EFFECTS OF USING NEEM LEAF (*Azadirachta indica*) IN BROILER RATION AS AN ALTERNATIVE TO ANTIBIOTIC FOR THE PRODUCTION OF SAFE MEAT

TOUFIK AHMED

DEPARTMENT OF POULTRY SCIENCE
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

JUNE, 2019

EFFECTS OF USING NEEM LEAF (*Azadirachta indica*) IN BROILER RATION AS AN ALTERNATIVE TO ANTIBIOTIC FOR THE PRODUCTION OF SAFE MEAT

by
TOUFIK AHMED
Reg. No. 12-04750

A Thesis
Submitted to the Department of Poultry Science
Sher-e-Bangla Agricultural University, Dhaka
In Partial Fulfillment of the Requirements
for the degree of

MASTER OF SCIENCE (MS) IN POULTRY SCIENCE

SEMESTER: Jan-Jun/2019

APPROVED BY

Prof. Dr. Md. Anwarul Haque Beg
Supervisor
Department of Poultry Science
Sher-e-Bangla Agricultural University

Dr. K.B. M. Saiful Islam
Co-Supervisor
Chairman & Associate Professor
Department of Medicine & Public Health
Sher-e-Bangla Agricultural University

Prof. Dr. Md. Anwarul Haque Beg
Chairman
Department of Poultry Science
Sher-e-Bangla Agricultural University

Prof. Dr. Md. Anwarul Haque Beg
Chairman
Department of Poultry Science
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
Mobile No: +8801718026200
E-mail: mahbegsau@yahoo.com

CERTIFICATE

This is to certify that the thesis entitled "EFFECTS OF USING NEEM LEAF (Azadirachta indica) IN BROILER RATION AS AN ALTERNATIVE TO ANTIBIOTIC FOR THE PRODUCTION OF SAFE MEAT" submitted to the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of Master of Science in Poultry Science, embodies the result of a piece of bona fide research work carried out by Toufik Ahmed, Registration No. 12-04750 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: June, 2019

Place: Dhaka, Bangladesh

Prof. Dr. Md. Anwarul Haque Beg
(Supervisor)
Chairman
Department of Poultry Science
Sher-e-Bangla Agricultural University

Dedicated
To
My Beloved Parents

ACKNOWLEDGEMENT

All praises are due to Almighty Allah, the Great, Gracious and Merciful, Whose blessings enabled the author to complete this research work successfully.

The author like to express his deepest sense of gratitude sincere appreciation to his respected supervisor ***Prof. Dr. Md. Anwarul Haque Beg****, Chairman, Department of Poultry Science, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, for his scholastic guidance, support, encouragement and invaluable suggestions and constructive criticism throughout the study period and gratuitous labour in conducting and successfully completing the research work and in the preparation of the manuscript writing.*

The author also expresses his gratefulness and best regards to his respected co- Supervisor, ***Dr. K .B. M. Saiful Islam****, Chairman and Associate Professor, Department of Medicine & Public Health, Sher-e-Bangla Agricultural University, Dhaka for his inestimatable help, valuable suggestions and constant inspiration throughout the research work and preparation of the thesis.*

The author expresses his sincere respect to ***Md. Aftabuzzaman,*** *Assistant Professor, Department of Poultry Science, Sher-e-Bangla Agricultural University &* ***Dr. Maksuda Begum,*** *Assistant Professor, Department of Poultry Science, Sher-e-Bangla Agricultural University, Dhaka for valuable suggestions and cooperation during the study period.*

The author also expresses heartfelt thanks to all the staffs of the Department of Poultry Science, SAU Dhaka for their co-operation during the period of the study.

The author expresses his sincere appreciation to his brothers, sister, relatives, well wishers and friends for their inspiration, help and encouragement throughout the study period.

The Author

LIST OF CONTENTS

LIST OF CONTENTS (CONT'D)

LIST OF CONTENTS (CONT'D)

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
A.M	=	Ante meridian
ACTH	=	Adreno Corticotropic Hormone
AGPs	=	Antibiotic growth promoters
ANOVA	=	Analysis of Variance
BANSDOC	=	Bangladesh National Scientific And Technical Documentation Centre
BARC	=	Bangladesh Agricultural Research Council
BLRI	=	Bangladesh Livestock Research Institute
Ca	=	Calcium
CAT	=	Catalase
CF	=	Crude Fibre
CFU	=	Colony Forming Units
Cm	=	Centimeter
cm^2	=	Squre Centimeter
CONTD.	=	Continued
CP	=	Crude Protein
CRD	=	Complete Randomized Design
DNLP	=	Dried Neem Leaf Powder
Dr.	=	Doctor
e.g.	=	For Example
EDTA	=	Ethylene Diethyle Tetraacitic Acid
et al.	=	Associates
FC	=	Feed Consumption
FCR	=	Feed Conversion Ratio
FOS	=	Fructo-oligosaccharides
g	=	Gram
GSH	=	Glutathione
Hb	=	Haemoglobin
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines
kcal	=	Kilo-calorie
Kg	=	Kilogram
LSD	=	Least Significant Difference
Ltd.	=	Limited
M.S.	=	Master of Science
ME	=	Metabolizable Energy
MOS	=	Mannan-oligosaccharides

ACRONYMS AND ABBREVIATIONS (CONT'D)

