COMPARATIVE STUDY OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID SUPPLEMENTATION ON ANTIOXIDANT STATUS AND IMMUNE RESPONSE IN BROILER CHICKEN

THESIS

By

Dr. Rakesh Kumar

Admission No.: BVC/M/ANN/003/2017-18

Submitted to

BIHAR ANIMAL SCIENCES UNIVERSITY

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IN

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DEPARTMENT OF ANIMAL NUTRITION

BIHAR VETERINARY COLLEGE, PATNA - 14

Bihar Animal Sciences University, Patna, Bihar

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CERTIFICATE - I

This is to certify that the thesis entitled **" Comparative study of *Moringa oleifera* aqueous leaf extract and Ascorbic acid supplementation on Antioxidant status and Immune response in Broiler chicken "** submitted in partial fulfillment of the requirement for the award of **Master of Veterinary Science (Animal Nutrition)** of the faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is the original work carried out by **Dr. RAKESH KUMAR, Admission No.: BVC/M/ANN/003/2017-18** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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Date: **(Rakesh Kumar)**

Place: Patna

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ABBREVIATIONS

ADF	-	Acid Detergent Fibre
ADG	-	Average Daily Gain
ALA	-	Alpha- Linoleic Acid
ALP	-	Alkaline Phosphatase
ALT	-	Alanine Transaminase
AMOLE	-	Aqueous *Moringa oleifera* Leaf Extract
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemist
AST	-	Aspartate Transaminase
BUN	-	Blood Urea Nitrogen
BW	-	Body weigh
BHT	-	Butylated Hydroxytolune
Ca	-	Calcium
CF	-	Crude Fibre
CP	-	Crude Protein
DMSO	-	Dimethyl sulfoxide
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
DTNB	-	5,5-dithiobis-(2-Nitrobenzoic Acid
DM	-	Dry Matter
DMI	-	Dry Matter Intake
EDTA	-	Ethylene Diamine Tetra Acetate
EE	-	Ether Extract
ELISA	-	Enzyme-linked immune sorbent assay
FI	-	Feed Intake
FAO	-	Food and Agricultural Organization
FCR	-	Feed Conversion Ratio
GSH	-	Glutathione
g	-	Gram
HA	-	Haemagglutination

Hb	-	Haemoglobin
HI	-	Haemagglutination Inhibition
HDL	-	High Density Lipoprotein
HSP	-	Heat Shock Protein
IFCC	-	International Federation of Clinical Chemistry
IU	-	International Unit
Kcal	-	Kilocalorie
Kg	-	Kilogram
LDL	-	Low Density Lipoprotein
LDH	-	Lactate dehydrogenase
LPO	-	Lipid peroxidation
ME	-	Metabolizable Energy
MJ	-	Mega joul
MOALE	-	Moringa oleifea Aqueous Leaf Extract
MOLE	-	Moringa oleifera Leaf Extract
MOLM	-	Moringa oleifera Leaf Meal
MOLP	-	Moringa oleifera Leaf Powder
ml	-	Millilitre
MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration
MLM	-	Moringa Leaf Meal
MOL	-	Moringa oleifera Leaf
Na	-	Sodium
NFE	-	Nitrogen Free Extract
NDV	-	Newcastle Disease Vaccine
ND	-	Newcastle Disease
NDF	-	Neutral Detergent Fibre
OM	-	Organic Matter
P	-	Phosphorus
PCV	-	Packed Cell Volume
PBS	-	Phosphate-Buffered Saline

PI	-	Performance index
ROS	-	Reactive Oxygen Species
RBC	-	Red Blood Corpuscles
SOD	-	Superoxide dismutase
SEM	-	Standard Error of Mean
SGOT	-	Serum Glutamic Oxaloacetic Transaminase
SBM	-	Soybean Meal
SPSS	-	Statistical Packages for Social Science
SGPT	-	Serum Glutamic Pyruvic Transaminase
TA	-	Total Ash
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TEC	-	Total Erythrocyte Count
TLC	-	Total Leucocytes Count
U/L	-	Unit Per Liter
VLDL	-	Very Low Density Lipoprotein
WHO	-	World Health Organization
WBC	-	White Blood Cell

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ABSTRACT

Livestock farming is one of the key components of Indian agriculture and contributes on large scale in the income of rural farmers. Poultry sector plays important role in minimising the protein and calorie deficiency of large human population of our country. Ascorbic acid is an important scavenger of free radical produced in the system during stress condition however, *Moringa oleifera* is nutrient rich high nutritional value species. The study was planned to see the comparative effect of water supplementation of *Moringa oleifera* aqueous leaf extract (MOALE) and ascorbic acid as an antioxidant sources in broiler chicken. All 135 day-old broiler chicks were divided into 3 different treatment groups. T1 served as control, T2 were fed basal ration with MOALE (90 ml/L drinking water) and T3 offered basal ration with ascorbic acid (15 mg/L drinking water) for 35 days experiment. The growth performance and feed efficiency was better in MOALE group followed by ascorbic acid supplemented birds without affecting the metabolism of nutrients. Most of the haemato-biochemical profiles and HSP70 gene expression were unaffected by the treatment except creatinine, while antioxidant profile was improved in treatment group. Immunity status of broiler chicken against NDV was enhanced in both treatment group. Abdominal fat deposit was significantly reduced without affecting carcass quality, however, maximum profit obtained in MOALE group followed by ascorbic acid supplemented birds.

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(Rakesh Kumar)

Chapter-I

Introduction

In present scenario allied agricultural farming is popularized with fast pace of development in developed and developing countries both and in India 67% of the population is dependent upon agriculture and its allied sector for their livelihood security. As we are aware that the Poultry farming in India is a fastest growing sector and provide employment to larger section of population. However, poultry production has a great potential to mitigate the challenges of nutritional security, poverty alleviation, women empowerment, employment generation, improvement in living standard, etc. Poultry population in India is 729.2 million numbers (12.39% increase) and Bihar having 12.75 million, rank 15th and state wise percentage share of poultry population of 1.75 percent (Livestock Census, 2012). Feeding management is one of the most important factor in poultry rearing which may cost up to 60-70% of the total cost of broiler production. Due to rising human population, availability of conventional ingredients for feeding livestock is decreasing day by day and rising the feed prices so, considerable attention has been made on the search towards non-conventional feed resources to take-up the future scarcity.

The leaves of Moringa species having high crude protein with higher nutritive value in terms of energy content and organic matter digestibility (Aberra et al., 2011). Presence of vital nutrients Moringa leaves referred that it could be utilized for improving growth performance and health status of poultry and the leaves of Moringa plant could be used as potential feed supplements for ruminants, non-ruminants and poultry feeding of India and other developing countries for sustainable production. Moringa tree is fast growing drought resistant, native to the Himalayas in northwest India and widely cultivated in tropical and subtropical areas. *Moringa oleifera* leaf is good source of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids and also possesses medicinal properties (Moyo et al., 2012; Teixeira et al., 2014). Moreover, due to induce prebiotic effects, anti-bacterial and immunomodulatory effect (Ghazalah and Ali, 2008) enhancement in productivity of broiler chickens recorded. The crude protein content of Moringa leaves is higher than most tropical forage legumes as reported by Babayemi (2007) and contains 13.2, 28.9, 6.73, 8.51, 42.6, 16.7, 12.1, 6.49, 5.59, 4.66, 16.8 as TA, CP, EE, CF, NFE, NDF, ADF, ADL, cellulose, hemi-cellulose and gross energy (MJ/kg DM) respectively (Aberra et al., 2011). However,

differences in agro-climatic conditions or varying ages of trees, soil conditions and stages of maturity, chemical composition of MOL may vary whereas, Yang et al. (2006) revealed that mature leaves contained more CP than young shoots.

The nutrient profile of Moringa leaf reflects that it has potential for alternative animal feed resources in tropical countries during scarcity. However, the suitability and digestibility of Moringa leaves in feeding ruminants, non-ruminants and poultry under India conditions is less documented. The dietary application of MOLM in the laying hens, significant increase in good quality trait found. Moreover, Kumar et al. (2017) reported that growth significant improvement in performance and feed conversion efficiency with MOLM supplemented birds as well as remarkable decreased in serum and meat cholesterol level noted. The antioxidant compounds like polyphenolics, Vitamin C, Vitamin E, β-carotene, minerals, flavonoids etc. in MOL reported to improve storage life and quality of meat products during pre-slaughter or post-slaughter stages (Valeria and Williams, 2011).

Production of free radicals (reactive oxygen species, ROS) played multiple important roles in tissue damage and one may affect the function of multiple tissues and organs (Zheng and Huang, 2001). Antioxidants reduces the oxidative damage of the tissues indirectly by enhancing natural defences of cell and/or directly by scavenging the reactive oxygen species. Several epidemiological studies shown that carotenoids, tocopherols, ascorbates and phenolics antioxidants could reduce the risk of life-threatening problems like cancers, cardiovascular diseases, neuro-degenerative diseases, aging, asthma and inflammatory diseases (Triantaphyllou et al., 2001). Therefore, in present problem more emphasis has been given to use of phytochemical source as natural antioxidant products. The beneficial effects of Vitamin C supplements given either in diets or drinking water enhanced the performance of broiler chickens, reduced stress related response and improve immunity of the birds however, practical relevance of such findings is yet to be cleared.

The highly reactive free radicals and oxygen species present in biological systems may oxidize nucleic acids, proteins and lipids which leads to initiating degenerative diseases (Hossain et al., 2008). Plant vegetation having high amount of several redox-active antioxidants such as polyphenols, carotenoids, tocopherols, glutathione, ascorbic acid and enzymes, which fight against hazardous oxidative damage of plant cells, however, in animal cells, antioxidant production is much more limited and oxidative damage involved in the pathogenesis of most chronic degenerative diseases and aging. Therefore, plant origin available antioxidants could be a potential factor to reduce risk of several diseases.

Ascorbic acid is a 6-carbon lactone which synthesized from glucose in poultry including many animals. Ascorbic acid is synthesized in the kidney in birds and reptiles, and in the liver in some mammals. Poultry having ability to synthesize ascorbic acid but under stress conditions such as high or low AT, RH, high productive rate and parasite infestation these ability is insufficient. However, ascorbic acid is the most important water-soluble antioxidant which protect biological membranes against lipid peroxidation by eliminating free radicals in the aqueous phase before peroxidation begins. Ascorbic acid cannot directly scavenge lipophilic radicals formed in membranes, but decreases the number of tocopheroxyl radicals bound with the membrane during the lipid aqueous phase transition (Ellinger and Stehle, 2009).

Moringa oleifera leaf extract increased Haem-agglutination Inhibition (HI) titres in the NDV vaccinated chickens (Younis et al., 2016). It is well proven that the absorption of nutrient through drinking water is faster and more than feed and addition of materials to water is easier than to feed however, limited study available in application of growth promoting substances through drinking water. Most of the work done on dietary MOLM through feed on different parameters in poultry but water supplementation of *Moringa oleifera* aqueous leaf extract (MOALE) is yet unknown. Immunomodulatory substances stimulate or regulates both innate and adaptive immune responses. Therefore, modulation of the immune system by various plant products has become a subject for scientific investigation (Sherwood and Toliver, 2004). The various doses of *Moringa oleifera* extract caused a significant increase in white blood cell counts and immunoglobulin levels which might be due to ginseng in Moringa which have antioxidant, anti-inflammatory, anti-apoptotic and immune-stimulant properties, so it hypothesised that Moringa plant and its extracts could play a part in immunomodulation. However, Al-Majali et al. (2017) indicated that Moringa leaf extract supplementation caused a significant increase in the body weight, weight and number of cells of spleen and lymph nodes of the treated mice whereas, the count of RBCs, WBCs, platelets, hemoglobin concentration and PCV % were increased by the extract treatment in a dose-dependent manner and enhancement of the proliferative responses of splenic lymphocytes reported for both T cell and B-cell mitogens. Oral administration of MOL extracts significantly increased PFCs/106 spleenocytes in a dose-dependent manner therefore, we can say that it has significant potential as an immunomodulatory agent. Moreover, Deshmukh et al. (2015) investigated to evaluate immunomodulatory property of hot aqueous and ethanolic extracts of *Moringa oleifera* in albino rats and revealed that it possesses immunomodulatory property.

Nfambi et al. (2015) found that the methanolic leaf extract of *M. oleifera* caused a significant immune stimulatory effect on both the cell mediated and humoral immune systems

in the Wistar albino rats whereas, similar studies showed immunomodulatory activity of MOLE in cyclophosphamide immunosuppressed rats of same species (Gaikward et al., 2011). Moreover, Faluyi and Agbede (2018) indicated that MOLE application moderately boost immunological responses to ND vaccinations, though increasing the dose did not enhance the suggested immunomodulatory activity and also improved productive performance in the broiler chickens. Paul et al. (2018) investigate the effects of aqueous MOLE supplementation in broiler chicken and suggested that the inclusion in the drinking water reduced feed intake and improved feed conversion efficiency and it can be considered as an alternate to synthetic antibiotics as growth promoter to fight the emergence of antibiotic resistance phenomena in poultry industry.

However, there is no systematic study has been done on *Moringa oleifera* aqueous leaf extract (MOALE) and ascorbic acid in chickens, which may help to take-up the future feeding strategies for healthy animal origin food production. Now a day's people are very conscious about the quality of product. Therefore, keeping in view of above facts the present experiment was formulated on comparative study of *Moringa oleifera* aqueous leaf extract and ascorbic acid supplementation on antioxidant status and immune response in broiler chicken during five weeks of age with following objectives given below;

Objectives:

1. To evaluate the total antioxidant capacity of *Moringa oleifera* aqueous leaf extract (MOALE).
2. To access the performance parameters of broiler chicken offered MOALE and Ascorbic acid.
3. To evaluate haemato-biochemical parameters, antioxidant status and immune response in broiler chicken on above supplementation.
4. To study the effect on carcass characteristics and production cost analysis.

Chapter-II

Review of Literature

Agriculture and allied sector plays a pivotal role in improving rural community income and livelihood and livestock is the main stake of farmer's community. Due to technological interventions, there is rapid expansion happens in the livestock sector and mainly in poultry industry. However, poultry farming is one of the fast growing subsectors of animal husbandry in our country. Poultry production and its produce plays important socio-economic roles and economic source of animal protein for major population in both developed and developing countries (Melesse et al., 2013). The contribution of these products are significant in maintaining the nutritional status of the human population in worldwide and Indian context also. Available feed resources play major role in expansion of the poultry sectors mainly depends on the sufficient availability of quality feed at affordable prices to both producer and consumers.

The aim of developing modern poultry production systems is to obtain maximum profit at minimum production cost, however, feed cost represents about 60-70 % of the production cost (Tesfaye et al., 2013). Now a day's poultry sectors are facing problems of increasing feed cost of commonly use protein source ingredients which affect the economy of production (Gadzirayi et. al., 2012; Abbas, 2013; Moreki and Gabanakgosi, 2014). Corn and soybean meal are the ingredients of choice for the energy and protein source in poultry ration. However, both ingredients are getting expensive due to higher consumption rates by human population and increased demand resulting from expansion in livestock industry and biofuel. This situation has to look forward for cheap, locally available and less competitive ingredient substitutes of poultry feed and in particular of protein sources (Gadzirayi et al., 2012). Thus, the high cost of cereals and protein supplements as well as uncertainty of their sustainable supply, present the need to search for other potential nonconventional feed sources, which are relatively less used for human consumption. In this respect, an alternative cost effective source of protein can be used in poultry nutrition are the leaves of tropical legumes such as *Moringa oleifera* (Melesse et. al., 2013; Tesfaye et al., 2013). *Moringa oleifera* is an excellent plant having high nutritional value and good biomass production, which can be used as a nutritional supplement (Sanchez et al., 2006).

Moringa oleifera belongs to the mono generic family Moringaceae, which includes another 12 species of shrubs and trees (Olson, 2002). *Moringa oleifera* plant is native to the Indian subcontinent and widely adopted in the tropical and subtropical areas around the globe. The leaves of *Moringa oleifera* are highly nutritious having good source of protein, β-carotene, vitamins A, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals, antioxidant and various phenolics compounds (Mbikay, 2012; Moyo et al., 2012; Jung, 2014) and less concentration in anti-nutritional factors (Makkar and Becker, 1997) thus it can play a great role in poultry nutrition. Leaf extracts exhibit the greatest antioxidant activity, and various safety studies in animals involving aqueous leaf extracts indicate a high degree of safety.

Antioxidants are known to be helpful agents that can combat the effect of stress and health. The most popular antioxidants are ascorbic acid as natural component of different plants. Khan et al. (2012) revealed that vitamin C supplementation to the diets of birds removed the oxidative injuries of chicks raised under stressed climatic conditions. Luqman et al. (2012) and William et al. (2014) found that Moringa leaves are a rich source of vitamins C, E and polyphenolic compounds and considered as important agents in combating the free radicals which also influence the performance of birds by affecting the nervous system and immune system (Habibian et al., 2014). Present study designed to evaluate the effects of supplementing MOALE as antioxidant agent with other antioxidants such as ascorbic acid in broiler diets.

One of the practical solutions to some of the problems of poultry production in the tropics is to attain attention related with nutrient requirements of broiler chicken for better and healthy production. Healthy and economic poultry production is the most logical step for solving the shortage and supply of quality material towards utilizing plants by-products and wastes for feeding poultry birds.

2.1 NUTRITIONAL VALUE AND HARMFUL COMPOUNDS IN *MORINGA OLEIFERA*

The nutritional compositions are strongly emphasized in different literature for various trees and shrubs which presents their importance for livestock (D'Mello and Devendra, 1995) and recommendation is based on their proximate principles including crude protein, crude fibre, ash, mineral contents, etc. The high protein content is one of the most significant advantages of moringa leaves. The different report (Stelwagen, 2003; Chandan, 2006) revealed that cow, buffalo, goat, and sheep milk provide average CP contents of 3.4%, 4.7%, 4.1% and

6.3%, respectively, while fresh and dry moringa leaves exhibit CP contents of 67.0 and 271.0 g kg^{-1}, respectively. Likewise, Soliva et al. (2005) and Ferreira et al. (2008) reported 332.5 g kg^{-1}. So, these comparisons reflect as moringa leaves contain higher amounts of CP in comparison with milk. Mendieta-Araica et al. (2011) reported 292 g kg^{-1} CP contents in moringa leaves, while different mixtures composed by different parts of moringa plants have different nutritional value. Similarly, NDF and ADF contents recorded in lower concentration in moringa fodder leaves, which show better fodder quality.

Moringa leaves are rich source of all amino acids that required for children, according to FAO reference protein levels (Makkar and Becker, 1996). Moreover, moringa leaves are also a good source of oxalic acid contents (11.2 mg g^{-1}), which are not harmful to the immune system (Makkar and Becker, 1997). Arginine, valine and leucine contents were found to be higher in dry moringa leaves and fresh pods, while serine, glutamate, aspartate, proline, glycine and alanine could not be detected in these moringa parts (Freiberger et al., 1998; Fuglie, 2000). The total carotenoids concentration is 40,139 µg 100 g^{-1} of fresh moringa leaves, out of which 47.8% (19,210 g kg^{-1}) corresponded to β-carotene (Seshadri and Nambiar, 2003). Moringa leaves contain 25 times more iron than spinach (Mathur, 2006) as Spinach leaves iron absorption is very limited, while in moringa leaves, the absorption level is better than in other leafy vegetables. Methionine and cysteine contents in raw moringa leaves and extracted moringa leaves are 14.14 and 8.36 mg g^{-1} of DMI, respectively, whereas, non-fat dry milk and dry whole milk contain 12.41 and 9.03 mg g^{-1} (methionine + cysteine), respectively (Ferreira et al., 2008). Moringa leaves contain iron, calcium, phosphorous, zinc as 379.83, 18,747.14, 1121, 22.05 mg kg^{-1}, respectively on dry matter basis (Nouman et al., 2012a).

Moringa oleifera plant regarded as a “miracle tree” being used in the treatment of numerous diseases (Matthew et al., 2001) including heart disease and obesity due to its hypocholesterolemic property (Olugbemiet al., 2010). As reported, *Moringa oleifera* leaves have the calcium equivalent of 4 glasses of milk, 3 times the iron of spinach, 4 times the amount of vitamin A in carrot and 2 times protein in milk (Loren, 2007). The extract of *Moringa oleifera* may contain antibiotic metabolites, such as carboxylic acid, 2,4-diacetyl phloroglucinol, and cell wall-degrading enzymes and chitinases (Jabeen et al., 2008). Chumark et al. (2008) specified that *Moringa oleifera* leaves displayed anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities. Sreelatha and Padma (2009) found that phenolic and flavonoid compound present in moringa leaf, affect lipid oxidation potential and fatty acid composition and antioxidant activity is also due to higher amount of polyphenols present in leaf. Verma et al. (2009) reported that properties like anti-inflammatory,

hepato protective and antioxidant were linked to the presence of carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavanoids and phenolics. *Moringa oleifera* leaves several nutritionally important minerals namely magnesium, copper, vanadium, chlorine, aluminum, manganese, calcium, sodium, potassium and devoid of potential toxic elements such as mercury, cadmium, and arsenic (Donkor et al., 2013). El-Hack et al. (2018) reported that *Moringa oleifera* characterized as a potentially useful animal feed owing to its high content of protein, carotenoids, several minerals and vitamins (such as iron and ascorbic acid) and certain phytochemicals (kaempferitrin, isoquercitrin, rhamnetin, kaempferol and quercetin).

