids on growth performance and immune function in broilers challenged with lipopolysaccharide. *Animal Bioscience* **34**(7): 1169–1180.
- Yanli, L., Tian, Y., Zhong, W., Wang, N., Wang, Y., Zhang, Y., Zhang ,Z., Li, J., Ma, F., Zhao, Z. and Peng, Y. 2021. *Artemisia argyi* Essential Oil Inhibits Hepatocellular Carcinoma Metastasis via Suppression of DEPDC1 Dependent Wnt/ β -Catenin Signaling Pathway. *Frontiers in Cell and Developmental Biology* **9**:664791.
- Yu Niu., Zhao, Y., He, J., Yun, Y., Shi, Y., Zhang, L. and Wang, T. 2020. Effect of diet supplemented with enzymatically treated *Artemisia annua* L. on intestinal digestive function and immunity in weaned pigs. *Italian Journal of Animal Science* **19**(1):1170-1179.
- Zhao, F., Shi, B., Sun, D., Chen, H., Tong, M., Zhang, P., Guo, X. and Yan, S. 2016. Effects of dietary supplementation of *Artemisia argyi* aqueous extract on antioxidant indexes of small intestine in broilers. *Animal Nutrition* **2**(3):1-5.
- Zhou, Y., Jiang, Z., Lv, D. and Wang, T. 2009. Improved energy utilizing efficiency by enzyme preparation supplement in broiler diets with different metabolizable energy levels. *Poultry Science* **88**:316-322.



Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Livestock Production and Management,
Faculty of Veterinary Sciences & Animal Husbandry, Shuhama

CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Ramesh Sharma, Professor & Head, Division of Animal Nutrition, SKUAST-J** during viva-voce examination held on **16-06-2022** have been incorporated in the final manuscript entitled **“Performance of Broiler chicken fed diets supplemented with *Artemisia absinthium* with or without enzyme”** submitted by **Dr. Shaista Saeedatu Nissa (Regd. No. 2018-763-D).**

(Prof. M. T. Bandy)
Chairman
Advisory Committee