Anti-nutritional factors are defined as substances generated in natural food items by the normal metabolism of species and by different mechanisms exert an effect opposite to optimum nutrition (Kumar, 1992). Although, moringa leaves have saponins, which gives a bitter taste to livestock, but these do not always have harmful effects on animals or human beings (Makkar and Becker, 1997). The extracts of moringa leaves contains saponins ranging between 4.7 and 5 $gkg^{-1}$of DM, so they can be consumed by livestock and human beings without any adverse effects (Makkar and Becker, 1997; Foidl et al., 2001). Moringa leaves have higher amount of available calcium and insoluble oxalates, which are not harmful for human beings or animals (Noonan and Savage, 1999; Radek and Savage, 2008) so, moringa leaves can be eaten as a richer calcium source, especially by mothers and children, without the fear of kidney stone formation. Many fodder trees are not selected as the first option for livestock feeding due to the presence of harmful compounds in leaves and other palatable parts. Moringa leaves are lack in lectins, trypsin and amylase inhibitors (Ferreira et al., 2008). A few derivatives of glucosinolates, like thiocarbamates, isothiocyanates and carbamates by the action of myrosinase reported in moringa leaves, but their concentration is very low as compared with other phytochemicals and somehow cannot be found in moringa tissues (Newton et al., 2010). There is no study available on the presence of anti-vitamin agents or activities in moringa leaves (Nuhu, 2010; Olugbemi et al., 2010) so, due to negligible anti-nutritional factors, moringa leaves are palatable for human beings, livestock and poultry.

2.2 MODE OF ACTION OF *MORINGA OLEIFERA* LEAF EXTRACT

Antimicrobial and antioxidant effects of *Moringa oleifera* were discussed by some researchers and suggested that extracts of *Moringa oleifera* may contain antibiotic metabolites, such as carboxylic acid, 2,4-diacetylphloroglucinol, and cell wall-degrading enzymes and chitinases. However, antioxidant effect of *Moringa oleifera* leaf extract and fruit was explained

by Luqman et al. (2012) and noticed that due to the presence of polyphenols, tannins, anthocyanin, glycosides and thiocarbamates, which remove free radicals, activate antioxidant enzymes and inhibit oxidases.

2.3 EFFECT OF *MORINGA OLEIFERA* AQUOUS LEAF EXTRACT AND ASCORBIC ACID ON PRODUCTIVE PERFORMANCE OF BROILER CHICKEN

Vathana et al. (2002) studied on water supplementation of Vitamin C on the productivity of broiler chickens and found that 40mg/bird/day in drinking water reduces the impact of heat stress significantly and improves the productivity of broilers under the tropical conditions.

Olugbemi et al. (2010) reported that the inclusion of MOLM beyond 5% in feed there were slight reduction in performance of broiler, final weight, FCR and ADG on the other hand were not significantly affected. Melesse et al. (2011) studied on the effects of feeding *Moringa stenopetala* leaf meal on nutrient intake and growth performance of Rhode Island Red chicks under tropical climate and reported that significant increase weight gain, feed efficiency ratio and protein efficiency ratio as compared to control diet. Authors co-related these findings to the presence of higher levels of methionine and other essential amino acids when compared to the soybean meal of a control diet which is convenient for mono-gastric animals. He also suggested that inclusion of *Moringa stenopetala* leaf meal up to 6% in the diet of growing chicks to replace expensive conventional protein sources has no negative effects on the chicks.

Moreover, Banjo (2012) revealed that inclusion of *Moringa oleifera* leaf meal enhanced the weight gain of the birds and also concluded that broilers can tolerate *Moringa oleifera* leaf meal up to 3% without adverse effect on their growth however, feed conversion ratio was significantly improved in all the treatments of the broiler birds. Ebenebe et al. (2012) studied and found that chicks fed on moringa based diets performed significantly ($P<0.05$) better than the birds of control group in term of higher weight gain and better feed conversion ratio and such improvement may be attributed to rich content of nutrients in MOLM and anti-microbial properties of moringa.

Paguia et al. (2014) studied on the utilization and evaluation of *Moringa oleifera* leaf meal as poultry feeds and found that the addition of moringa leaf powder on broiler diets did not significantly influence the broiler's feed intake, ADG, feed conversion ratio (FCR), final weight, feed cost per kg of broiler produced and income over feed and chick cost. However, Etalem et al. (2013) worked on the *Moringa oleifera* leaf meal as an alternative protein feed ingredient in broiler ration and they found that it can be substituted to SBM in broilers diet up

to a level of 5% inclusion in the total ration without negative effect on biological performance of birds. Tesfaye et al. (2013) worked on MOLM as an alternative protein feed ingredient in broiler ration and found that there was significantly increase in feed intake, weight gain, FCR with supplemented groups as compared to the control group. However, Akhouri et al. (2013) worked on *Moringa oleifera* leaf extract and reported as better feed utilization, feed conversion efficiency and body weight gain in the broiler chicks.

Akhouri et al. (2014) found that the supplementation of aqueous extract and dried powder of *Moringa oleifera* leaf enhances feed conversion ratio, feed conversion efficiency and body weight gain in vaccinated broiler chicken. Nkukwana et al. (2014) studied on supplementation of *Moringa oleifera* leaf meal as an additive in broiler chicken and found that its supplementation had no adverse effect on broiler performance. Safa (2014) revealed that birds fed on MOLM gained significantly ($P<0.05$) higher weight and superior feed conversion ratio than the birds fed on control diet. However, Divya et al. (2014) found that the addition of MOL powder at any level slightly non-significant decrease in BW and feed intake on 21 and 42d of age as compared to control and finding of result suggested that MOL powder could be a potential growth promoter for broiler. Imad khan et al. (2015) observed that the addition of *Moringa oleifera* leaf meal in the diet of the broilers chicken significantly ($p<0.05$) enhanced their weight gain as compared to the control group**.**

The effect water supplementation of *Moringa oleifera* aqueous extract on performance, carcass characteristics, immune response and blood antioxidant level were rarely studied. Study showed that *Moringa oleifera* treated Ross breed and Cobb breed chicken recorded significantly better performance as compared to control group (Younis and Elbestawy, 2017). Alabi et al. (2017) investigate the effect of aqueous *Moringa oleifera* leaf extracts (AMOLE) on growth performance and carcass characteristics of broiler chicken and found that inclusion of 90 ml/litre AMOLE in drinking water of broiler chicken can reduced feed intake (12.83 %) and improved feed conversion efficiency (9.11 %). Agashe et al. (2017) study on dietary inclusion of *Moringa oleifera* leaf powder in the diet of broiler bird and results showed that the improvement in live body weight, slightly reduced feed intake and better feed conversion ratio was found in comparison to control. Paul et al. (2018) revealed that the aqueous extract of *Moringa olefiera* leaf inclusion in drinking water of broiler chicken reduced feed intake and improved feed conversion efficiency and it can be considered as an alternate to synthetic antibiotics as growth promoter to fight the emergence of antibiotic resistance phenomena in poultry industry.

2.4 EFFECT OF *MORINGA OLEIFERA* AQUOUS LEAF EXTRACT AND ASCORBIC ACID ON BLOOD BIOCHEMICAL PROFILE OF BROILER CHICKEN

Olugbemi et al. (2010) stated that erythrocytes are responsible for the transportation of oxygen and carbon dioxide in the blood as well as manufacture of haemoglobin hence higher values indicate a greater potential for this function and a better state of health of broiler chickens. However, Zanu et al. (2012) worked on application of *Moringa oleifera* leaf meal as a partial substitute of fish meal in broiler chicken diets and they found that triglycerides, VLDL and LDL values in blood serum of broilers were significantly different in treatment groups ($P<0.05$), whereas, total cholesterol, HDL-cholesterol, total protein and glucose values were not significantly ($P<0.05$) affected. There was negative relationship observed between cholesterol and triglyceride values. The triglycerides and VLDL values of the groups fed with 5% MLM were the lowest and total cholesterol of control group found highest. Moreover, the mean corpuscular haemoglobin, all the other haematological indices were unaffected, which indicates the diets were nutritionally adequate to meet the nutrient needs of the birds. However, Ogbe and John (2012) worked on proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves and he observed low level of cholesterol and triglyceride in serum as well as enhances the mineral level of product.

Aderinola et al. (2013) observed that the values for PCV was significantly higher for the diet containing 0% MOLM and inversely proportional to MOLM concentration in diet and also found that treatment group having highest percentage *Moringa oleifera* have highest value of WBC. However, all the serum indices were found to be highest in those group, who fed maximum level of *Moringa oleifera*, except total cholesterol and triglyceride whereas, SGPT and SGOT values were not found to be significantly different among the groups. Donkor et al. (2013) worked on nutritional value of the leaves of *Moringa oleifera* on poultry and they found that MOL lowered serum triglyceride level and increased serum calcium, sodium, potassium, albumen and chloride and latter justified by the levels of these and other minerals detected in MOL powder.

A study was conducted by Divya et al. (2014) on dietary supplementation of *Moringa oleifera* leaves powder on growth performance, blood chemistry, meat quality and gut microflora of broiler chicks and expressed that significant ($P<0.05$) decrease in total protein, triglycerides, cholesterol, albumin and uric acid which might be due to higher fibre content in MOLP responsible for less absorption of triglycerides and cholesterol from the intestinal tract of the birds. They also found that significant reduction in creatinine level in serum with increasing level of MOPL concentration in diet. Decreasing creatinine level indicates retarded

catabolism rate in broilers and is perhaps reason for non-significant reduction in body weight of birds whereas, uric acid and liver enzymes (ALT and AST) in moringa fed bird were significantly lower than that of control.

Mahmood et al. (2015) worked on water supplementation of extract of *Azadirachta indica* @ 4% and *Moringa oleifera* @ 6% in birds and found significant ($P<0.05$) decrease in blood glucose and cholesterol whereas, Red blood cell count significantly ($P<0.05$) increased in treatment group and haemoglobin, white blood cell and packed cell volume remained unaffected due to the addition of these herbal plants leaf extracts. Tijani et al. (2015) studied on dietary inclusion of MOLM on blood biochemical profile and they reported that haemoglobin values were unchanged among the groups up to 5-15% MOLM based diets, but reduced significantly in birds fed 20% MOLM and white blood cell count observed lowest in 20% MOLM. However, significant reductions in albumin, total protein, uric acid, aspartate amino transferase and alanine amino transferase in birds fed 20% MOLM whereas, creatinine content was significantly ($P<0.05$) higher in birds fed 20% MOLM based diet. So, authors concluded that MOLM can be incorporated into broiler diets at 15% level without adverse effects on the haematological and serum biochemical indices of the birds.

Nihad et al. (2016) studied on effect of Moringa leaf meal supplementation on broiler production and health and they found that more improvement and effective treatment were 20% of MOL of blood biochemical, lipid profile (triglycerides, total cholesterol, HDL, LDL and VLDL) and haematological parameters (Hb, RBC and WBC), comparing with normal diets so, it is recommended to add *Moringa oleifera* at 15% and 20% in broiler diets to improve performance and health. Allam et al. (2016) worked on effect of *Moringa oleifera* leaf extract in broiler chickens on hemato-biochemical, bacteriological and pathological parameters and they found that Moringa leaf extracts (watery & alcoholic) induced significant increase in body weight gain, RBCs, Hb, PCV, WBCs, total proteins, albumin, globulins, SOD beside significant decrease in MDA and non-significant increase in AST, ALT, ALP but urea and creatinine non-significantly decreased and improved in feed conversion rate.

Sigolo et al. (2018) studied on to evaluate growth performance and blood serum parameters of Japanese quails fed diets containing different supra-nutritional levels of vitamin E and C and they found that lowest serum concentrations of uric acid and creatinine ($P \leq 0.01$) and high HDL ($P = 0.01$) and low LDL, triglycerides, aspartate amino transferase, alanine amino transferase and albumin, whereas increased total protein, calcium, phosphorous, thyroid stimulating hormone, red blood cells, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration.

2.5 EFFECT OF *MORINGA OLEIFERA* AQUOUS LEAF EXTRACT AND ASCORBIC ACID ON ANTIOXIDANT ACTIVITY AND IMMUNE STATUS IN BROILER CHICKEN

Moyo et al. (2012) investigated the antioxidant potency of *Moringa oleifera* leaves in different in vitro systems using standard phytochemical methods and MOL extract depicted higher percentage inhibition against DPPH, ABTS and nitric oxide radicals which were comparable with reference standard antioxidants (vitamin C and BHT). However, MOLE increased the antioxidant activity of GSH (186%), SOD (97.8%) and catalase (0.177%) whereas, lipid peroxidation was significantly reduced. Kulkarni et al. (2012) worked on dietary supplementation of ascorbic acid in broiler chicken and reported that higher cellular and humoral immunity recorded in AA supplemented birds in comparison with control whereas, serum lipid peroxidation was comparable but higher reduced glutathione and significantly down regulated expression of HSP70 gene was noted. Elagib and Omer (2012) studied on the effect of supplementation of ascorbic acid in broiler chicks and reported that antibody titre against Newcastle virus was increased in birds supplemented with different levels of ascorbic acid.

Elangovan et al. (2014) reported that *Moringa oleifera* leaf possesses a good antioxidant, it has a scavenging property against ROS and also it has good antibacterial properties so, it can be used to synthesize a new drug preparation against various diseases responsible for severe illness. Charoensin (2014) reported that *M. oleifera* leaves possess antioxidant activity, cytotoxic and chemo preventive properties which might be beneficial for alternative novel anticancer drugs and nutraceutical products.

Karthivashan et al. (2015) worked on the effect of *Moringa oleifera* leaf extract supplementation on the growth performance and antioxidant status in broiler birds and found that high expression of isoflavones and fatty acids from soy and corn source, which antagonistically inhibit the expression of the flavonoids/phenols in the MOLE thereby masking its antioxidant effects. AbouSekken (2015) investigate the effect of adding graded levels of *Moringa oleifera* leaves meal or its extract on productive performance, anti-oxidative potentials and immune response in broilers and found that aqueous leaf extract given via drinking water appeared to be a good feed additive in order to obtain the best growth, improve the immune response and cause better disease resistance and feed utilization as well as the overall better health of broiler. Hajati et al. (2015) reported that HSP70 gene expression in heart and liver of broilers reduced by vitamin C supplementation during chronic heat stress condition.

Alhusnan et al. (2016) studied on aqueous extract of Moringa leaf population which showed antimicrobial activity against tested bacteria, fungi and yeasts at different concentrations. Allam et al. (2016) studied on Moringa leaf extracts and found significantly enhanced SOD value beside significant decrease in MDA and insignificant increase in AST, ALT, ALP but urea and creatinine level decreased and improved in feed conversion rate. They also reported that Moringa leaf extract have beneficial effect on immunity and hemato-biochemical parameters. Liaqat et al. (2016) studied on the effect of *Moringa oleifera* leaf powder on growth performance, blood haematology, and immune response in broilers and suggested that MOLP as a vegetable protein source can enhance the immune response to Newcastle disease and infectious bursal disease vaccination without any change in performance parameters of broiler birds. Tamzil et al. (2016) studied on expression of HSP70 gene by ascorbic acid supplementation in broiler chickens exposed to transportation stress and found that supplementation of ascorbic acid for 2 hr before the broilers were transported and for 2 hr immediately after they were transported decreased HSP 70 gene expression. Bhatti et al. (2016) reported that supplementation of vitamin C and E combination in drinking water at the time of vaccination against NDV improve humoral immune response against NDV. Erian et al. (2016) worked on phytochemical activity of *Moringa oleifera* leaf extract and found that methanolic extract produced (LPO, OH, DPPH and ABTS) highest antioxidants activity and highest growth inhibition (20 and 17mm) for against *Escherichia coli* and *Bacillus subtillis* at 4mg/ml, respectively while, the aqueous extracts highest growth inhibition (13mm) of against *S. aureus* at 4mg/ml.

The total Lactobacillus count and immunity against Newcastle disease virus vaccine as compared to control groups. However, no significant differences between treatments in carcass characteristics and blood total antioxidant capacity (Younis and Elbestawy, 2017). Khan et al. (2017) reported after conducting *In-vitro* trials and found that aqueous extract remarkably inhibited the activity of a-amylase and a-glycosidase and it displayed improved antioxidant capacity. Falowo et al. (2017) examined the leaf extracts of *Moringa oleifera* for presence of bioactive phytochemicals and their antioxidant activities on pH and lipid oxidation of fresh ground beef and found that lower pH and thiobarbituric acid-reactive substances values compared with control and BHT treatments (0.2 g/kg) during the storage period and suggested that the antioxidant activities of the extracts indicate *M. oleifera* leaf can be used as nutraceuticals or preservative agents in food industry. Musa et al. (2017) stated that phytonutrients are reliable and effective source concern to public health on antibiotic resistance and adverse effects of synthetic growth promoters.

Ramadan et al. (2017) worked on supplementation of MOLM on broiler performance and immune response and found that birds supplemented with MOLM had significant higher antibody titre against ND compared to un- supplemented birds which results in improved performance and immune response against ND. Kumar et al. (2017) conducted an experiment to determine the effect of betaine and ascorbic acid on ducks exposed to heat stress during the grower phase and reported that betaine or betaine plus ascorbic acid significantly reduced gene expression of heat shock protein HSP70 in the liver in comparison to control group. Naresh et al. (2017) conducted an experiment to evaluate comparative effects of supplementation of poly herbal formulation and synthetic ascorbic acid on the performance of environmental heat stressed broilers birds and noticed that antibody titre was significantly higher in the treated groups as compared to untreated control. Mishra et al. (2017) studied on to explore and quantify the relative mRNA expression of HSP70 gene in relation to ascorbic acid supplementation in White Leghorn egg type growers exposed to heat stress and reported that expression patterns of HSP70 gene provide an indication that AA may be useful in combating rigors of heat stress in chickens.

Arafat et al. (2018) worked on *Moringa oleifera* leaves extract (1% and 0.5%) supplementation against gentamicin induced toxicity in chicken and found that reversed the severity of gentamicin drawbacks, nephrotoxicity and hepatotoxicity by normalized the altered serum levels of liver and kidney biomarkers and reduced tissue nitric oxide and oxidative stress in chicken. Cui et al. (2018) reported that plasma total anti-oxidative capacity, total superoxide dismutase, glutathione peroxidase activities increased quadratically ($P < 0.01$), whereas, MDA decreased quadratically ($P < 0.001$), in response to dietary MOL supplementation. Faluyi et al. (2018) worked on *Moringa oleifera* leaf extracts and found that it served to moderately boost immunological responses to ND vaccinations, however, increasing the dose did not enhance the suggested immunomodulatory activity. Paul et al. (2018) worked on aqueous leaf extract of Moninga and suggested that inclusion of MOALE in the drinking water of broiler chicken can be considered as an alternate to synthetic antibiotics as growth promoter to fight the emergence of antibiotic resistance phenomena in poultry industry.

Faluyi and Agbede (2018) investigate the immuno-modulatory activity of aqueous leaf extract of *Moringa oleifera* on immune response of broiler chickens to Newcastle disease vaccinations and reported that the plant extract had slight immune stimulatory effects on response to ND vaccinations and improved the growth performance of broiler chickens. Gan et al. (2018) studied on the effects of dietary inclusion of ascorbic acid on old hen performance, immunity and its antioxidant status and found that improvement in the health of old layers by

enhancing immunity and antioxidant capacity noted. Tekayev et al. (2018) reported that aqueous extract of *Moringa oleifera* reduces the oxidative stress in a unilateral cryptorchidism induced rats, and HSP expression and germ cell apoptosis.

Mansour et al. (2019) studied and revealed that Moringa possess various medicinal properties including hypoglycemic, analgesic, anti-inflammatory, hypolipidemic, and antioxidant activities as well as antimicrobial and anticancer activity. Ramadan et al. (2019) studied on the effects of dietary supplementation of different levels of vitamin C in broiler chicken on the growth performance, blood biochemical parameters and the expression of heat shock protein genes and found that improved final body weight and total feed intake, decreased the mortality % and down regulated liver HSP70 expression level.

2.6 EFFECT OF *MORINGA OLEIFERA* AQUOUS LEAF EXTRACT AND ASCORBIC ACID ON CARCASS CHARATERISTICS OF BROILER CHICKEN

Ayssiwede et al. (2011) reported that no any adverse effect on carcass cuts due to inclusion of MOLM up to 24% in the diet of and produced yellow coloration of the skin and abdominal fat of growing indigenous chickens. Moyo et al. (2011) worked on polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver in goat and reported that antioxidant compounds assist in the prevention of meat degradation by oxidation. Zanu et al. (2012) worked on the possibilities of using *Moringa oleifera* leaf meal as a partial substitute for fishmeal in broiler chicken diets and he found that meat quality and composition (moisture, crude protein and fat) were significantly ($P<0.05$) affected by the dietary treatments. However, Gadzirayi et al. (2012) reported that there was significant difference on carcass yield between the different treatments of birds fed on different inclusion level of MOLM and also carcass parts, liver, head, neck, wing, back, thighs, shanks, breast, gizzard and lung, were weighed and exposed to significant tests.

Aderinola et al. (2013) worked on the utilization of *Moringa oleifera* leaf as feed supplement in broiler diets and they observed that cholesterol levels and abdominal fat reduced with increased MOL inclusion in the diets of broiler birds. However, Etalem et al. (2013) reported that the slaughter weight, dressed weight, eviscerated weight, breast weight, thigh weight, drumstick weight and giblet weight were lower ($P<0.05$) in birds that received dietary MOLM than those in control. Madukwe et al. (2013) found that birds placed on diets supplemented with *Moringa oleifera* aqueous extract exhibit a less acceptable coloration despite the high beta-carotene and vitamin C (6.26mg/100ml extract) content of the leaf.

Safa (2014) reported that the inclusion of MOLM in broiler diets significantly (P<0.05) improved hot and cold eviscerated carcass weight, dressing percentage, tenderness and juiciness scores for both breast and thigh meat. However, Ologhobo et al. (2014) found that higher slaughter weight, dressed weight were recorded for birds fed diets containing *Moringa oleifera* leaf meal as compared to those fed on the control diet. Onunkwo and George (2015) studied to evaluate the effects of *Moringa oleifera* leaf meal on growth performance and carcass characteristics of boiler chicks and found that significant difference (P<0.05) in organ weights (wings, shank, drumsticks, kidney, liver, gizzard) and cut parts between the experimental and control groups and suggest that *Moringa oleifera* leaf meal can replace protein source (soya bean and groundnut cake) up to 10% in broiler diets without any adverse effects on growth and carcass qualities which could marginally reduce feed cost in broiler production.

Voemesse et al. (2016) observed that similarity between feed intake, liver relative weight while significant differences (p<0.05) between treated groups and the control one were observed on body weight, daily weight gain, feed conversion ratio and gizzard relative weight, however, total protein, albumin, calcium, magnesium and iron levels were significantly increased (p<0.05) in chickens fed MOLM as compared to control. Younis and Elbestawy (2017) studied on the effect of water Supplementation of *Moringa oleifera* on performance, blood antioxidant and immune response of broiler and found that there were no significant differences occurred between treatments in carcass characteristics and blood total antioxidant capacity.

Cui et al. (2018) studied on dietary supplementation of *Moringa oleifera* leaf on performance, meat quality and oxidative stability of meat in broiler chicken and they found that increased feed conversion ratio and decreased abdominal fat linearly in response to the supplementation of MOL in diets. Kumar et al. (2018) reported that carcass characteristics were significantly (P<0.05) affected in diet containing MOLM, however, sensory evaluation parameters like appearance, flavor, tenderness, juiciness and palatability were highly significant (P<0.01) with increasing level of MOLM which inferred that inclusion of MOLM will improve meat quality.

2.7 EFFECT OF *MORINGA OLEIFERA* LEAF MEAL AND ASCORBIC ACID ON PRODUCTION ECONOMY OF BROILER CHICKEN

Onibi et al. (2008) study the partial replacement of soya bean meal with MOLM in the diets of broiler finisher found reduction in the cost of feed consumed at higher inclusion of leaf

meals. However, with increasing the level of MOLM in the diets net income from birds reduced. Ayssiwede et al. (2011) reported that the inclusion of MOLM in diets of growing Indigenous Senegal chickens that the lowest feed cost/kg carcass was achieved when 8% and 16% of MOLM was introduced into the diets of the birds.

Zanu et al. (2012) noticed that partial replacement of fish meal with MOLM decreased the feed cost and also decreased the net revenue for broilers due to reduction in weight gain however, economic analysis indicated that the cost of feed reduced with increasing levels of MOLM in the diet. Talha (2013) reported that levels of inclusion of *Moringa* leaf meal can be expected to be cost effective at 10% to replace fish meal in broiler diet, and 8% and 16% introduction in the diet of indigenous chicken.

Makanjuola et al. (2014) reported that the inclusion of *Moringa oleifera* leaf meal in broiler chickens lower the production cost per kilo gram weight gain as compared to control diet. Karthivashan et al. (2015) reported that the supplementation of *Moringa oleifera* leaf extract would provide an efficient and cost-effective feed supplement for broiler production by altering the soy and corn gradients in conventional nutrition feed.

Kumar et al. (2018) reported that the supplementation of 5% followed by 10% *Moringa oleifera* leaf meal in birds shown significant improvement in overall performance achieving maximum profit and healthy meat production for human consumption. Tesfaye et al. (2018) assess the feeding value of *Moringa oleifera* leaf meal (MOLM) in layer ration and suggested that 5% inclusion of MOLM as an additive in the poultry industry may serve the sector by enhancing the product quality besides serving as protein feed. Sigolo et al. (2019) observed that offering vitamin E and C at different levels can be a good management practice in Japanese quail nutrition to promote growth performance and egg production traits under thermo neutral condition.

Chapter-III

Materials and Methods

The present study was intended to investigate the supplementation of *Moringa oleifera* aqueous leaf extract (MOALE) and ascorbic acid on growth performance, antioxidant status, immune response, carcass traits, blood biochemical profile and economics of production on broiler chicken for a period of five weeks at Poultry Nutrition Research Unit, Bihar Veterinary College, Bihar Animal Sciences University Patna, India.

3.1 EVALUATION OF TOTAL ANTIOXIDANT CAPACITY OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT

3.1.1 Preparation of *Moringa oleifera* aqueous leaf extract (MOALE)

Fresh green *Moringa oleifera* leaves were harvested from Bihar Veterinary College Campus, Patna, India. Stems were cut from the mature moringa trees over twelve months old. All plucked twigs were spread out on a floor and allowed to dry for a period of 3-5 days under shady and aerated conditions. Thereafter, branches were jerked carefully to separate leaves from twigs before grinding. The separated leaves were dried in hot air oven at 45±2º C for proper grinding. The dried leaves were milled to make in powder form. The leaf powder was stored air tightly in the nylon bags for further analysis.

After that took 2 litre capacity conical flask and added 1 litre distilled water. Then, took 60 gm MOL powder and kept in a shaker machine for 24 hrs for homogeneously mixing. After 24 hrs took it out and filtered in properly washed glass bottle having cap, using muslin cloth. All debris were discarded and final volume of filtrate was 450 ml and stored the filtrate at 4º C for further analysis.

3.1.2 Measurement of total antioxidant activity

The DPPH free radical scavenging activity in *Moringa oleifera* aqueous leaf extracts and ascorbic acid was determined as per method describe by Ahmed et al. (2015). The stock solution of the radical, prepared by dissolving 24 mg DPPH in 100 mL methanol and kept in a refrigerator till further use. The working solution of the radical was prepared by five tomes diluting the DPPH stock solution with methanol. Different dilution of MOALE (30 µl/ml, 60

µl/ml, 90 µl/ml) and ascorbic acid (5µg/ml, 10 µg/ml, 15 µg/ml) was prepared for estimation of DPPH free radical scavenging activity.

Further, 3 mL DPPH working solution was mixed with 100 µl of different dilution of MOALE and ascorbic acid and incubated for 30 minutes at room temperature. The absorbance of each tube was measured at 517 nm. The percent antioxidant or radical scavenging activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(Ac - As)/Ac] \times 100$$

Where, Ac and As are the absorbance of control (ascorbic acid) and sample (MOAL extract), respectively. The control contained 100 µL methanol in place of the plant sample or ascorbic acid.

3.2 EFFECT OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON PERFORMANCE PARAMETERS OF BROILER CHICKEN

3.2.1 Experimental techniques and period

The study was planned to see the comparative effect of water supplementation of *Moringa oleifera* aqueous leaf extract (MOALE) and ascorbic acid as an antioxidant sources in broiler chicken. One hundred thirty-five day-old broiler chicks were procured from local market reliable supplier during winter season and maximum temperature was approximately around 28˚C. The experimental birds were weighed and randomly divided into three experimental groups including control of 45 chicks in each group and further replicated with 15 chicks each as replicate and offered fresh water and crushed maize on first day and then given standard ration as per schedule. Chicks were reared on electrically heated brooder in early age under different treatment groups. All chicks were vaccinated against Ranikhet and Gumboro diseases following standard poultry vaccination protocol. The experiment was conducted for the period of 35 days. All the standard managemental practices were followed during experimental period

3.2.2 Housing

Deep litter system was adopted for rearing of broiler chicken. Properly dried saw dust was used as bedding material of 3-4 inch thick. By-weekly racking of deep litter was performed to prevent any cake formation in rearing pens. All chicks were served fresh clean drinking water *ad libitum* in properly disinfected drinker. Broiler chicks were reared under uniform condition of housing including brooding, feeding, watering, lighting and other

managements. During early periods of growth chicks were provided with artificial light during night, using 100-watt electric bulb and natural during the day.

3.2.3 Hygienic measures

All utensils, feeding and watering troughs were cleaned and disinfected. Fresh water bath with phenol solution, which was changed every morning, was maintained at the entrance of the experiment room throughout the experimental period as one of the hygienic measures.

3.2.4 Dietary treatment

All 135 broiler chicken were divided into 3 different treatment groups. Birds in T_1 group which served as control were fed only as basal ration. Birds in T_2 group were fed basal ration with MOALE (90 ml/L drinking water) and T_3 group were offered basal ration with ascorbic acid (15 mg/L drinking water).

Treatment group		Ration
T_1	:	Basal ration
T_2	:	Basal ration + MOALE (90 ml/L drinking water)
T_3	:	Basal ration + Ascorbic acid (15 mg/L drinking water)

3.2.5 Feed formulation

All the feed ingredients were procured in one lot before the start of experiment. The procured ingredients were analyzed for proximate principles (AOAC, 1995) as well as calcium and phosphorus using the method modified by Talapatra et al. (1940) and are presented in table-3.1. Based on the analyzed value of crude protein and standard published value of metabolizable energy of five experimental rations were formulated accordingly. The above formulated rations were again analyzed for their proximate principles in department laboratory. The composition of experimental ration and analytical values were presented in table 3.1 and 3.2.

3.2.6 Proximate analysis

The AOAC (1995) methods of analysis were followed for estimation of proximate composition, as follows:

3.2.6.1 Dry matter

Representative samples were weighed in moisture cups and kept in a hot air oven at 100±2°C until constant weight. Dried samples were cooled in desiccators, weighed, and DM was calculated as follows:

$$DM\ (\%) = (a/b)\ X\ 100$$

Where,

a = dry weight of sample

b = fresh weight of sample

3.2.6.2 Crude protein

Crude protein content of the sample was determined by the standard Kjeldahl method. One gram of sample was digested with 25 ml concentrated sulfuric acid and 2.5 g digestion mixture (copper sulfate: sodium sulfate in 1: 9 ratio) until it became clear. Volume was made to 250 ml with distilled water by transferring the content of digestion flask to volumetric flask with several washings with distilled water. 10 ml aliquot of digested samples was distilled in a Micro Kjeldahl assembly by adding 15 ml of 40% sodium hydroxide solution. Gaseous ammonia thus released was trapped in 15 ml boric acid containing Tashiro's indicator (10 ml each of methyl red and bromocresol green solution added to 1000 ml of 2% boric acid). The nitrogen trapped in boric acid was estimated by titrating it against N/100 sulphuric acid. A blank was also run, the value of which was subtracted from sample's reading. The normality of acid was checked by titrating against sodium carbonate using methyl orange as indicator. The crude protein content was determined as follows:

$$\text{Crude protein (\%)} = \frac{(B-B_1) \times 0.014 \times N \times Y \times 6.25}{X \times W} \times 100$$

Where,

B = Volume (ml) of N/100 H_2SO_4 consumed for titration of sample

B_1 = Volume (ml) of N/100 H_2SO_4 consumed for titration of blank distillate

N = Normality of N/100 H_2SO_4

Y = Volume (ml) made out of digested sample

X = Volume (ml) of aliquot taken for distillation

W = Weight (g) of oven dried sample taken for digestion

6.25 = Factor for converting nitrogen into protein of sample

3.2.6.3 Ether extract

Ether extract was determined by extracting weighed quantity (about 2-3 g) of ground moisture free sample with petroleum ether (B.P. 60-80^{0}C) in Soxhlet apparatus for 8-10 h. The extracted oil in the flask was dried to constant weight at 100^{0}C. The difference in the weight of oil flask before and after extraction gave the amount of ether extract and was expressed on DM basis by the formula:

$$\text{Ether Extract (\%)} = \frac{\text{(Weight of oil flask after extraction - Weight of oil flask before extraction)}}{\text{Weight of oven dried sample}} \times 100$$

3.2.6.4 Total ash

Approximately 3-4 g of sample (exactly weighed) was taken in a pre-weighed silica basin and decarbonized on heater to make it smoke free. The crucible along with the sample was ignited at 600^0C for 3 hr. The sample weight remained after ashing was taken as total ash and was expressed on DM basis. The percent total ash was calculated from the following formula:

Total Ash (%) = (a – b) / w X 100

Where,

a = weight (g) of silica basin plus oven dried sample

b = weight (g) of silica basin plus ash; w = weight (g) of oven dried sample

3.2.6.5 Organic matter

Per cent organic matter in the sample was calculated by deducting per cent TA from 100.

OM % = 100 - % TA

3.2.6.6 Crude fibre

The fat and moisture free samples were transferred to spotless beakers of 1.0 litre capacity marked to 200 ml and refluxed for 30 minutes with 25 ml each of 2.04 N H_2SO_4 and 2.50 N NaOH and make up the volume to 200 ml. The refluxed contents were filtered through muslin cloth with the help of Buchner funnel with suction pump with repeated hot water washings and transferred to a clean silica basin with the help of smooth steel spatula. The content of silica basin was oven dried at 100°C overnight and the weight of dried residue along with silica basin was recorded. The dried residue was decarbonized and then ashed in muffle furnace at 550°C for 2 hours. The percent crude fibre in the sample was calculated from the following formula:

Crude fibre (%) = (a – b) / w X 100

Where,

a = weight (g) of silica basin plus oven dried residue left after digestion

b = weight (g) of silica basin plus ash; w = weight (g) of oven dried sample

Table 3.1 Chemical composition of feed ingredients used in experiment (% on DM basis)

Ingredients	DM	OM	CP	EE	CF	TA	AIA	NFE	Ca	P	ME (kcal/kg)
Yellow maize	91.28	97.26	9.83	4.49	2.11	2.74	1.09	80.92	0.06	0.29	3340
Soyabean meal	92.76	93.02	45.08	0.29	5.53	6.98	1.16	41.86	0.28	0.64	2450
Moringa oleifera leaf powder (MOLP)	94.53	88.93	25.79	6.54	10.02	11.08	1.29	46.57	1.65	0.36	2878

Table 3.2 Percentage composition of different experimental diets

Ingredients	Pre-starter	Starter	Finisher
Yellow maize	53.00	54.00	58.00
Soya bean meal	39.50	36.50	32.00
Vegetable oil	3.00	5.10	5.90
Common salt	0.30	0.30	0.30
Di-calcium Phosphate	1.00	1.00	1.00
Calcite powder	1.00	1.00	1.00
DL-Methionine	0.40	0.40	0.30
Lysine	1.10	1.00	0.80
Mineral mixture	0.50	0.50	0.50
Premix	0.20	0.20	0.20
Total	100	100	100

Calculated value

Attributes	Pre-starter	Starter	Finisher
CP (%)	22.97	21.72	20.08
ME (kcal/kg)	2968.95	3090.55	3175.50
Ca (%)	0.80	0.79	0.78
Av. P (%)	0.66	0.64	0.62

Composition of mineral mixture

Vitamin A (7,00,000 I.U.), Vitamin D_3 (70,000 I.U.), Vitamin E (250 mg), Nicotinamide (1000 mg), Cobalt (150 mg), Copper (1200 mg), Iodine (325 mg), Iron (1500 mg), Potassium (100 mg), Magnesium (6000 mg), Manganese (1500 mg), Selenium (10 mg), Sodium (5.9 mg), Sulfur (0.72 %), Zinc (9600 mg), Calcium (25.5%) and Phosphorus (12.75%)

3.2.7 Growth parameters

3.2.7.1 Feed consumption

Feed consumption is the amount of feed consumed every week. It was calculated for each treatment group at weekly basis. At the end of the week, the residual amount of feed was weighed and subtracted from the weight of feed offered at the beginning of week. Difference in weight was divided by the total number of birds.

3.2.7.1 Body weight and body weight gain

During the initial phase of the experiment, body weight of individual chicks was recorded. Thereafter, body weight change was observed at weekly interval up to five weeks. Live weight gain was calculated by subtracting the live weight at the beginning of the week from the live body weight of the next week and whole body weight gain at the end of 5^{th} week from the initial body weight.

3.2.7.2 Feed conversion ratio

Feed conversion ratio (FCR) was calculated every week as the amount of feed consumption per unit of body weight gain.

FCR was calculated by using the formula;

$$FCR = \frac{\text{Feed consumed (g)}}{\text{Body weight gain (g)}}$$

3.2.7.3 Balance study of nutrients

After end of the experiment, a five days metabolic trial was conducted to observe the balance of major nutrients such as protein, energy, calcium and phosphorus. In each trial six birds from each group were randomly selected and transferred to metabolic cages. Preliminary feeding was given for adaptation of birds to the new system of housing. Polythene sheets of appropriate size were spread over the dropping trays for the collection of mixed excreta. The chicks were offered a weighed amount of experimental ration at a fixed morning hour every day during the trial period. The mixed droppings were also quantitatively collected at the end of 24 hrs at fixed hours and pooled to know the total amount of excreta voided for five days. Daily feed intake was collected after deducting weight of feed residue left from the feed offered. Representative feed samples were drawn from the bulk, finely ground and stored in bottles for proximate, Ca and P analysis in laboratory. Aliquots from dropping after thorough mixing with the help of spatula was drawn for dry matter and follow up analysis for nitrogen estimation. Aliquots of five days were pooled together for nutrient analysis.

3.3 EFFECT OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON HEMATO-BIOCHEMICAL PROFILE, ANTIOXIDANT STATUS AND IMMUNE RESPONSE IN BROILER CHICKEN

3.3.1 Blood biochemical parameters

3.3.1.1 Collection of blood samples

At the end of the experiment blood samples were collected from two birds per replicate, making six samples per treatment. Blood was collected in two set of vial, one with without anticoagulant and other with anticoagulant EDTA, from the wing vein using insulin syringes. Haematological study like Haemoglobin (Hb), Packed Cells Volume (PCV), Total erythrocyte count (TEC), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC) Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular

Haemoglobin Concentration (MCHC) was estimated in Blood samples with EDTA within 24 hours after collection.

Blood without anticoagulant were allow to clot then centrifuged for 15 min at 3000 rpm to separate the serum. The serum sample were stored at -20º C for the analysis of serum for total protein, albumin, globulin, blood urea nitrogen, creatinine, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). This serum sample were also used for estimation of antioxidant enzyme like Superoxide Dismutase (SOD), Catalase (CAT), Lactate Dehydrogenase (LDH), Reduced Glutathione (GSH), Lipid Peroxidation (LPO) and HI titre against (Newcastle disease virus) NDV.

3.3.1.2 Whole blood analysis

3.3.1.2.1 Haemoglobin

Haemoglobin (Hb) was estimated as per cyanmethemoglobin (Drabkin, 1932) method. In this method hemoglobin is oxidized to methaemoglobin by potassium ferricyanide; methaemoglobin in turn combines with potassium cyanide to form cyanmethaemoglobin. The standard absorbance was read before the start of the procedure. A 20 µl of the blood was added to 5.0 ml of Drabkin's solution. The diluted sample was allowed to stand for 10 minutes, it was then transferred to a cuvette and the optical density was observed at 540 nm against a blank of Drabkin's solution. The result was calculated from formula given below;

$$\text{Hb (g/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

3.3.1.2.2 Packed cell volume

Packed cell volume (PCV) was estimated by micro haematocrit method (Campbell, 1995). Packed cell volume was measured as micro-haematocrit with 75 x 16 mm capillary tubes. Capillary tube was filled with two-thirds to three-quarters full with well mixed blood. The one end of capillary tube was sealed with sealing wax. Filled capillaries were placed in the micro-hematocrit centrifuge, with the plugged end away from the centre of the centrifuge and centrifuged at 3000 rpm for 5 minutes. PCV reading was taken with the help of PCV reader and express as percentage.

3.3.1.2.3 Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC)

Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) were determined by the Natt and Herrick (1952) method using a Newbauer hemocytometer. TEC was expressed in million/µL and TLC was expressed in thousands/µL of blood.

3.3.1.2.4 Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV) was calculated using following formula and expressed in femtoliters (fl).

$$MCV = \frac{PCV}{RBC \text{ in million}} \times 10 \text{ cubic microns}$$

3.3.1.2.5 Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin (MCH) was calculated using following formula and expressed in Picograms (pg).

$$MCH = \frac{Hb \text{ (g/100 ml)}}{RBCs \text{ in millions}} \times 10 \text{ micro micrograms}$$

3.3.1.2.6 Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular hemoglobin concentration (MCHC) was calculated using following formula and expressed in g/dL.

$$MCHC = \frac{Hb \text{ (g/100 ml)}}{PCV} \times 100 \text{ percent}$$

3.3.1.3 Serum biochemical analysis

3.3.1.3.1 Total protein

Total protein was estimated by Biuret Method by using commercial kit of Coral Clinical System (a division of Tulip Diagnostics (P) Ltd.) and expressed in g/dl. Absorbance was

measured at 550 nm wavelength UV Visible Double Beam spectrophotometer 2205 (Systronic India).

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 8$$

3.3.1.3.2 Albumin

The collected samples of serum from each group were examined for albumin protein by Bromocresol green method using commercial kit of Coral Clinical System (a division of Tulip Diagnostics (P) Ltd.) and expressed in g/dl. Absorbance was measured at 550 nm wavelength using UV Visible Double Beam spectrophotometer 2205 (Systronic India).

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 4$$

3.3.1.3.3 Globulin

It was calculated by using following formula expressed in g/dl;

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

3.3.1.3.4 Albumin: Globulin Ratio

It was calculated by dividing albumin concentration with globulin concentration.

3.3.1.3.5 Blood urea nitrogen

Blood Urea Nitrogen (BUN) was estimated by glutamate dehydrogenase (GLDH) kinetic method by using commercial kit of Coral Clinical System (a division of Tulip Diagnostics (P) Ltd.) and expressed in mg/dl. Decrease in absorbance was measured at 340 nm wavelength using UV Visible Double Beam spectrophotometer 2205 (Systronic India).

$$\text{Urea (mg/dl)} = \frac{\text{Change in absorbance of test}}{\text{Change in absorbance of standard}} \times 40$$

BUN = Urea (mg/dl) × factor (0.44650)

3.3.1.3.6 Creatinine

The collected samples of serum from each group were examined for creatinine by modified Jaffe's kinetic method using commercial kit of Coral Clinical System (a division of Tulip Diagnostics (P) Ltd.) and expressed in mg/dl. Decrease in absorbance was measured at 520 nm wavelength using UV Visible Double Beam spectrophotometer 2205 (Systronic India).

$$\text{Creatinine (mg/dl)} = \frac{\text{Change in absorbance of test}}{\text{Change in absorbance of standard}} \times 2$$

3.3.1.3.7 Aspartate transaminase

The collected samples of serum from each group were examined for AST by Modified IFCC method using commercial kit of Coral Clinical System (a division of Tulip Diagnostics (P) Ltd.) and expressed in U/L. Decrease in absorbance was measured at 340 nm wavelength using UV Visible Double Beam spectrophotometer 2205 (Systronic India).

AST(U/L) = Mean absorbance change/minute ×1746

3.3.1.3.8 Alanine transaminase

The collected samples of serum from each group were examined for ALT by IFCC method using commercial kit of Coral Clinical System (a division of Tulip Diagnostics (P) Ltd.) and expressed in U/L. Decrease in absorbance was measured at 340 nm wavelength using UV Visible Double Beam spectrophotometer 2205 (Systronic India).

ALT (U/L) = Mean absorbance change/minute × 952

3.3.1.3.9 Serum lipid profile

Total cholesterol, Triglyceride and HDL were estimated by using commercial kit Coral Clinical System (a division of Tulip Diagnostics (P) Ltd.) and expressed in mg/dl. absorbance was measured at 505nm wavelength in UV Visible Double Beam spectrophotometer 2205 (Systronic India).

$$\text{(a) Total cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

$$\text{(b) Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

$$\text{(c) HDL cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 50 \times 2^*$$

*(2 = dilution factor, as sample was diluted 1:1)

(d) LDL cholesterol = Total cholesterol – $\frac{\text{Triglyceride}}{5}$ – HDL cholesterol

(e) VLDL Cholesterol = $\frac{\text{Triglyceride}}{5}$

3.3.2 Antioxidant status

3.3.2.1 Catalase activity

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

0.05 change in absorbance = 3.45 µmoles of H_2O_2 disappeared

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

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

= 1380 A

3.3.2.2 Lipid peroxidation

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

MDA produced (µmoles/ml) = Absorbance X dilution factor/ $(1.56 \text{ X } 10^5)$.

3.3.2.3 Reduced glutathione

Reduced glutathione (GSH) level in serum was estimated using the method described by Lin et al. (1988) with some modifications. An aliquot of serum (400 µL) was mixed with 400 µl of tris-EDTA buffer followed by addition of 40 µl of 10 mM 5,5'- dithiobis (2-nitro

benzoic acid) [DTNB] and 3.16 ml of absolute methanol. Colour was developed after incubation at 37^0C for 30 minutes. Now, the suspension was centrifuged at 3500 rpm for 10 minutes. The absorbance of the supernatant was measured at 412 nm (A) and subtracted from a DTNB blank (B) and a blank containing the sample without DTNB. In agreement with Sedlak and Lindsay (1968), a value of 0.03 at 412 nm for the sample blank was consistently obtained. Consequently, individual sample blanks were not critical and were taken as 0.03. GSH levels were conveniently calculated using an absorptivity of 13600 $cm^{-1}M^{-1}$ as follows.

(A-B-0.03) X (4.0/0.4) / 13.6 = (A-B-0.03) X 0.735 mM

3.3.2.4 Superoxide dismutase activity

Serum superoxide dismutase (SOD) activity was measured using the method as given by Madesh and Balasubramanian (1997) with some modifications. In the micro-titre plate method, the assay mixture in a total volume of 300 µl per well consisted of 120 µl PBS, 10 µl serum sample, 5 µl of 1.25 mM MTT and 15 µl of freshly prepared 1mM pyragallol solution to be added at the end. Sample was replaced with PBS in the blank. After an incubation period of 15 minutes, 150 µl DMSO was added and absorbance was taken in ELISA reader at 570 nm.

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

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

Where, A_T= Absorbance for test.

And A_B= Absorbance for blank.

3.3.2.5 Lactate dehydrogenase

The collected samples of serum from each group were examined for lactate dehydrogenase by Modified IFCC method using commercial kit of Coral Clinical System (a division of Tulip Diagnostics (P) Ltd.) and expressed in U/L. Decrease in absorbance was measured at 340 nm wavelength using UV Visible Double Beam spectrophotometer 2205 (Systronic India).

LDH (U/L) = Mean absorbance change/minute × 8095

3.3.3 Haemagglutination and Haemaglutination inhibition assay

3.3.3.1 Haemagglutination (HA)

3.3.3.1.1 Preparation of chicken red blood cell (RBC) suspension

4 ml freshly collected whole blood of broiler chicken taken in a 15 ml centrifuge tube and make final volume up to 15 ml with PBS solution

⇩

Centrifuged at 800 rpm for 10 minutes

⇩

Aspirate the supernatant without disturbing the blood cells

⇩

Added 12 ml PBS solution and mix by manual tilting (do not vortex)

⇩

Centrifuged at 800 rpm for 5 minutes and repeat washing 2-3 times

⇩

Aspirate supernatant & add enough PBS solution to made a 10% solution of RBC

⇩

Made a final working solution of 0.5% RBCs in PBS solution for HI test

3.3.3.1.2 Viral Dilution and Assay

Took a round bottomed 96-well dish

⇩

Added 50 µL PBS solution in each well with the help of micro pipette

⇩

Added 50 µL of virus sample in the first column

⇩

Mix each well with the help of pipette and transfer 50 µL from first column to the next well for serial dilution and finally discard 50 µL from last well

⇩

Added 50 µL of 0.5% red blood cell working solution to each well & mix gently

⇩

Kept at room temperature for 30-60 minutes to develop. Negative results appeared as dots in the center of round-bottomed plates. Positive result formed a uniform reddish color across the well

⇩

Virus's HA titer is a simple number of the highest dilution factor that produced a positive reading

3.3.3.2 Haemaglutination inhibition (HI) assay

3.3.3.2.1 Preparation of chicken red blood cell (RBC) suspension

First of all, 2 ml chicken blood was collected with the help of disposable syringe and dispensed in an anticoagulant vials containing 1 ml of sodium citrate (4% solution). Blood sample was centrifuged at 1500 rpm for 15 minutes and the plasma and buffy coat were removed with a pipette. After washing three times with phosphate buffered saline (PBS) solution, 1% suspension in PBS was prepared for HI test.

3.3.3.2.2 Viral Dilution and Assay

HI test was performed as per the procedure of OIE (2002). Briefly, two-fold serial dilution of 50 µL serum was made with PBS in round bottom plates. Then, 50 µl of Newcastle disease virus or antigen was added up to 11^{th} well. The plates were kept at room temperature for more than 30 minutes to facilitate antigen-antibody reaction. Then, 50 µL of 1% (v/v) chicken RBC suspension was added to each well. However, 11^{th} well contains antigen and RBCs as the positive control and the 12^{th} well contains only RBCs as the negative control. After gentle mixing, the RBCs were allowed to settle at room temperature for 40 minutes and agglutination was assessed by tilting the plates. Samples showing peculiar central button shaped settling of RBCs were recorded as positive and maximum dilution of each sample causing haemagglutination inhibition was considered as the end point, which was used to estimate the HI titer. The HI titer of each serum sample was expressed as reciprocal of the serum dilution (Hossain et al., 2010).

3.4 EFFECT OF SUPPLEMENTATION OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON CARCASS CHARACTERISTIC AND PRODUCTION COST ANALYSIS IN BROILER CHICKEN

3.4.1 Carcass study

At the end of five weeks, six birds from each group and two from each replicate were randomly selected for slaughter following standard protocol and processing. The birds were starved 24 hours before slaughter without withdrawing of drinking water. Each bird was weighed twice, just prior to starvation and again immediately before slaughter. The birds were bled by clean incision at the base of ear lobes and allowed to bleed. The birds were emerged in hot water (70º C) for 30 second (hard scalding). The scalded birds were hand plucked to remove body feathers perfectly. The head was removed by severing cutting between the first cervical vertebra and optical bone. The feet and shank were cut at the tibio-tarsal joints, wings tips were removed and dressed weight of the carcass was recorded. The birds were then

eviscerated by removing the crop, gullet, trachea and viscera. The lungs were scrapped off. The giblets (heart, liver and gizzard) were removed from the viscera. Gall bladder was removed from liver and gizzard was opened and contents were washed out and lining was pulled off and the contents were washed. The heart was free from blood clot and adhering vessels. The weight of the carcass along with giblets was recorded as eviscerated weight. The dressing percentage and eviscerated percentage were calculated on the basis of pre-slaughter live weight at 5th week of age.

The neck of carcasses was removed as closely to the clavicles as possible, weight of neck and giblet were recorded separately. The weight of heart, liver and gizzard were recorded and expressed as percentage of live weight.

$$\text{Dressing percentage} = \frac{\text{Dressed weight}}{\text{Pre-slaughtered weight}} \times 100$$

$$\text{Giblet percentage} = \frac{\text{Weight of giblet}}{\text{Dressed weight}} \times 100$$

$$\text{Eviscerated percentage} = \frac{\text{Eviscerated weight}}{\text{Pre-slaughter weight}} \times 100$$

3.4.2 Production cost analysis

The economics of chicken production was calculated on the cost of feed per kg live weight gain. The economics is thus dependent on the cost of different feed ingredients used in the experiment along with feed efficiency of various treatments. Actual cost of feed was calculated on the basis of rates on which the different feed ingredients were purchased from the local market.

Total output/bird : Total weight of bird (kg) × sale price / kg

Total input/bird : Cost of feed + Cost of chicks + Cost of medicine etc.

Net profit /bird : Total output/bird - Total input/bird.

3.5 STATISTICAL ANALYSIS

All statistical analyses were performed as per standard method (Snedecor and Cochran, 1989) by using SPSS (2015) computer package. For comparison of multiple groups Generalized Linear model ANOVA procedures and Duncan's multiple range tests were utilized.

Chapter-IV

Results and Discussion

Poultry sector in India plays key role in improving socioeconomic status and livelihood of rural community. However, in present situation the increase in cost of conventional cereals and protein sources due to increasing its demand by human population, sustainability of this sectors may have affected which results increase in cost of production. Nutritional security for human population is great challenge now a days and poultry plays important role in minimizing the protein and calorie deficiency of human population. Now a day's people are more concern about heath and quality food for consumption which emphasizes to produce quality and healthy animal food through dietary interventions. So, to combat the challenges of uncertainty about their sustainable supply and quality animal product production, need to search for nutrient rich potential nonconventional feed sources, which are relatively less used for human consumption. Ascorbic acid is an important scavenger of free radical produced in the system during stress condition. *Moringa oleifera* is a well-known widely available plant species having high nutritional value and good biomass production. Hence, supplementation of ascorbic acid and Moringa tree leaf extract could be a possible alternative to improve the production performance by reducing the stress of broiler birds with quality product production.

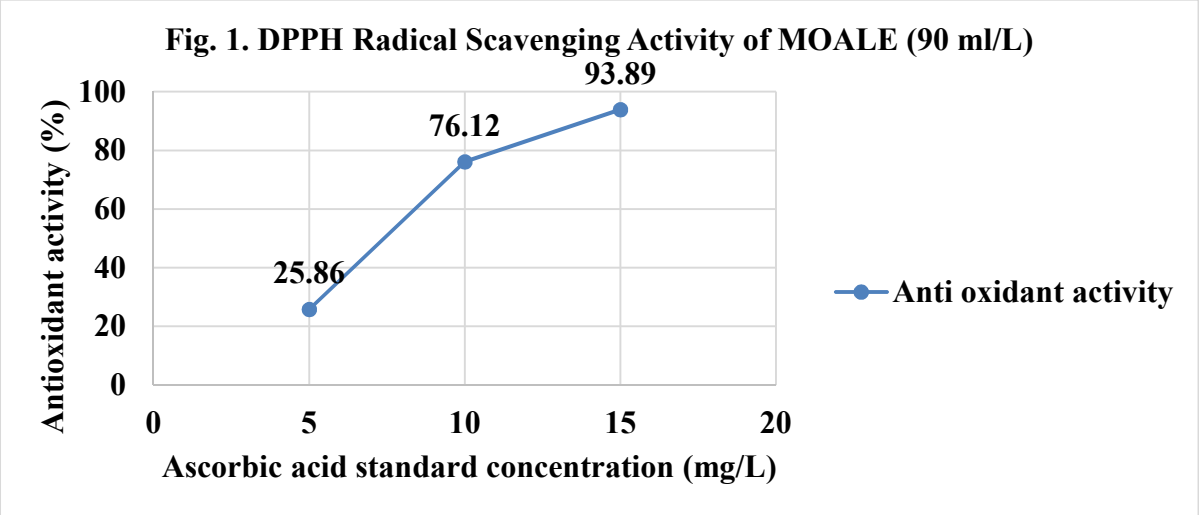
The limited studies on these aspect has been done and in the perspective of Bihar state no study was performed. So, to address the problems we planned the comparative effect of *Moringa oleifera* aqueous leaf extract and ascorbic acid supplementation on production performance, antioxidant status and immune response in broiler chicken.

4.1 EVALUATION OF TOTAL ANTIOXIDANT CAPACITY OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID

Result of total antioxidant capacity of *Moringa oleifera* aqueous leaf extract (MOALE) and ascorbic acid is presented in table 4.1. The percentage antioxidant activity of MOALE (90 µl/ml) was 93.89 % and ascorbic acid (15 mg/ml) was 98.76 %, respectively as the data shown nearer to each other. So, 90 ml MOALE and 15 mg ascorbic acid per litre drinking water, respectively were used for supplementation in boiler chicken throughout the experiment.

Table 4.1 Determination of total antioxidant capacity of *Moringa oleifera* aqueous leaf extract and ascorbic acid

Moringa oleifera aqueous leaf extract (MOALE)		Ascorbic acid	
Concentration	**Antioxidant activity (%)**	**Concentration**	**Antioxidant activity (%)**
30.00 µl	25.86	5.00 mg	31.88
60.00 µl	76.12	10.00 mg	82.55
90.00 µl	93.89	15.00 mg	98.76



The inclusion of 90 ml/L aqueous *Moringa oleifera* leaf extract in the drinking water of broiler chicken improved production performance as reported by Alabi et al. (2017). In present experiment DPPH radical scavenging activity of MOALE was calculated with reference to different ascorbic acid concentration as standard. At 15 mg/L ascorbic acid concentration, MOALE extract shown 93.89% activity which was nearer to cent percent. So, the antioxidant activity of MOALE was similar to ascorbic acid 15 mg/L ascorbic acid (Fig.1).

4.2 EFFECT OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON PERFORMANCE PARAMETERS OF BROILER CHICKEN

4.2.1 Feed intake

The feed intake at weekly interval and 5th weeks of age in broiler chicken is presented in table-4.2, showed non-significant effect of supplementation of *Moringa oleifera* aqueous

leaves extract (MOALE) and ascorbic acid on feed intake in experimental birds. Average feed intake during experiment varied from 2983.03 in T2 to 3055.52 g in T1 group, respectively. Week wise feed intake varied from 113.35 g in T2 to 122.30 g in T1 at first week, which was significantly differed, whereas it ranged from 411.65 g in T2 to 424.89 g in T1 group at second week, from 658.10 g in T2 to 679.72 g in T3, from 780.28 g in T3 to 825.0 g in T1, from 991.59 g in T2 to 1018.27 g in T3 in 3rd, 4th and 5th week, respectively. A marginal fluctuation was observed in feed intake in every week among different groups except first week where significant difference was noted.

Analysis of variance for the effect of treatment on feed intake in broiler chicken were found to be non-significant ($P>0.05$). Average feed intake at the end of first week in T2 group was found to be the lowest (113.35 g) which was significantly ($P<0.05$) lower than T1 and T3 groups. In second, third, fourth and fifth week feed intake in all groups were found to be non-significant ($P>0.05$) and comparable among the groups. As a whole after the end of 5th week it was found that supplementation of *Moringa oleifera* aqueous leaves extract (MOALE) and ascorbic acid had no any significant effect on feed intake in comparison to control (Fig. 2), however, MOALE supplemented group consumed 2.37 % less feed as compared with control group during the experiment.

Paguia et al. (2013) found that the addition of *Moringa oleifera* leaf powder on broiler diets did not significantly influence the feed intake as compared to control. However, study of Divya et al. (2014) revealed that the addition of MOL powder at any level slightly non-significant decrease in feed intake compared to control. Alabi et al. (2017) investigate the effect of aqueous *Moringa oleifera* leaf extracts (AMOLE) and found that inclusion of 90 ml/litre AMOLE in drinking water of broiler chicken can reduced feed intake in treated group. Agashe et al. (2017) found that dietary inclusion of *Moringa oleifera* leaf powder in the diet of broiler bird, slightly reduced feed intake in comparison to control. Paul et al. (2018) also revealed that the aqueous extract of *Moringa olefiera* leaf inclusion in drinking water of broiler chicken slightly reduced feed intake as control group.

The result of our study was an agreement with the above study investigated by various researchers on poultry birds. The depressed feed intake might be due to nutrient satisfaction in broiler which could be a result of improved digestion and metabolism activities of MOALE, thus meeting the nutrient requirements at lower feed intake.

4.2.2 Body weight

The result of body weight at weekly interval in broiler bird is presented in table-4.3. The average body weight ranged from 193.82 g in T2 to 211.94 g in T1 at first week, whereas it

varied from 525.22 g in T2 to 549.72 g in T1 at second week. It varied from 951.50 g in T3 to 978.99 g inT2, from 1287.06 g in T2 to 1320.55 g in T3, from 1645.92 g in T1 to 1785.35 g in T2 during 3rd, 4th and 5th week, respectively.

Analysis of variance for the effect of treatment on body weight changes in broiler chicken was found to be non-significant (P>0.05), except in first week the body weight in T2 group was significantly lower than control (P<0.05). Average body weight at the end of 1st week to 5th week was found to be higher side in T2 followed by T3 group as compared to control but changes were non-significant (Fig. 2). There was marginal variation in body weight among the group throughout the experiment except first week where significant reduction in weight was noted in T2 group. However, in MOALE supplemented group 8.47% higher body weight in broiler found as compared with control group.

Ebenebe et al. (2012) reported that chicks fed on moringa based diets performed higher weight gain such improvement may be attributed to rich content of nutrients in MOLM and anti-microbial properties of moringa. Paguia et al. (2013) revealed that the supplementation of *Moringa oleifera* leaf meal in broiler diets did not significantly influence the final body weight. However, Akhouri et al. (2014) reported that the supplementation of *Moringa oleifera* leaf aqueous extract enhances body weight in vaccinated broiler chicken. Moreover, Nkukwana et al. (2014) noticed that supplementation of *Moringa oleifera* leaf meal in broiler chicken had no adverse effect on its performance. Agashe et al. (2017) found improvement in live body weight in *Moringa oleifera* leaf powder supplemented broiler bird in comparison to control.

So, the finding of present study was an agreement with the above observation reported by several researches. The body weight changes were marginally enhanced in MOALE and ascorbic acid supplemented birds in respect of control. The increment in body weight might be the result of different phytochemicals present in extract which may affect broad aspect of physiology such as nutrient absorption and availability in broiler chicken.

4.2.3 Body weight gain

The result of body weight gains at weekly interval and at the end of fifth week in broiler chicken is presented in table-4.4. Average body weight gain varied from 193.82 g in T2 to 211.94 g in T1 during 1st week. At the end of 2nd week it ranged from 331.39 g in T2 to 337.78 g in T1. In 3rd, 4th and 5th week, minimum and maximum change in body weight gain ranged from 417.50 g in T1 to 453.77 g in T2, 308.07 g in T2 to 369.05 g in T3 and 326.48 g in T1 to 498.30 g in T2, respectively. Final body weight gains at the end of experiment varied between 1645.92 g in T1 to 1785.35 g in T2.

Analysis of variance for the effect of treatment on body weight gain in broiler birds showed non-significant (P>0.05) effect on change in body weight. Average weight gain at the end of 1st week in T2 group was found to be lowest (193.82 g) which was significantly (P<0.05) lower than control, however, T3 group was comparable with each other. In 2nd, 3rd, 4th and 5th week body weight gain was found to be non-significant (P>0.05) and are comparable among the groups. There was fluctuation in weekly body weight gain noted during the experiment. However, the average body weight gain of T2 group was found to be 8.47% higher, though the changes was non-significant (P<0.05) in comparison with control group (Fig.1).

Olugbemi et al. (2010) found that with increasing inclusion level MOLM in broiler chicken diet results in depressed body weight gain. Ebenebe et al. (2012) noticed that chicks fed on moringa based diets attain higher weight gain than the birds of control group. Such increment in body weight gain may be attributed to rich in nutrient concentration of MOLM and its anti-microbial properties. Imad khan et al. (2015) revealed that the weight gain in broiler chickens after addition of *Moringa oleifera* leaf meal in the diet of the broilers significantly (p<0.05) enhanced as compared to the control group**.** Study showed that *Moringa oleifera* aqueous leaf extract supplemented Ross and Cobb breed chicken having significantly better performance as compared to control group (Younis and Elbestawy, 2017). Alabi et al. (2017) found that aqueous *Moringa oleifera* leaf extracts (AMOLE) supplemented broiler chicken group had better growth performance.

So, present report was an agreement with the above observation reported by several researches. The average body weight gain was higher side in MOALE and ascorbic acid supplemented birds in respect of control. The increment in body weight might be the result of different bio-active molecules present in aqueous extract which may affect physiology of nutrient absorption and its availability in broiler chicken.

4.2.4 Feed conversion ratio

The result of feed conversion ratio (FCR) at weekly interval and at the end of fifth week in broiler bird is presented in table-4.5. The FCR varied from 1.69 in T3 to 1.80 in T1 at 1st week, whereas it ranged from 1.64 in T2 to 1.96 in T1. In 3rd week FCR varied from 1.62 in T2 to 2.0 in T1. In 4th week it ranged from 1.63 in T2 to 1.90 in T1, whereas FCR in 5th week ranged from 1.63 in T1 to 1.80 in T2 group, respectively. The average FCR of fifth week of experiment varied from 1.68 in T2 to 1.86 in T1 group.

Analysis of variance for the effect of treatment on FCR in broiler chicken showed significant (P<0.05; P<0.01) effect on change in FCR between the groups. Feed conversion

ratio at the end of 1st week in T3 group was found to be lower (1.69) than T1 group. In 2nd week FCR of T2 group was significantly ($P<0.05$) lower than T1 group and comparable with T3 group in comparison of control. In 3rd week FCR in T2 and T3 group was comparable but significantly ($P<0.01$) lowest than T1 group. In 4th week FCR in T2 group was found to be lowest which was significantly ($P<0.05$) decreased than T1, whereas FCR in T3 and T1 found comparable. In 5th week FCR was varied between the groups but differences were non-significant ($P>0.05$). The overall FCR for T2 group was found to be significantly ($P<0.01$) lowest than all other treatment group followed by T3 group in comparison with control (Fig. 3). However, 9.67% better FCR was noted in MOALE supplemented group followed by T3 group (5.91%) in comparison with control.

Ebenebe et al. (2012) noticed that moringa based diets supplementation in chicks performed significantly ($P<0.05$) better than control group in term of feed conversion ratio and such reduction in FCR may be attributed to rich content of multi nutrients in MOLM and anti-microbial properties. Safa (2014) suggested that *Moringa oleifera* leaf meal offered broiler chicks shown significantly ($P<0.05$) superior effect on FCR in comparison with control diet. However, Akhouri et al. (2013) reported that treatment group offered *Moringa oleifera* leaf extract having better feed conversion efficiency in the broiler chicks. Akhouri et al. (2014) revealed that the supplementation of aqueous extract and dried powder of *Moringa oleifera* leaf enhances feed conversion ratio and feed conversion efficiency in vaccinated broiler chicken. Alabi et al. (2017) found that the aqueous *Moringa oleifera* leaf extracts supplementation in broiler chicken through drinking water improved feed conversion efficiency and similar finding observed by Paul et al. (2018) that the AEMOL inclusion in drinking water of broiler chicken improved FCR.

Present finding was an agreement with the several observations recorded by different researchers around the globe. The average FCR was found to be lowest in MOALE followed by ascorbic acid supplemented broiler chicken in respect of control. The better feed conversion efficiency shown in these treatment might be because of presence of phytogenic constituents and immunomodulatory properties of *Moringa oleifera* plant and antioxidant properties of both supplements.

Table 4.2 Effect of MOALE and ascorbic acid on an average feed intake (g)/bird at weekly interval and 5th week in broiler chicken

Week	T_1	T_2	T_3	SEM	P-value
1st	122.30 [b] ±1.36	113.35 [a] ±3.04	116.19 [ab] ±0.34	2.733	0.042
2nd	424.89 ±9.22	411.65 ±15.01	416.72 ±6.29	15.27	0.698
3rd	673.14 ±12.69	658.10 ±15.44	679.72 ±4.61	16.75	0.464
4th	825.00 ±2.10	808.33 ±18.79	780.28 ±16.12	20.29	0.464
5th	1010.18 ±32.13	991.59 ±17.54	1018.27 ±33.02	40.25	0.800
1 - 5th week	3055.52 ±18.01	2983.03 ±38.55	3011.19 ±24.66	40.16	0.267

[ab] Values with different superscripts in a row differ significantly (P<0.05)

Table 4.3 Effect of MOALE and ascorbic acid on an average body weight (g) at weekly interval in broiler chicken

Week	T_1	T_2	T_3	SEM	P-value
1st	211.94 [b] ±5.30	193.82 [a] ±4.40	196.46 [ab] ±4.90	6.897	0.078
2nd	549.72 ±4.09	525.22 ±11.81	533.22 ±6.40	11.47	0.174
3rd	967.22 ±19.63	978.99 ±19.46	951.50 ±12.29	24.70	0.568
4th	1319.44 ±31.94	1287.06 ±57.42	1320.55 ±24.92	57.38	0.809
5th	1645.92 ±74.04	1785.35 ±91.61	1733.52 ±103.78	128.18	0.577

[ab] Values with different superscripts in a row differ significantly (P<0.05)

Table 4.4 Effect of MOALE and ascorbic acid on an average body weight gain (g) at weekly interval and 5th week in broiler chicken

Week	T_1	T_2	T_3	SEM	P-value
1st	211.94 [b] ±5.30	193.82 [a] ±4.40	196.46 [ab] ±4.90	6.897	0.078
2nd	337.78 ±1.82	331.39 ±12.90	336.76 ±5.88	11.67	0.845
3rd	417.50 ±21.48	453.77 ±9.95	418.28 ±16.62	23.61	0.289
4th	352.22 ±21.57	308.07 ±38.16	369.05 ±36.86	46.76	0.453
5th	326.48 ±49.81	498.30 ±34.26	412.96 ±86.85	86.40	0.219
1 - 5th week	1645.92 ±74.04	1785.35 ±91.61	1733.52 ±103.78	128.18	0.577

[ab] Values with different superscripts in a row differ significantly (P<0.05)

Table 4.5 Effect of MOALE and ascorbic acid on FCR at weekly interval and 5th week in broiler chicken

Week	T_1	T_2	T_3	SEM	P-value
1st	1.80 ±0.05	1.71 ±0.05	1.69 ±0.04	0.067	0.293
2nd	1.96 [b] ±0.08	1.64 [a] ±0.07	1.78 [ab] ±0.06	0.096	0.045
3rd	2.00 [b] ±0.05	1.62 [a] ±0.05	1.73 [a] ±0.06	0.078	0.007
4th	1.90 [b] ±0.03	1.63 [a] ±0.05	1.83 [b] ±0.07	0.070	0.020
5th	1.63 ±0.10	1.80 ±0.06	1.70 ±0.07	0.112	0.398
1 - 5th week	1.86 [c] ±0.02	1.68 [a] ±0.01	1.75 [b] ±0.02	0.025	0.001

[abc] Values with different superscripts in a row differ significantly (P<0.05; P<0.01)

Fig. 2. Effect of MOALE and ascorbic acid on feed intake, body weight and body weight gain

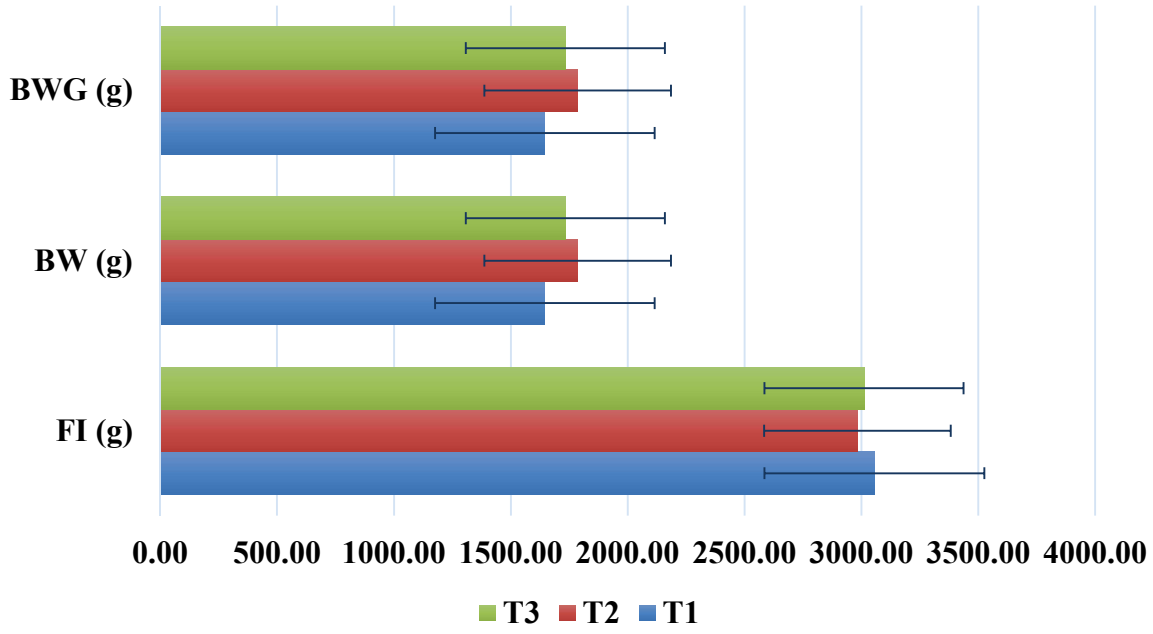
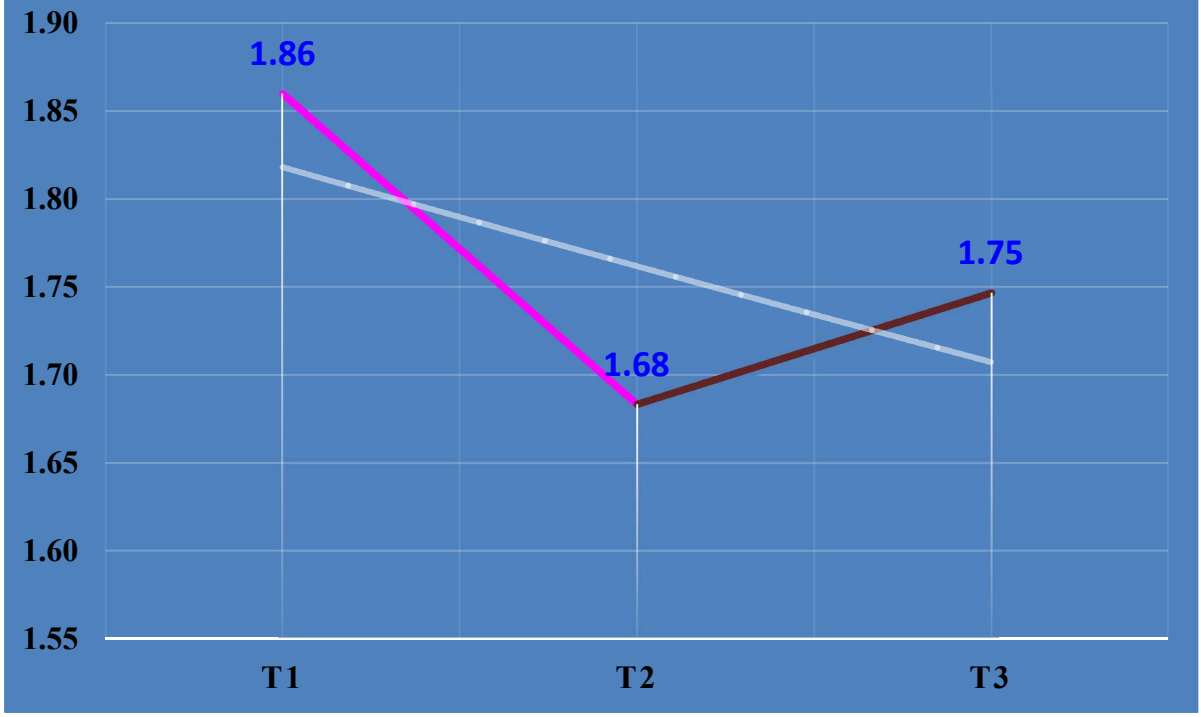


Fig.3. Effect of MOALE and ascorbic acid on FCR



4.2.5 Balance of nutrients

4.2.5.1 Nitrogen retention

Nitrogen retention percentage in broiler birds at 5th week of age is depicted in table-4.6. Nitrogen retention percentage ranged from 51.50 in T1 group to 54.01 in T2 group. Analysis of variance for the effect of treatment on nitrogen retention in broiler chicken differed significantly (P<0.05). In MOALE supplemented group birds shown higher nitrogen retention (4.87 %) followed by ascorbic acid fed group (3.51%) in comparison with control (Fig. 4). This might have happened due to increased absorption and availability of nutrient at tissues level from offered feed.

4.2.5.2 Energy metabolizability

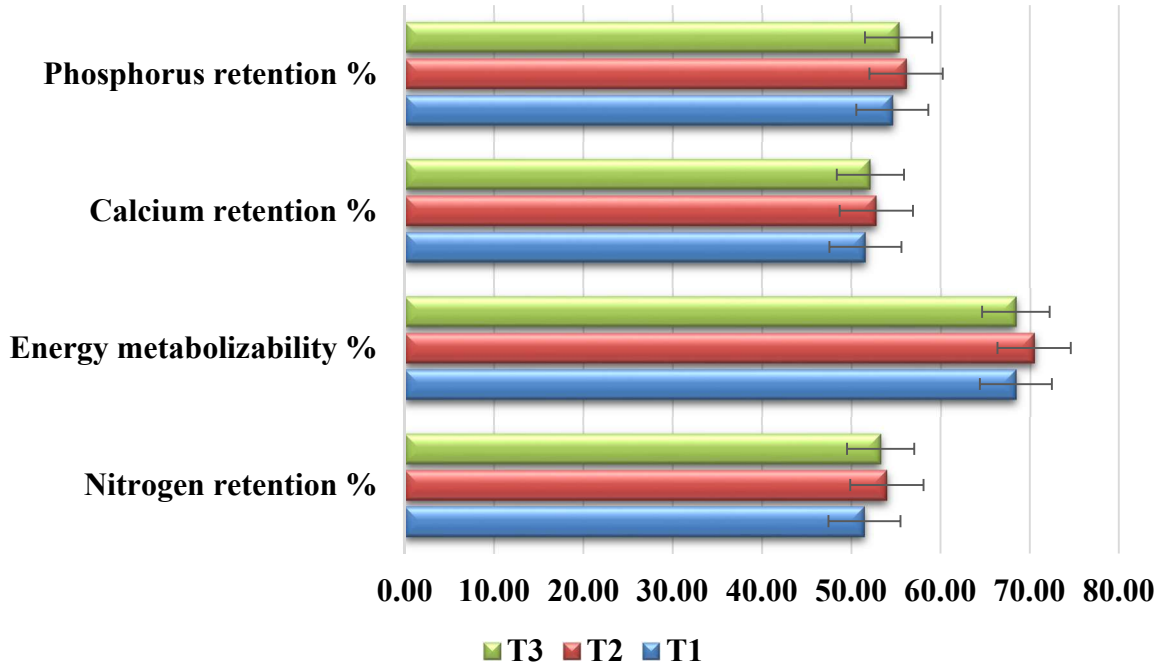
The result of average metabolizability of experimental birds during balance study is mentioned in table-4.6. Analysis of variance for the effect of treatment was found to be non-significant (P>0.05) in either of the treatment groups. It ranged from 68.45 in T1 group to 70.49 in T2 group, respectively (Fig. 4).

Table 4.6 Effect of MOALE and ascorbic acid on nitrogen retention, energy metabolizability, calcium and phosphorus retention percentage at 5th week in broiler chicken

Attributes	T_1	T_2	T_3	SEM	P-value
Nitrogen retention %	51.50 [a] ±0.46	54.01 [b] ±0.40	53.31 [b] ±0.81	0.827	0.023
Energy metabolizability %	68.45 ±0.74	70.49 ±0.57	68.46 ±1.14	1.204	0.182
Calcium retention %	51.62 [a] ±0.39	52.82 [b] ±0.21	52.16 [ab] ±0.38	0.477	0.070
Phosphorus retention %	54.62 ±0.54	56.15 ±0.87	55.33 ±0.77	1.043	0.364

[ab] Values with different superscripts in a row differ significantly (P<0.05)

Fig.4. Effect of MOALE and ascorbic acid on nutrient balance



4.2.5.3 Calcium retention

The result of calcium retention percentage at 5th week of age is presented in table-4.6. The calcium retention in birds ranged from 51.62 in T1 to 52.16 in T3 group. Analysis of variance for the effect of treatment on calcium retention percentage was significantly differed ($P<0.05$) between the groups. The retention of calcium was significantly ($P<0.05$) higher (2.32%) in T2 group followed by T3 group (1.04%) as compared with control (Fig. 4). Such changes might have due to presence of bioceutical agent in MOALE and antioxidant properties of ascorbic acid leads to better absorption and availability of nutrient from offered feed at tissues level.

4.2.5.4 Phosphorus retention

Phosphorus retention percentage in broiler birds at 5th week of age is given in table-4.6. Phosphorus retention percentage ranged from 54.62 in T1 to 56.15 in T2 group, respectively. Analysis of variance for the effect of treatment on phosphorus retention in broiler chicken was non-significantly ($P>0.05$) and found comparable among the groups (Fig. 4).

4.3 EFFECT OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON HAEMATO-BIOCHEMICAL PROFILE, ANTIOXIDANT STATUS AND IMMUNE RESPONSE IN BROILER CHICKEN

4.3.1 Haematological parameters

4.3.1.1 Haemoglobin

The result of haemoglobin (Hb) level in whole blood at 5th week of age in broiler chicken is depicted in table-4.7. Average haemoglobin level varied from 10.93 g/dl in T3 group to 11.19 g/dl in T1 group. Statistical analysis for the effect of treatment on haemoglobin level in birds showed non-significant changes (P>0.05) and found comparable among the groups. Result showed no any untoward effect of MOALE and ascorbic acid on physiological status of birds.

4.3.1.2 Packed cell volume

The result of packed cell volume (PCV) level in blood at 5th week of age in broiler birds is presented in table-4.7. Average PCV level in whole blood sample varied from 27.75 percent in T1 group to 29.72 percent in T2 group. Statistical analysis for the effect of treatment on PCV level in birds shown non-significant changes (P>0.05) and it was comparable among the groups. However, PCV level was slightly higher side in T2 followed by T3 in comparison with control group.

4.3.1.3 Total erythrocyte count

The result of total erythrocyte count (TEC) level in blood sample at 5th week of age in broiler bird is presented in table-4.7. Average TEC varied between 2.82 x 10^6µL in T1 group to 2.96 x 10^6µL in T2 group. There were no any significant differences (P>0.05) found among the treatment groups as compare to control. However, TEC level in MOALE group was slightly higher side followed by ascorbic acid fed group in comparison with control.

4.3.1.4 Total leucocyte count

The result of total leucocyte count (TLC) level in blood sample at 5th week of age in broiler bird is depicted in table-4.7. Average TLC varied between 12.74 x 10^3µL in T3 group to 15.50 x 10^3µL in T1 group. There were no any significant differences (P>0.05) found and comparable among the treatment groups as compare to control.

4.3.1.5 Mean corpuscular volume

The result of mean corpuscular volume (MCV) level in whole blood at 5th week of age in broiler bird is presented in table-4.7. Average MCV level varied between 4.49 fL in T2 group to 5.81 fL in T1 group. MCV level was found to be highest in control group and lowest

in MOALE group. There was no significant difference (P>0.05) found and comparable among the treatment group.

4.3.1.6 Mean corpuscular haemoglobin

The result of mean corpuscular haemoglobin (MCH) level in blood sample at 5th week of age in broiler bird is given in table-4.7. Average MCH level in whole blood varied from 3.78 pg in T2 group to 4.13 pg in T1 group. Statistical analysis for the effect of treatment on MCH level in bird was non- significant (P>0.05) and found comparable among the groups.

4.3.1.7 Mean corpuscular haemoglobin concentration

The result of mean corpuscular haemoglobin concentration (MCHC) level in blood sample at 5th week of age in broiler chicken is presented in table-4.7. Average MCHC level in blood varied from 72.28 g/dl in T1 group to 89.53 g/dl in T3 group. Statistical analysis for the effect of treatment on MCHC level in broiler birds was non-significant (P>0.05) and found comparable among the groups.

4.3.1.8 Neutrophil

The result of neutrophil level in whole blood sample of broiler chicken at 5th week of age is presented in table-4.7. Average neutrophil level varied between 23.33 % in T1 group to 25.00 % in T3 group. The data were not significantly differed (P>0.05) among various treatment groups as comparable to control group. However, numerical value of T3 group was higher side followed by T2 group than control.

4.3.1.9 Lymphocyte

The result of lymphocyte percentage in blood sample at 5th week of age in broiler bird is presented in table-4.7. Average lymphocyte level varied between 53.83 % in T1 group to 60.50 % in T2 group. Lymphocyte level was found to be significantly higher (P<0.01) in MOALE supplemented group followed by ascorbic acid offered group in comparison with control. The enhanced level of lymphocyte reflected better immunity of birds in treatment group as compared with control which might be due to immunomodulatory effect and antioxidant effect of *Moringa oleifera* and ascorbic acid.

4.3.1.10 Monocyte

The result of monocyte percentage in whole blood samples of broiler chicken at 5th week of age is given in table-4.7. Average monocyte level ranged between 5.17 % in T1 to 6.00 % in T2 group. There was no significant difference (P>0.05) among various treatment groups and also between control and treatment group. However, numerical value of T2 group was higher side.

Table 4.7 Effect of MOALE and ascorbic acid on haematological indices in broiler chicken

Attributes	T_1	T_2	T_3	SEM	P-value
Hb (g/dl)	10.98 ±0.39	11.19 ±0.50	10.93 ±0.49	0.658	0.920
PCV (%)	27.75 ±2.50	29.72 ±1.08	28.47 ±1.37	2.492	0.731
TEC ($x10^6$ µL)	2.82 ±0.12	2.96 ±0.29	2.91 ±0.27	0.335	0.908
TLC ($x10^3$ µL)	15.50 ±1.11	13.26 ±0.82	12.74 ±1.40	1.605	0.221
MCV (fL)	5.81 ±0.60	4.49 ±0.30	4.57 ±0.65	0.760	0.185
MCH (pg)	4.13 ±0.40	3.78 ±0.17	3.92 ±0.37	0.465	0.747
MCHC (g/dl)	72.28 ±4.72	85.77 ±6.04	89.53 ±7.72	8.886	0.159
Neutrophil (%)	23.33 ±1.86	24.00 ±2.22	25.00 ±1.13	2.536	0.806
Lymphocyte (%)	53.83 [a] ±1.58	60.50 [b] ±1.23	58.50 [b] ±1.06	1.849	0.008
Monocyte (%)	5.17 ±0.70	6.00 ±0.45	5.33 ±0.67	0.871	0.609
Eosinophil (%)	3.00 ±0.37	2.83 ±0.40	3.33 ±0.67	0.702	0.772
Basophil (%)	0.50 ±0.22	0.67 ±0.33	0.67 ±0.33	0.426	0.904

[ab] Values with different superscripts in a row differ significantly (P<0.05; P<0.01)

4.3.1.11 Eosinophil

The result of eosinophil level in broiler chicken blood sample at 5th week of age is presented in table-4.7. Average eosinophil level ranged between 2.83 % in T2 to 3.33 % in T3 group. There was no significant difference (P>0.05) among various treatment groups as

compared to group. However, numerical value of ascorbic acid supplemented group was found to be higher side.

4.3.1.12 Basophil

The result of basophil level in broiler chicken blood sample at 5th week of age is presented in table-4.7. The average basophil level ranged from 0.50% in T1 to 0.67 % in T2 and T3, respectively. Statistical analysis shown non-significant difference between the treatment and comparable among the groups.

Zanu et al. (2012) reported that offering *Moringa oleifera* leaf meal as a partial substitute of fishmeal in broiler chicken ration the mean corpuscular haemoglobin (MCH) and other haematological indices were not significantly affected that indicated the diets offered were nutritionally adequate to meet the nutrient needs of the birds. Moreover, haemoglobin, white blood cell and packed cell volume remained unaffected due to the addition of extract of *Azadirachta indica* and *Moringa oelifera* leaf extracts (Mahmood et al., 2015). Tijani et al. (2015) found that inclusion of MOLM in birds shown unchanged haemoglobin values among the groups but reduced significantly in white blood cell count in 20% MOLM. However, Nihad et al. (2016) found no significant effect of Moringa leaf meal supplementation on broiler haematological parameters (Hb, RBC and WBC) compared with normal diets. Moreover, Sigolo et al. (2018) studied on Japanese quails fed diets containing different supra-nutritional levels of vitamin E and C and they found that RBCs, MCV, MCH and MCHC were higher side in treatment group as compared with control.

4.3.2 Serum biochemical parameters

4.3.2.1 Total protein

The result of total protein level in serum at 5th week of age in broiler chicken is presented in table-4.8. Average total protein level varied between 3.73 g/dl in T1 group to 4.54 g/dl in T2 group. Statistical analysis for the effect of treatment on total protein level in bird was significantly not different ($P>0.05$) and found comparable among the groups. However, the serum protein level was found to be higher side in MOALE supplemented group in comparison with control.

4.3.2.2 Albumin

The result of average albumin level in serum at 5th week of age in broiler bird is depicted in table-4.8. Average total albumin level varied between 1.62 g/dl in T3 group to 1.76 g/dl in T2 group. Average albumin level was found to be highest in T2 group followed by T1

(1.72 g/dl) and lowest in T3 group. There was no significant difference (P>0.05) among various treatment group and comparable with control group.

4.3.2.3 Globulin

The result of total globulin level in serum at 5th week of age in broiler bird is presented in table-4.8. Average total globulin level varied between 2.01 g/dl in T1 group to 2.78 g/dl in T2 group. Statistical analysis for the effect of treatment on total globulin level in birds was non-significant (P>0.05) and found comparable among the groups. The highest globulin was noted in T2 group (2.78 g/dl) followed by T3 group (2.32 g/dl) and lowest in T1 group (2.01 g/dl).

4.3.2.4 Albumin globulin ratio

The result of average A:G ratio in serum at 5th week of age in broiler chicken is presented in table-4.8. Average A:G ratio ranged between 0.68 in T2 and 0.93 in T1 group. There was no significant difference (P>0.05) found among various treatment groups and comparable with control group. However, numerical value of T1 group was found to be highest and T2 group was lowest.

4.3.2.5 Blood urea nitrogen

The result of blood urea nitrogen (BUN) level in serum at 5th week of age in broiler chicken is presented in table-4.8. Average BUN level varied between 2.92 mg/dl in T3 group to 3.13 mg/dl in T1 group. Statistical analysis for the effect of treatment on BUN level in bird was found to be non-significant (P>0.05) comparable among the groups. The highest BUN level was estimated in T1 group (3.13 mg/dl) and lowest in T3 group (2.92 mg/dl), however T2 group have 2.97 mg/dl BUN.

4.3.2.6 Creatinine

The result of serum creatinine level in broiler chicken at 5th week of age is presented in table-4.8. Average serum creatinine level varied between 0.37 mg/dl in T2 group to 0.45 mg/dl in T1 followed by T3 group (0.40 mg/dl). Statistical analysis for the effect of treatment on creatinine level in bird was found to be significantly different (P=0.10). The highest creatinine level was noted in T1 and lowest in T2 group. The creatinine level noted in MOALE group was 17.77% lower followed by 11.11% in ascorbic acid groups in comparison with control group. During protein metabolism generation of creatinine takes place as a waste molecule and reduction in creatinine indicates retarded catabolism rate in broiler birds.

4.3.2.7 Aspartate transaminase

The result of average aspartate transaminase (AST) level in serum at 5th week of age in broiler chicken is presented in table-4.8. Average serum total AST level varied between 241.73

U/lit in T2 group to 267.88 U/lit in T1 group. Average AST level was found to be highest in T1 followed by T3 (250.85 U/dl) and lowest in T2 group. There was no significant difference (P>0.05) noted among the treatment group in comparison with control group.

4.3.2.8 Alanine transaminase

The result of average alanine transaminase (ALT) level in serum at 5th week of age in broiler chicken is presented in table-4.8. Average total ALT level varied between 53.85 U/lit in T2 group to 57.71 U/lit in T1 group. Average ALT level was found to be highest in T1 group followed by T3 group (55.48 U/lit) and lowest in T2 group. There was no significant difference (P>0.05) found among treatment group in comparison with control group.

4.3.2.9 Total cholesterol

The result of total cholesterol level in serum at 5th week of age in broiler chicken is presented in table-4.8. Average total cholesterol level varied between 108.88 mg/dl in T2 group to 122.15 mg/dl in T1 group (Fig. 5). Statistical analysis for the effect of treatment on total cholesterol level in birds was non-significantly (P>0.05) differed. However, the total cholesterol level in T1 group was found to be highest followed by T3 group (112.70 mg/dl) and lowest in T2 group. As reduction in total cholesterol might be due to presence of β-sitosterol a bioactive compound present in *Moringa oleifera* leaf and that β-sitosterol causes lowering of plasma concentration of LDL which results deceased cholesterol percentage in blood serum.

4.3.2.10 Triglyceride

The result of total triglyceride level in the serum sample at 5th week of age in broiler chicken is depicted in table-4.8. Average total triglyceride level varied between 99.72 mg/dl in T2 group to 104.11 mg/dl in T3 group (Fig. 5). Statistical analysis for the effect of treatment on total triglyceride level in birds was non-significantly differed (P>0.05) and found comparable among the groups. The highest triglyceride level was noted in T1 group followed by T3 group (104.11 mg/dl) and lowest in T2 group.

4.3.2.11 High density lipoprotein

The result of high density lipoprotein (HDL) level in serum at 5th week of age in broiler bird is presented in table-4.8. Average serum HDL level ranged from 25.22 mg/dl in T2 and 28.54 mg/dl in T1 group followed by 104.11 mg/dl in T3 group (Fig. 5). There was no significant difference (P>0.05) found among treatment groups in comparison with control group. However, numerical value of HDL for T1 group was highest and lowest for T2 group.

Table 4.8 Effect of MOALE and ascorbic acid on serum biochemistry in broiler chicken

Attributes	T_1	T_2	T_3	SEM	P-value
Total protein (g/dl)	3.73 ±0.24	4.54 ±0.33	3.95 ±0.21	0.379	0.121
Albumin (g/dl)	1.72 ±0.09	1.76 ±0.08	1.62 ±0.08	0.114	0.484
Globulin (g/dl)	2.01 ±0.23	2.78 ±0.30	2.32 ±0.23	0.359	0.135
A:G ratio	0.93 ±0.14	0.68 ±0.10	0.75 ±0.10	0.166	0.334
BUN (mg/dl)	3.13 ±0.25	2.97 ±0.09	2.92 ±0.14	0.243	0.668
Creatinine (mg/dl)	0.45 [a] ±0.02	0.37 [b] ±0.02	0.40 [ab] ±0.03	0.033	0.105
AST (U/lit)	267.88 ±12.53	241.73 ±8.10	250.85 ±12.37	15.82	0.275
ALT (U/lit)	57.71 ±4.67	53.85 ±6.01	55.48 ±4.86	7.374	0.872
Cholesterol (mg/dl)	122.15 ±15.78	108.88 ±5.92	112.70 ±9.53	15.80	0.695
Triglyceride (mg/dl)	102.12 ±3.67	99.72 ±1.19	104.11 ±5.54	5.512	0.732
HDL (mg/dl)	28.54 ±3.95	25.22 ±1.48	26.18 ±2.38	3.952	0.695
LDL (mg/dl)	73.19 ±11.53	63.72 ±4.55	65.70 ±7.18	11.69	0.700
VLDL (mg/dl)	20.43 ±0.73	19.95 ±0.24	20.82 ±1.11	1.103	0.734

[ab] Values with different superscripts in a row differ significantly ($P<0.05$)

4.3.2.12 Low density lipoprotein

The result of low density lipoprotein (LDL) level in serum at 5th week of age in broiler chicken is given in table-4.8. Average total LDL level varied between 63.72 mg/dl in T2 group

to 73.19 mg/dl in T1 group. Statistical analysis for the effect of treatment on total LDL level in broiler was non-significant (P>0.05) and found comparable between the groups. The highest LDL level was noted in T1 group followed by T3 group (65.70 mg/dl) and lowest in T2 group.

4.3.2.13 Very low density lipoprotein

The result of very low density lipoprotein (VLDL) level in serum at 5th week of age in broiler bird is presented in table-4.8. Average VLDL level varied between 19.95 mg/dl in T2 group to 20.43 mg/dl in T3 group. Statistical analysis for the effect of treatment on total VLDL level in broiler was non-significant (P>0.05) and found comparable among the groups. The highest VLDL level was estimated in T3 group followed by T1 group (20.43 mg/dl) and lowest in T2 group (Fig. 5).

Zanu et al. (2012) found that the triglycerides, VLDL and LDL values in blood serum of broiler chickens were significantly different in *Moringa oleifera* leaf meal fed group (P<0.05), whereas no significant changes in total cholesterol, HDL, total protein and glucose values were noted. Aderinola et al. (2013) reported that *Moringa oleifera* leaf supplementation in broiler ration triglycerides, total cholesterol and urea in blood serum of broilers were significantly different among the groups, however, SGPT and SGOT values were comparable among the group. Divya et al. (2014) found that with increased level of MOL powder in treatment a significant reduction in serum creatinine level which indicates retarded catabolism rate in broilers. Nihad et al. (2016) revealed that the supplementation of Moringa leaf meal in broiler production and health found more improvement of blood biochemical, lipid profile (triglycerides, total cholesterol, HDL, LDL and VLDL) in comparing with normal diets. Moreover, Allam et al. (2016) found that the effect of *Moringa oleifera* leaf extract in broiler chickens induced significant increase in total proteins, albumin, globulins and slight increase in AST, ALT, ALP whereas, decreased creatinine level noted. Sigolo et al. (2018) reported that supplementation of vitamin E and C in Japanese quails reduced serum uric acid, creatinine, LDL, triglycerides, aspartate amino transferase, alanine amino transferase and albumin concentration, whereas increased HDL, total protein, calcium and phosphorous level. Present report was an agreement with the different observations recorded by various researchers around the globe and effect of treatment shown good impact on physiological status and health.

4.3.3 Serum antioxidant status

4.3.3.1 Lipid peroxidation

The result of lipid peroxidation level in serum at 5th week of age in broiler chicken is depicted in table-4.9. Average lipid peroxidation level varied between 3.24 nM MDA/ml in T2

group to 4.41 nM MDA/ml in T1 group (Fig. 5). Statistical analysis for the effect of treatment on lipid peroxidation level in chicken was significantly different ($P<0.05$) in treatment groups. The lipid peroxidation concentration in MOALE group was found to be significantly lower (26.53%) followed by ascorbic acid group (25.00%) in comparison with control group. The lipid peroxidation level was highest in T1 group and lowest in T2 group followed by T3 group.

4.3.3.2 Super oxide dismutase

The result of super oxide dismutase (SOD) activity in serum at 5th week of age in broiler chicken is given in table-4.9. Average SOD level in birds varied between 201.69 U/L in T1 group to 229.58 U/L in T2 group (Fig. 5). Statistical analysis for the effect of treatment on SOD level in broiler chicken was significantly not different ($P>0.05$) in treatment groups. However, SOD level in MOALE group was found to be highest followed by ascorbic acid group (214.58 U/L) and lowest in control group. The SOD level in T2 group was 13.82 % higher followed by T3 group (6.39 %) in comparison with control. The increased level of SOD shown in T2 and T3 group having better impact of treatment in reduction of stress in birds.

4.3.3.3 Reduced glutathione

The result of reduced glutathione (GSH) in serum at 5th week of age in broiler chicken is given in table-4.9. Average reduced glutathione level in birds varied between 0.31 mM/ml in T1 group to 0.36 mM/ml in T2 group (Fig. 5). Statistical analysis for the effect of treatment on GSH level in broiler chicken was significantly not different ($P>0.05$) in treatment groups. Moreover, GSH concentration in MOALE group was found to be highest followed by ascorbic acid group (0.34 mM/ml) and lowest in control group. The GSH concentration was 16.12 % higher in T2 group followed by T3 group (9.67%) in comparison with control. The increased concentration of GSH in serum of birds shown in treatment group T2 and T3 having better impact on stress reduction.

4.3.3.4 Lactate dehydrogenase

The result of lactate dehydrogenase (LDH) activity in serum at 5th week of age in broiler chicken is presented in table-4.9. Average lactate dehydrogenase level in birds varied between 506.55 U/L in T2 group to 538.32 U/L in T1 group (Fig. 5). Statistical analysis for the effect of treatment on LDH activity in broiler chicken was significantly not different ($P>0.05$) between treatment groups. However, LDH activity in MOALE group was found to be lowest followed by ascorbic acid group (530.58 U/L) and highest in control group. The LDH level was 5.90% lower in T2 group followed by T3 group (1.43 %) in comparison with control. The decreased pattern of GSH serum concentration in of birds shown in treatment group T2 and T3 having better health impact in chicken.

4.3.3.4 Catalase

The result of catalase activity in serum at 5th week of age in broiler chicken is presented in table-4.9. Average catalase activity in birds varied between 26.04 U/ml in T1 group to 34.58 in T2 group (Fig. 5). Statistical analysis for the effect of treatment on catalase activity in broiler chicken was significantly not different (P>0.05) between treatment groups. However, catalase level in MOALE group was found to be highest followed by ascorbic acid group (28.99 U/ml) and lowest in control group. The catalase level was 32.00% higher in T2 group followed by T3 group (11.32%) in comparison with control. The increasing pattern of serum catalase activity in of birds shown in treatment group T2 and T3 having better productivity impact in chicken.

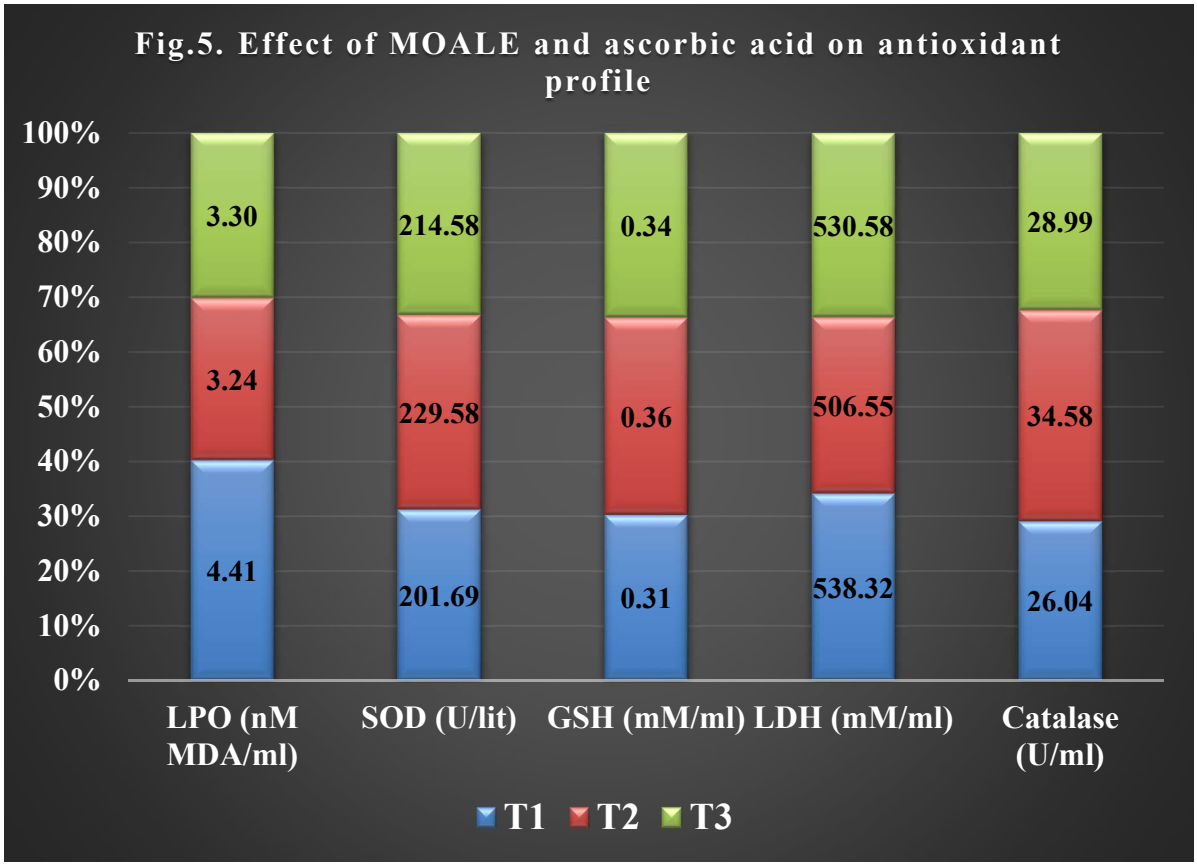


Table 4.9 Effect of MOALE and ascorbic acid on antioxidant profile, immune status and HSP70 gene expression analysis in broiler chicken

Attributes	T_1	T_2	T_3	SEM	P-value
Antioxidant parameters					
Lipid Peroxidation (nM MDA/ml)	4.41 [a] ±0.34	3.24 [b] ±0.12	3.30 [b] ±0.41	0.449	0.03
Super Oxide Dismutase (U/lit)	201.69 ±15.41	229.58 ±4.71	214.58 ±7.81	14.62	0.196
Reduced Glutathione (mM/ml)	0.31 ±0.02	0.36 ±0.03	0.34 ±0.02	0.035	0.284
Reduced Glutathione (mM/ml)	538.32 ±45.98	506.55 ±26.39	530.58 ±31.01	50.15	0.806
Catalase (U/ml)	26.04 ±4.69	34.58 ±1.43	28.99 ±4.25	5.298	0.292
Immunity parameter					
HI titre	5.67 ±2.09	50.00 ±24.74	20.00 ±8.94	21.54	0.145
HSP70 gene expression analysis					
Fold chain	4.24 ±0.66	0.02 ±0.00	2.84 ±1.65	1.454	0.129

[ab] Values with different superscripts in a row differ significantly ($P<0.05$)

Moyo et al. (2012) investigated the antioxidant potency of *Moringa oleifera* leaves and found that MOLE increased the antioxidant activity of GSH, SOD and catalase, whereas, lipid peroxidation was significantly reduced. Kulkarni et al. (2012) noticed that the dietary supplementation of ascorbic acid in broiler chicken had comparable on serum lipid peroxidation but higher reduced glutathione activity. The antioxidant compound like polyphenols, tannins, anthocyanin, glycosides present in MOLE may attach to the cytoplasmic membrane and remove free radicals, activate antioxidant enzymes, and inhibit oxidases thus, making these elements more available for the birds to use (Luqman et al., 2012). However,

AbouSekken (2015) reported that aqueous *Moringa oleifera* leaf extract given via drinking water appeared to be a good feed additive in order to obtain the best growth, better disease resistance as well as the overall better health of broiler. Allam et al. (2016) revealed that Moringa leaf extracts had significantly enhanced SOD value beside significant decrease in MDA in birds. Moreover, Khan et al. (2017) reported that aqueous extract remarkably inhibited the activity of amylase and glycosidase and it displayed improved antioxidant capacity. Cui et al. (2018) revealed that plasma total anti-oxidative capacity, total superoxide dismutase, glutathione peroxidase activities were increased, whereas, MDA decreased in response to dietary MOL supplementation. However, Gan et al. (2018) reported that dietary inclusion of ascorbic acid on old hen performance, immunity and its antioxidant status and found that improvement in the health of old layers by enhancing immunity and antioxidant capacity. So, the observation of present study was an agreement with the finding of different workers in poultry throughout the world as the effect of treatment had positive impact in birds.

4.3.4 Immune status

4.3.4.1 Haemaglutination inhibition (HI) assay

The result of haemaglutination inhibition (HI) titre in serum at 5th week of age in broiler chicken vaccinated against NDV antigen is presented in table-4.9. Average HI titre in birds varied between 5.67 in T1 group to 50.00 in T2 group (Fig. 5). Statistical analysis for the effect of treatment on HI titre in broiler chicken was significantly not different ($P>0.05$) between treatment groups. However, result showed that HI titre in MOALE group was found to be highest followed by ascorbic acid group (20.00) and lowest in control group. The higher pattern of HI titre in treatment group birds reflected better immunity in comparison to control which shown good health impact.

Kulkarni et al. (2012) revealed that dietary supplementation of ascorbic acid in broiler chicken had higher cellular and humoral immunity in comparison with control. Moreover, Elagib and Omer (2012) reported that antibody titre against Newcastle virus was increased in birds supplemented with different levels of ascorbic acid in birds. Aqueous *Moringa oleifera* leaf extract given via drinking water, improved the immune response and cause better disease resistance of broiler (AbouSekken, 2015). Allam et al. (2016) reported that Moringa leaf extract have beneficial effect on immunity in broiler birds. Liaqat et al. (2016) noticed enhanced immune response to Newcastle disease vaccination without any change in performance parameters of broiler birds fed on *Moringa oleifera* leaf powder. However, Bhatti

et al. (2016) reported that supplementation of vitamin C and E combination in drinking water at the time of vaccination against NDV improve humoral immune response against NDV. Ramadan et al. (2017) found that birds supplemented with MOLM had significant higher antibody titre against ND compared to un-supplemented birds. Naresh et al. (2017) noticed that antibody titre was significantly higher in the treated groups (poly herbal formulation and synthetic ascorbic acid) as compared to untreated control. Faluyi et al. (2018) found that *Moringa oleifera* leaf extracts supplementation moderately boost immunological responses to ND vaccinations, however, increasing the dose did not enhance the suggested immunomodulatory activity. Faluyi and Agbede (2018) reported that the aqueous leaf extract of *Moringa oleifera* had slight immune stimulatory effects on response to ND vaccinations in broiler chickens. So, the result of present study was an agreement to the finding of various researchers as the MOALE and ascorbic acid had immunomodulatory effect in birds which enhanced performance.

4.3.5 HSP70 gene expression analysis

Result of HSP70 gene expression at 5th week of age in broiler chicken is presented in table-4.9. HSP 70 gene expression analysis not influenced by treatment and showed non-significant difference ($P>0.05$) in expression level however, in MOALE supplemented group HSP 70 gene expression was found minimum followed by ascorbic acid offered group and maximum in control group.

Kulkarni et al. (2012) reported that dietary supplementation of ascorbic acid in broiler chicken significantly down regulated expression of HSP70 gene. Moreover, Hajati et al. (2015) found that HSP70 gene expression in heart and liver of broilers reduced by vitamin C supplementation during chronic heat stress condition. Kumar et al. (2017) determined that betaine or betaine plus ascorbic acid supplementation significantly reduced gene expression of heat shock protein HSP70 in broiler liver in comparison to control group. Mishra et al. (2017) reported that the expression patterns of HSP70 gene provided an indication that AA may be useful in combating rigors of heat stress in chickens. Tekayev et al. (2018) reported that aqueous extract of *Moringa oleifera* reduces the oxidative stress in a unilateral cryptorchidism induced rats, and HSP expression and germ cell apoptosis. However, Ramadan et al. (2019) reported that the supplementation of different levels of vitamin C in broiler chicken, down regulated liver HSP70 expression level.

Fig. 6. Amplification Plots

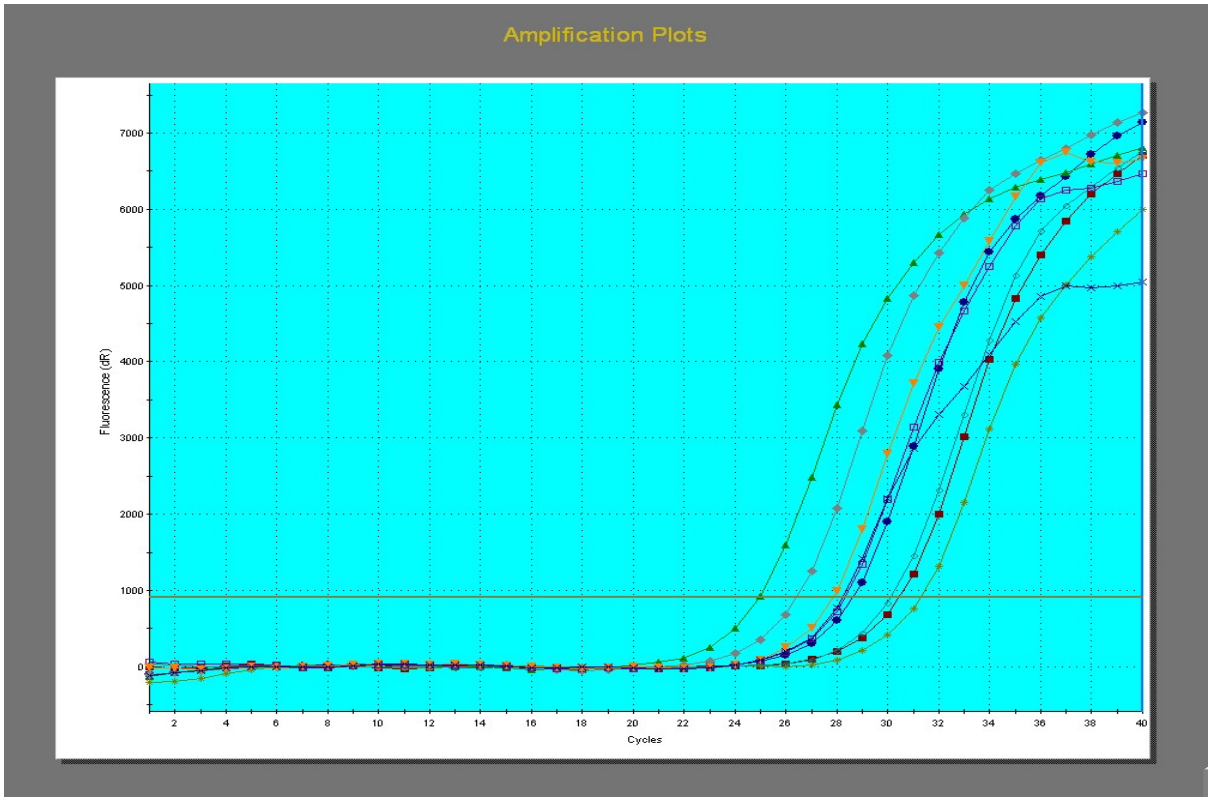
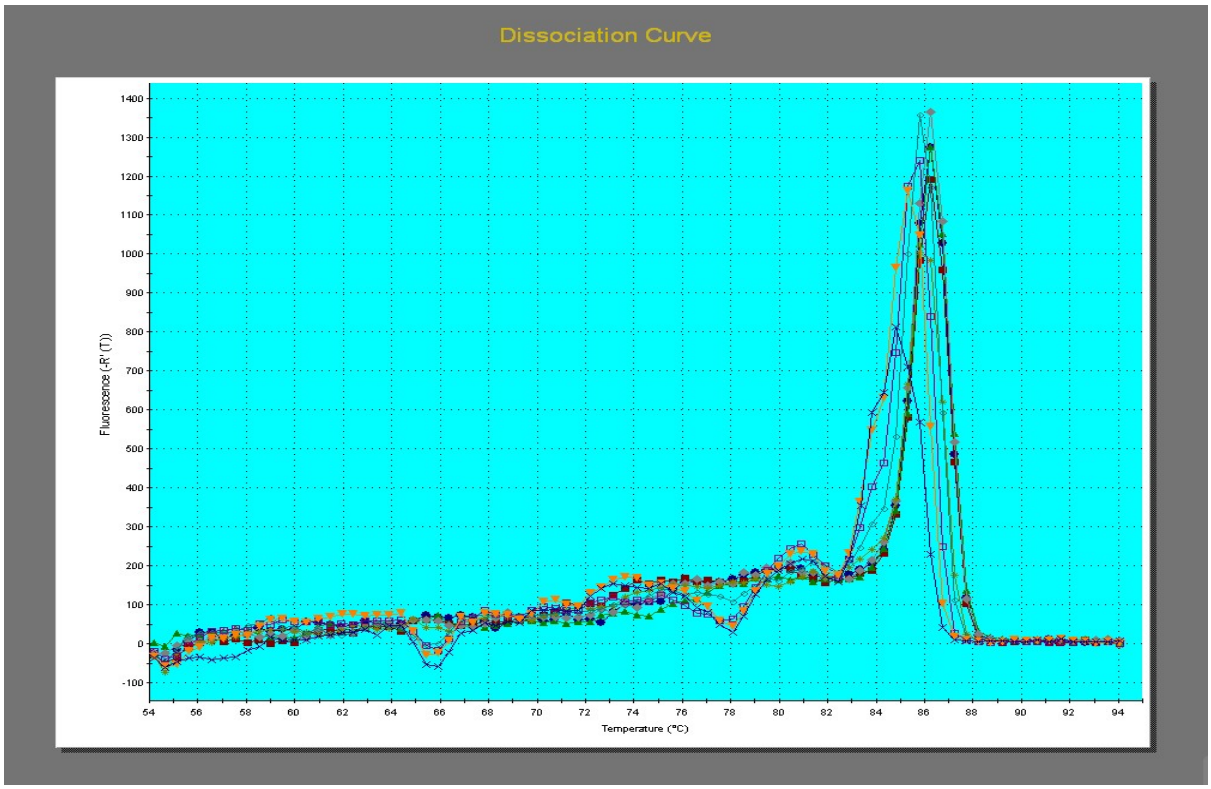


Fig. 7. Dissociation Curve



4.4 EFFECT OF SUPPLEMENTATION OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON CARCASS CHARACTERISTIC OF BROILER CHICKEN

4.4.1 Carcass characteristics

4.4.1.1 Liver

Result of relative weight of liver at 5th week of age in broiler chicken is presented in table-4.10. The weight of liver varied between 44.92 g in T1 group to 47.50 g in T2 group. Statistical analysis for the effect of treatment on relative weight of liver in birds were found to be non-significant (P>0.05) and comparable among the groups. The relative weight of liver in T1 group was found to be lowest while highest in T2 group followed by T3 group (46.00 g), respectively.

4.4.1.2 Heart

Result of relative weight of heart at 5th week of age in broiler chicken is presented in table-4.10. The weight of heart varied between 10.25 g in T1 group to 11.58 g in T3 group. Statistical analysis for the effect of treatment on relative weight of heart in broiler birds were found to be non-significant (P>0.05) and data was comparable among the groups. The weight of heart in T1 group was found to be lowest while highest in T3 group followed by T2 group (10.75 g), respectively.

4.4.1.3 Gizzard

Result of weight of gizzard at 5th week of age in broiler chicken is presented in table-4.10. There was no any significant effect on weight of gizzard noted on treatment group and data was comparable among the groups. The weight of gizzard varied between 37.08 g in T3 group to 39.17 g in T1 group. The weight of gizzard in T1 group was found to be highest followed by T2 group (37.67 g) while lowest in T3 group, respectively.

4.4.1.4 Intestine

Result of intestine weight at 5th week of age in broiler bird is presented in table-4.10. Statistical analysis showed that there was no any significant (P>0.05) changed found in weight of intestine between the treatment group and found comparable among the groups. The weight of intestine varied between 91.67 g in T1 group to 95.17 g in T3 group. The weight of intestine in T1 group was found to be lowest followed by T2 group (91.92 g) while highest in T3 group, respectively.

4.4.1.5 Abdominal fat

Result of abdominal fat weight at 5th week of age in broiler bird is presented in table-4.10. The relative weight of abdominal fat was significantly affected by the treatment. The

weight of abdominal fat varied between 11.02 g in T2 group to 19.57 g in T1 group. Statistical analysis for the effect of treatment on relative weight of abdominal fat in chickens were found to be highly significant (P<0.01). The weight of abdominal fat in T2 group was found to be lowest followed by T3 group (17.30 g) while highest in T1 group, respectively. During the experiment abdominal fat deposits of MOALE group was found significantly (P<0.01) lower (43.68%) followed by ascorbic acid group (11.59%) in comparison with control group. The decreased abdominal fat deposits might be due to hypocholesteromic properties of moringa leaf.

4.4.1.6 Spleen

Result of spleen weight at 5th week of age in broiler bird is presented in table-4.10. The weight of spleen varied between 3.33 g in T2 group to 4.50 g in T1 group. Statistical analysis for the effect of treatment on relative weight of spleen in chickens non-significant (P>0.05) and found comparable among the groups. The weight of spleen in T2 group was found to be lowest followed by T3 group (4.17 g) and highest in T1 group, respectively.

4.4.1.7 Bursa

Result of relative weight of bursa at 5th week of age in broiler chicken is presented in table-4.10. The weight of bursa varied between 2.68 g in T3 group to 3.35 g in T1 group. Statistical analysis for the effect of treatment on relative weight of bursa in birds were non-significant (P>0.05) varied and found comparable among the groups. The weight of bursa in T1 group was found to be highest followed by T2 group (2.85 g) and lowest in T3 group, respectively. The decreased size of bursa might be due to antioxidant effect of ascorbic acid and presence of bioactive molecules in MOALE.

4.4.1.8 Giblet percentage

Result of giblet percentage at 5th week of age in broiler chicken is depicted in table-4.10. The giblet percentage varied between 7.33 in T3 group to 7.73 in T1 group. Statistical analysis for the effect of treatment on giblet percentage in broiler was non-significantly (P>0.05) varied and found comparable among the groups. The giblet percentage in T1 group was found to be highest followed by T2 group (7.46) while lowest in T3 group, respectively.

4.4.1.9 Dressing percentage

The result of dressing percentage at 5th week of age in broiler bird is mentioned in table-4.10. The dressing percentage varied between 70.40 in T2 group to 71.11 in T1 group. The dressing percentage was found to be highest in control group followed by ascorbic acid group and lowest in MOALE group. There was no significant difference (P>0.05) found among the various treatment group and also between control and treatment group.

Table 4.10 Effect of MOALE and ascorbic acid on carcass traits of broiler chicken

Attributes	T_1	T_2	T_3	SEM	P-value
Liver (g)	44.92 ±3.42	47.50 ±5.05	46.00 ±2.66	5.434	0.893
Heart (g)	10.25 ±1.42	10.75 ±1.42	11.58 ±0.79	1.764	0.751
Gizzard (g)	39.17 ±3.57	37.67 ±1.48	37.08 ±2.74	3.868	0.858
Intestine (g)	91.67 ±4.82	91.92 ±6.26	95.17 ±3.98	7.226	0.865
Abdominal fat (g)	19.57 [b] ±1.73	11.02 [a] ±0.73	17.30 [b] ±1.12	1.785	0.001
Spleen (g)	4.50 ±0.67	3.33 ±0.95	4.17 ±0.31	0.985	0.492
Bursa (g)	3.35 ±0.57	2.85 ±0.31	2.68 ±0.26	0.572	0.502
Giblet %	7.73 ±0.66	7.46 ±0.39	7.33 ±0.28	0.664	0.829
Dressing %	71.11 ±0.71	70.40 ±0.50	70.80 ±0.28	0.746	0.642
Eviscerated %	60.03 ±0.82	59.94 ±0.73	60.20 ±0.43	0.964	0.964

[ab] Values with different superscripts in a row differ significantly (P<0.05; P<0.01)

4.4.1.10 Eviscerated percentage

The eviscerated percentage at 5th week of age in broiler chicken is presented in table-4.10. It varied between 60.20 in T3 group to 59.94 in T2 group. Statistical analysis for the effect of treatment on eviscerated percentage in broiler was non-significant (P>0.05). Eviscerated percentage in T2 group was found to be lowest followed by T3 and highest in control group, respectively.

Zanu et al. (2012) found that *Moringa oleifera* leaf meal supplementation in broiler chicken diets had not any significant (P>0.05) effect on carcass characteristic parameters. Aderinola et al. (2013) reported that supplementation of *Moringa oleifera* leaf in broiler diets, decreased abdominal fat proportion was noted and the utilization of MOLM in broiler diet

could be adopted when the motive is production of broiler meat with low fat deposit is targeted. However, Safa (2014) revealed that the inclusion of MOLM in broiler diets significantly ($P<0.05$) improved hot and cold eviscerated carcass weight, dressing percentage, breast and drumstick percentages for both breast and thigh meat. Madukwe et al. (2013) observed and found that birds offered on *Moringa oleifera* aqueous extract diets exhibit a less acceptable colouration despite the high beta-carotene and vitamin C (6.26mg/100ml extract) content of the leaf. Moreover, Younis and Elbestawy (2017) reported that the water Supplementation of *Moringa oleifera* in broiler birds had no any significant effect between treatments in carcass characteristics. Cui et al. (2018) found that the dietary supplementation of *Moringa oleifera* leaf in broiler chicken had decreased abdominal fat linearly, whereas, Kumar et al. (2018) reported that carcass characteristics were significantly ($P<0.05$) affected in diet containing MOLM, however, sensory evaluation parameters like appearance, flavour, tenderness, juiciness and palatability were highly significant ($P<0.01$) with increasing level of MOLM.

4.4.2 Meat chemical composition

4.4.2.1 Moisture

The result of moisture percentage in broiler meat sample at 5th week of age is depicted in table-4.11. It varied between 73.19 in T2 group to 73.94 in T1 group. Statistical analysis showed that the moisture percentage in broiler meat was significantly not differed ($P>0.05$) and found comparable among the groups. Numerical data of moisture percentage showed highest in T1 group followed by T3 group and lowest in T2 group, respectively.

4.4.2.2 Dry Matter

The result of dry matter percentage in broiler chicken carcass sample at 5th week of age is presented in table-4.11. Dry matter percentage of meat sample varied between 26.06 in T1 group to 26.81 in T2 group. Statistical analysis showed that the dry matter percentage in broiler chicken was significantly not differed ($P>0.05$) and found comparable between the treatment groups. The dry matter percentage was found to be highest in T2 group followed by T3 group (26.10) and lowest in T1 group, respectively.

Table 4.11 Effect of MOALE and ascorbic acid on meat chemical composition (%) of broiler chicken

Attributes	T_1	T_2	T_3	SEM	P-value
Moisture	73.94 ±0.64	73.19 ±0.40	73.90 ±0.14	0.623	0.450
Dry Matter	26.06 ±0.64	26.81 ±0.40	26.10 ±0.14	0.623	0.450
Organic Matter	98.95 ±0.10	98.86 ±0.04	98.81 ±0.03	0.900	0.344
Crude Protein	22.09 ±0.63	22.02 ±0.70	22.41 ±0.58	0.090	0.901
Ether Extract	8.66 ±0.35	9.21 ±0.67	7.85 ±0.61	0.794	0.302
Total Ash	1.05 ±0.10	1.14 ±0.04	1.19 ±0.03	0.090	0.344

Values with different superscripts in a row differ significantly (P<0.05)

4.4.2.3 Organic matter

The result of organic matter percentage in broiler chicken carcass sample at 5th week of age is mentioned in table-4.11. Statistical analysis for the effect of treatment on organic matter in broiler bird was found to be non-significant (P>0.05) and comparable among the different groups. Organic matter in T1 group was found to be highest (98.95) followed by T2 group (98.86) and lowest in T3 group (98.81), respectively.

4.4.2.4 Crude protein

The result of crude protein percentage at 5th week of age in meat of broiler birds is presented in table-4.11. The crude protein level of carcass varied between 22.41 in T3 group to 22.02 in T2 group. There was no significant difference (P>0.05) found among various treatment group and also between control and treatment group. However, numerical value of T3 group was higher side than T2 and T1 groups.

4.4.2.5 Ether extract

The result of fat percentage at 5th week of age in broiler meat sample is presented in table-4.11. The percentage of fat in broiler carcass was varied between 7.85 in T3 group to 9.21

in T2 group. Statistical analysis for the effect of treatment on ether extract in meat sample was found to be non-significant (P>0.05). Ether extract in MOALE fed group was found to be highest followed by control group (8.66 %) and lowest in ascorbic acid group, respectively.

4.4.2.5 Total Ash

The result of total ash percentage at 5th week of age in broiler meat sample is depicted in table-4.11. The total ash percentage in broiler meat sample varied from 1.05 in T1 group to 1.19 in T3 group. Statistical analysis for the effect of treatment on total ash in meat of broiler was found to be non-significant (P>0.05) and data was comparable among the treatment group and control. However, total ash in T3 group was found to be highest followed by T2 group (1.14 %) and lowest in T1 group, respectively.

The redness values were higher in the diet which had MOLM inclusion treatment and the reason could be due to the iron consumed by the broilers on the MOLM diet, which could have increased haemoglobin and myoglobin concentrations (Sreelatha and Padma, 2009). Tavarez et al. (2011) reported that inclusion of antioxidant in broiler diet improved meat quality and extended shelf life so, supplementation of antioxidant source in poultry feed is an efficient method for increasing meat oxidative stability. Moreover, the feeding of *Moringa oleifera* leaf meal as an additive in broiler chicken showed higher values for lightness which could be due to the antioxidant activities in *M. oleifera*, such as vitamin C, E and selenium (Moyo et al., 2011). However, Ologhobo et al. (2014) reported that the supplementation of *Moringa oleifera* leaf meal in the diet of broiler chickens had no significant effect on carcass characteristics (flavor and color). Kumar et al. (2018) reported that MOLM supplementation in birds improved overall meat quality and sensory parameters.

4.5 EFFECT OF SUPPLEMENTATION OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON ECONOMICS OF BROILER CHICKEN PRODUCTION

The production economics influenced by different dietary treatment is shown in table-4.12. Total input cost per bird was calculated on the basis of total feed cost and cost of chicks, vaccines and miscellaneous expenditure. The water supplementation of MOALE and ascorbic acid in broiler, decreased the production cost by enhancement of live weight gain. However, the profit per kg live weight was maximum in MOALE supplemented group followed by ascorbic acid group and least profit noted in control group.

Table 4.12 Production economics influenced by MOALE and ascorbic acid supplementation in broiler chicken

Attributes	T_1	T_2	T_3
Feed consumed/bird (kg)	3.06	2.98	3.01
Feed cost per kg (Rs.)	34.60	34.60	34.60
Feed cost per bird (Rs.)	105.72	103.21	104.19
Cost per Chicks	38.00	38.00	38.00
Miscellaneous cost per bird (Rs.)	12.00	15.00	13.50
Total cost per bird (Rs.)	155.72	156.21	155.69
Average live weight per bird (kg)	1.65	1.79	1.73
Market price per bird (@ Rs. 100/- per kg)	164.59	178.54	173.35
Net profit per bird (Rs.)	8.87	22.32	17.66
Profit per kg live weight (Rs.)	5.39	12.50	10.19

Ayssiwede et al. (2011) reported that incorporation of MOLM in the diets of growing Indigenous Senegal chickens achieved lowest feed cost/kg carcass when 8% and 16% of *Moringa oleifera* leaf meal was introduced into the diets of the birds. Talha (2013) found that the inclusion of *Moringa* leaf meal could be cost effective at 8% and 16% introduction in the diet of indigenous chickens. Makanjuola et al. (2014) reported that the inclusion of MOLM in broiler chickens, lower the production cost per kilo gram weight gain as compared to control diet. Karthivashan et al. (2015) revealed that the supplementation of *Moringa oleifera* leaf extract could be an efficient and cost-effective feed supplement for broiler production. Kumar et al. (2018) reported that the supplementation of 5% followed by 10% *Moringa oleifera* leaf meal in cross breed indigenous birds shown significant improvement in overall performance achieving maximum profit. Tesfaye et al. (2018) assessed the feeding value of MOLM in layer ration and suggested that 5% inclusion of MOLM as an additive in the poultry industry may serve the sector by enhancing the product quality besides serving as protein feed. However, Sigolo et al. (2019) reported that supplementation of vitamin E and C at different levels could enhanced Japanese quail production and got more profit.

Chapter-V

Summary and Conclusions

Livestock farming is one of the key components of Indian agriculture and contributes on large scale in the income of rural farmers. The increasing price of conventional cereals and protein sources sustainability of this sectors may have affected which results increase in cost of production. Poultry sector plays important role in minimizing the protein and calorie deficiency of large human population of our country. Ascorbic acid is an important scavenger of free radical produced in the system during stress condition however, *Moringa oleifera* is nutrient rich high nutritional value species. Hence, supplementation of ascorbic acid and Moringa tree leaf extract could be a possible alternative to improve the production performance by reducing the stress of broiler birds with quality product production. The limited studies on these aspect has been done and in the perspective of Bihar state no study was performed. So, experiment was conducted on the comparative effect of *Moringa oleifera* aqueous leaf extract and ascorbic acid supplementation on production performance, antioxidant status and immune response in broiler chicken.

All the standard managemental practices were followed during experimental period. 135 day-old broiler chicks were weighed and randomly divided into three experimental groups including control of 45 chicks in each group and further replicated with 15 chicks each as replicate. In this study different parameter like feed intake, body weight gain, feed conversion ratio, blood bio-chemicals, antioxidant activity, immune status, HSP70 gene expression analysis, carcass traits and economy of production of broiler chickens were observed, respectively during 35 days of experiment.

5.1 EVALUATION OF TOTAL ANTIOXIDANT CAPACITY OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID

The DPPH radical scavenging activity of MOALE was calculated with reference to different ascorbic acid concentration as standard. The percentage antioxidant activity of MOALE (90 µl) was 93.89 % and ascorbic acid (15 µg) was 98.76 %, respectively, which was nearer to cent percent. So, 90 ml MOALE and 15 mg ascorbic acid per litre drinking water, respectively were used for supplementation in boiler chicken.

5.2 EFFECT OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON PERFORMANCE PARAMETERS OF BROILER CHICKEN

The supplementation of *Moringa oleifera* aqueous leaves extract (MOALE) and ascorbic acid on feed intake in experimental birds have non-significant effect. Average feed intake during experiment varied from 2983.03 in T2 to 3055.52 g in T1 group, respectively. At the end of experiment, it was found that the supplementation of *Moringa oleifera* aqueous leaves extract (MOALE) and ascorbic acid had no any significant effect on feed intake in comparison to control, however, MOALE supplemented group consumed 2.37% less feed as compared with control group.

Similar pattern was observed in average body weight changes at the end of trial. There was marginal variation in body weight among the group throughout the experiment except first week of age, where significant reduction in body weight was noted in T2 group. Moreover, in MOALE supplemented group 8.47% higher body weight in broiler chicken found as compared with control group. There was fluctuation in weekly body weight gain noted during the experiment. However, the average body weight gain in MOALE group was found to be 8.47% higher, though the changes was non-significant in comparison with control group.

The average FCR of fifth week of experiment varied from 1.68 in T2 to 1.86 in T1 group. The overall FCR for MOALE group was significantly ($P<0.01$) lowest followed by ascorbic acid group in comparison with control. However, 9.67% better FCR was noted in MOALE supplemented group followed by ascorbic acid group (5.91%) in comparison with control.

Effect of treatment on nitrogen retention in broiler chicken differed significantly ($P<0.05$) and it ranged from 51.50 in T1 group to 54.01 in T2 group. However, MOALE supplemented group birds had higher nitrogen retention (4.87 %) followed by ascorbic acid fed group (3.51%) in comparison with control group. Similar trend was noted in calcium retention percentage which was significantly ($P<0.05$) higher (2.32%) in MOALE group followed by ascorbic acid group (1.04%) as compared with control. However, energy metabolizability and phosphorus retention were unaffected between the treatment and found comparable among the groups.

5.3 EFFECT OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON HAEMATO-BIOCHEMICAL PROFILE, ANTIOXIDANT STATUS AND IMMUNE RESPONSE IN BROILER CHICKEN

Statistical analysis for the effect of treatment on haemoglobin level in birds showed non-significant variation and found comparable among the groups whereas, average haemoglobin level varied from 10.93 g/dl in T3 group to 11.19 g/dl in T1 group. Similar trend was noticed for the effect of treatment on PCV level in birds and had no significant changes ($P>0.05$) occurred among the groups. However, TEC level in MOALE group was slightly higher side followed by ascorbic acid fed group in comparison with control but the changes was non-significant between the treatment. Average TLC varied between 12.74 x 10^3µL in T3 group to 15.50 x 10^3µL in T1 group whereas, no any significant differences ($P>0.05$) found between treatment groups as compare to control.

Similarly, MCV, MCH and MCHC were significantly not affected by the treatment and found comparable among the groups. The neutrophil, monocyte, eosinophil and basophil level in whole blood of broiler chicken were unaffected by the treatment and found comparable among the groups. However, Lymphocyte level was significantly higher ($P<0.01$) in MOALE supplemented group followed by ascorbic acid treated group in comparison with control. Such enhancement in lymphocyte count reflected better immunity of birds in treatment group which might be due to immunomodulatory and antioxidant effect of *Moringa oleifera* and ascorbic acid.

Average total protein level in serum sample varied between 3.73 g/dl in T1 group to 4.54 g/dl in T2 group and it was significantly not affected ($P>0.05$) between the treatment and found comparable among the groups. Similarly, albumin, globulin, A:G ratio and blood urea nitrogen were also not significantly affected by the treatment and found comparable among the groups. However, serum creatinine level was significantly different ($P=0.10$) and found lowest (17.77%) in MOALE group followed by ascorbic acid group (11.11%) and highest in control group and reduction in creatinine indicates retarded catabolism rate in broiler birds. Likewise, serum ALT and AST level were non-significantly differed between the treatment and found comparable among the group.

Average total cholesterol level varied between 108.88 mg/dl in T2 group to 122.15 mg/dl in T1 group. The serum total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were significantly not affected ($P>0.05$) by the treatment and found comparable among the groups.

The effect of treatment on lipid peroxidation level in serum sample was significantly differed ($P<0.05$) between the treatment groups and its concentration in MOALE group was found to be significantly lower (26.53%) followed by ascorbic acid group (25.00%) in comparison with control group. However, SOD level in broiler chicken serum was significantly

not affected (P>0.05) by the treatment but SOD level in MOALE group was 13.82 % higher followed by ascorbic acid group (6.39%) in comparison with control. Likewise, reduced glutathione level (GSH) in serum sample varied between 0.31 mM/ml in T1 group to 0.36 mM/ml in T2 group but the changes were significantly not different (P>0.05) whereas, GSH concentration was 16.12% higher in MOALE group followed by ascorbic acid group (9.67%) in comparison with control. Similarly, statistical analysis for the effect of treatment on LDH activity in the serum of broiler chicken was significantly not different (P>0.05) between treatment groups but LDH level was 5.90% lower in MOALE group followed by ascorbic acid group (1.40%) in comparison with control. However, the effect of treatment on catalase activity in broiler chicken serum sample was significantly not different (P>0.05) between treatment groups, whereas, its level was 32.00% higher in MOALE group followed by ascorbic acid group (11.32%) in comparison with control which reflected better productivity impact in chicken.

The average HI titre in birds varied between 5.67 in T1 group to 50.00 in T2 group and the effect of treatment on HI titre in broiler chicken was significantly not different (P>0.05) between treatment groups, whereas, HI titre in MOALE group was found to be highest followed by ascorbic acid group (20.00) and lowest in control group which reflected better immunity in treatment groups in comparison to control that shown good health impact.

HSP 70 gene expression analysis in broiler chicken was not influenced by treatment and shown non-significant difference (P>0.05) in expression level however, in MOALE supplemented group HSP 70 gene expression was down regulated followed by ascorbic acid offered group in comparison with control group.

5.4 EFFECT OF SUPPLEMENTATION OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON CARCASS CHARACTERISTIC OF BROILER CHICKEN

The effect of MOALE and ascorbic acid on carcass traits were found non-significant (P>0.05) as the weight of liver varied between 44.92 g in T1 group to 47.50 g in T2 group and relative weight of liver in birds were found to be non-significant (P>0.05) and comparable among the groups. Similarly, the effect of treatment on relative weight of heart, gizzard, intestine, spleen, bursa, giblet percentage, dressing percentage and eviscerated percentage in broiler chicken were shown non-significant (P>0.05) changes between the treatment and comparable among the groups. However, the relative weight of abdominal fat was significantly differed (P<0.01) between the treatment groups and abdominal fat deposits of MOALE group

was found to be lowest than ascorbic acid group and control group which might be due to hypocholesteromic properties of moringa. The chemical composition of broiler meat sample was significantly unchanged (P>0.05) and found comparable among the groups. The moisture level, dry matter, organic matter, crude protein, ether extract and total ash were found to be comparable between the treatment and control groups.

5.5 EFFECT OF SUPPLEMENTATION OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID ON ECONOMICS OF BROILER CHICKEN PRODUCTION

The production economics of broiler chicken was measured and found that water supplementation of MOALE and ascorbic acid in bird, reduced production cost by improving live weight gain and profit per kg live weight was maximum in MOALE supplemented group followed by ascorbic acid group and least profit was noted in control group.

The results of present study can be concluded as follows;

1. The growth performance and feed efficiency was better in MOALE group followed by ascorbic acid supplemented birds without affecting the metabolism of nutrients.
2. Most of the haemato-biochemical profiles and HSP70 gene expression were unaffected by the treatment except creatinine, while antioxidant profile was improved in treatment group.
3. Immunity status of broiler chicken against NDV was enhanced in both treatment group.
4. Abdominal fat deposit was significantly reduced without affecting carcass quality, however, maximum profit obtained in MOALE group followed by ascorbic acid supplemented birds.

RECOMMENDATIONS AND FUTURE STRATEGIES

1. Considering the above finding we can suggest that aqueous MOALE may be incorporated in broiler chicken farming practices for economical production.
2. There is need to do more studies on aqueous supplementation of *Moringa oleifera* leaf extract in different species for dose standardization, mass awareness among industries & farmers to explore its nutritional importance.

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APPENDIX

1. Analysis of variance for the effect of treatment on growth performance of broiler chicken during experiment

Source of Variance	Degree of freedom	Sum of square	Mean square	F
Feed Intake				
Between groups	2	8012.827	4006.413	1.656
Within groups	15	14515.079	2419.180	
Body weight				
Between groups	2	29800.357	14900.178	0.605
Within groups	15	147873.774	24645.629	
Body weight gain				
Between groups	2	29800.357	14900.178	0.605
Within groups	15	147873.774	24645.629	
Feed conversion ratio				
Between groups	2	0.048	0.024	25.151
Within groups	15	0.006	0.001	

*Significant at 5 % level (P<0.05); **Significant at 1 % level (P<0.01)

2. Analysis of variance for the effect of treatment on nutrient balance in broiler chicken during experiment

Source of Variance	Degree of freedom	Sum of square	Mean square	F
Nitrogen retention				
Between groups	2	20.183	10.091	4.915
Within groups	15	30.798	2.053	
Energy metabolizability				
Between groups	2	16.647	8.323	1.913
Within groups	15	65.273	4.352	
Calcium retention				
Between groups	2	4.360	2.180	3.189
Within groups	15	10.253	0.684	
phosphorus retention				
Between groups	2	7.067	3.533	1.082
Within groups	15	48.974	3.265	

*Significant at 5 % level (P<0.05); **Significant at 1 % level (P<0.01)

3. Analysis of variance for the effect of treatment on blood biochemical profile in broiler chicken during experiment

Source of Variance	Degree of freedom	Sum of square	Mean square	F
Hb				
Between groups	2	0.218	0.109	0.084
Within groups	15	19.467	1.298	
PCV				
Between groups	2	11.942	5.971	0.320
Within groups	15	279.452	18.630	
TEC				
Between groups	2	0.066	0.033	0.098
Within groups	15	5.061	0.337	
TLC				
Between groups	2	25.812	12.906	1.670
Within groups	15	115.933	7.729	

*Significant at 5 % level (P<0.05); **Significant at 1 % level (P<0.01)

4. Analysis of variance for the effect of treatment on serum biochemical profile in broiler chicken during experiment

Source of Variance	Degree of freedom	Sum of square	Mean square	F
Total Protein				
Between groups	2	2.110	1.055	2.445
Within groups	15	6.474	0.432	
BUN				
Between groups	2	0.147	0.074	0.414
Within groups	15	2.665	0.178	
Creatinine				
Between groups	2	0.018	0.009	2.630
Within groups	15	0.050	0.003	
Cholesterol				
Between groups	2	560.013	280.007	0.373
Within groups	15	11246.635	749.776	
Triglyceride				
Between groups	2	58.120	29.060	0.319
Within groups	15	1367.126	91.142	
VLDL				
Between groups	2	2.305	1.152	0.316
Within groups	15	54.725	3.648	

*Significant at 5 % level (P<0.05); **Significant at 1 % level (P<0.01)

5. Analysis of variance for the effect of treatment on antioxidant profile and immune status of broiler chicken during experiment

Source of Variance	Degree of freedom	Sum of square	Mean square	F
Lipid peroxidation				
Between groups	2	5.217	2.609	4.314
Within groups	15	9.070	0.605	
SOD				
Between groups	2	2338.266	1169.133	1.823
Within groups	15	9620.514	641.368	
GSH				
Between groups	2	0.010	0.004	1.369
Within groups	15	0.057	0.005	
LDH				
Between groups	2	3293.890	1646.945	0.218
Within groups	15	113175.039	7545.003	
Catalase				
Between groups	2	225.499	112.750	1.339
Within groups	15	1263.187	84.212	
HI titre				
Between groups	2	6141.778	3070.889	2.205
Within groups	15	20891.333	1392.756	

*Significant at 5 % level (P<0.05); **Significant at 1 % level (P<0.01)

6. Analysis of variance for the effect of treatment on carcass traits of broiler chicken during experiment

Source of Variance	Degree of freedom	Sum of square	Mean square	F
Liver				
Between groups	2	20.194	10.097	0.114
Within groups	15	1328.708	88.581	
Heart				
Between groups	2	5.444	2.722	0.292
Within groups	15	139.958	9.331	
Abdominal Fat				
Between groups	2	235.396	117.698	12.313
Within groups	15	143.383	9.559	
Spleen				
Between groups	2	4.333	2.167	0.744
Within groups	15	43.667	2.911	
Bursa				
Between groups	2	1.419	0.710	0.722
Within groups	15	14.740	0.983	
Dressing %				
Between groups	2	1.520	0.760	0.642
Within groups	15	25.013	1.668	

*Significant at 5 % level (P<0.05); **Significant at 1 % level (P<0.01)

7. Analysis of variance for the effect of treatment on chemical composition of meat in broiler chicken during experiment

Source of Variance	Degree of freedom	Sum of square	Mean square	F
Dry Matter				
Between groups	2	1.064	0.532	0.914
Within groups	15	3.492	0.582	
Organic Matter				
Between groups	2	0.031	0.015	1.281
Within groups	15	0.072	0.012	
Crude Protein				
Between groups	2	0.256	0.128	0.106
Within groups	15	7.284	1.214	
Ether Extract				
Between groups	2	2.783	1.391	1.471
Within groups	15	5.677	0.946	
Total Ash				
Between groups	2	0.031	0.015	1.281
Within groups	15	0.072	0.012	

*Significant at 5 % level (P<0.05); **Significant at 1 % level (P<0.01)