Abbreviation		Full meaning
MCHC	=	Mean Corpuscular Hemoglobin Concentration
ml	=	Mililitre
mm	=	Milimeter
mmol	=	Milimol
MT	=	Metric ton
N	=	Nitrogen
NDV	=	Newcastle Disease Vaccine
No.	=	Number
NS	=	Non-significant
P	=	Phosphorus
PCV	=	Packed Cell Volume
Pp	=	Page to page
ppm	=	Parts per Million
PRP	=	Parboiled Rice Polish
RBC	=	White Blood Cell
SAU	=	Sher-e-Bangla Agricultural University
SED	=	Standard Error Difference
SPSS	=	Statistical Package for Social Sciences
viz.	=	Such as
Vs	=	Versus
WBC	=	White Blood Cell
WHO	=	World Health Organization
WPSA	=	World's Poultry Science Association

LIST OF SYMBOLS

Symbols		Full meaning
:	=	Ratio
@	=	At the rate of
+	=	Plus
<	=	Less than
>	=	Greater than
°C	=	Degree Celcius
°F	=	Degree Fahrenheit
%	=	Percentage
&	=	And
*	=	5% level of significance
**	=	1% level of significance
/	=	Per

EFFECTS OF USING NEEM LEAF (*Azadirachta indica*) IN BROILER RATION AS AN ALTERNATIVE TO ANTIBIOTIC FOR THE PRODUCTION OF SAFE MEAT

BY
TOUFIK AHMED

ABSTRACT

The experiment was conducted at Sher-e-Bangla Agricultural University poultry farm, to evaluate the productive performance of commercial broiler chicks fed Dried Neem Leaf Powder (DNLP) containing diets comparison to antibiotic based diet. A total of 180 day old Cobb-500 broiler chicks were divided randomly into 6 experimental groups of 3 replicates each with 10 chicks per replications. Commercial starter and grower feed were used as basal diet which contained minimum 21% CP, 3000 ME Kcal/Kg and 19% CP, 3200 ME Kcal/Kg respectively. One of the 6 experimental group was fed as control diet (basal) while, the remaining five groups were fed basal diet along with DNLP (1%, 1.5%, 2%, 2.5% and antibiotic). Significantly ($P<0.05$) highest hemoglobin (16.33 gm/dl) was found in 2.0% DNLP fed group of broiler chicken than other groups. No significant ($P>0.05$) difference was found in glucose and cholesterol for any treatment groups but significantly lowest ($P<0.05$) uric acid was observed in 1.5% DNLP treated group than antibiotic group. The DNLP treated fed groups broiler chicken showed no significant ($P>0.05$) difference in neutrophils, lymphocytes, monocytes and eosinophils counts comparing with antibiotic and control groups. Neem treated fed groups showed significantly ($P<0.05$) higher liver weight (43.67 ± 1.764^{a} to 46.67 ± 4.410^{a}) g than antibiotic treated group (31.0 ± 2.082^{b} g). Spleen weights were not affected ($P>0.05$) by any treatments. The highest ($P<0.05$) viable bacteria was found in control group (16.3×10^{5}) then antibiotic treated group (3.3×10^{5}). But, neem and antibiotic treated groups showed no significant ($P>0.05$) difference among them. There is no significant differences ($P>0.05$) in dressing percentage among all treated groups. Feed consumption is significantly less in 2% and 2.5% neem leaf treated groups but feed conversion ratio is comparatively greater in 2% and 2.5% neem leaf treated group than any other treated groups. The results showed that the birds fed 1.5% DNLP diets achieved superior body weights among all groups. The results of the study demonstrate the beneficial effects of supplementing DNLP on body weight gain and FCR in the treated groups in broiler chicken. DNLP is, therefore, suggested 2.5% to be used as an alternative of antibiotics on broiler chicken ration for higher profitability.

Keywords: Antibiotic alternative; broiler; growth performance; Neem (Azadirachta indica) leaf meal; hematological parameter.

CHAPTER I

INTRODUCTION

Poultry farming has emerged as one of the fastest growing agribusiness industries in the world, even in Bangladesh. Research on meat production globally indicates poultry as the fastest growing livestock sector especially in developing countries. It has triggered the discovery and widespread use of a number of 'feed additives'. The term feed additive is applied in a broad sense, to all products other than those commonly called feedstuffs, which could be added to the ration with the purpose of obtaining some special effects. The main objective of adding feed additives is to boost animal performance by increasing their growth rate, better-feed conversion efficiency, greater livability and lowered mortality in poultry birds. These feed additives are termed as "growth promoters" and often called as non-nutrient feed additives. (Ilango, 2013)

In poultry industry, antibiotic growth promoters (AGP) have been used as a feed additive to enhance gut health and control sub-clinical diseases. Synthetic growth enhancers and supplements in poultry nutrition are expensive, usually unavailable and possess adverse effects in bird and human (Mahady, 2005). Sub-therapeutic levels of antibiotics given to poultry as growth enhancer may result to the development of antibiotic-resistant of bacteria, which are hazardous to animal and human health (Sarica *et al.* 2005).

The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibits bacteria which is administered at a low subtherapeutic dose. The mechanism of action of antibiotics as growth promoters is related to interactions with intestinal microbial population (Dibner and Richards, 2005). Four hypotheses have been proposed to explain their action: (i) nutrients may be protected against bacterial destruction;

(ii) absorption of nutrients may improve because of a thinning of the small intestinal barrier;

(iii) the antibiotics may decrease the production of toxins by intestinal bacteria; and (iv) there may be a reduction in the incidence of subclinical intestinal infections and other pathogenic bacteria (Patterson and Burkholder, 2003).

However, the use of antibiotics as feed additives is under severe criticism. Growth stimulating antibiotics, by the spread of antibiotic resistant bacteria, are a threat to human health (Wray and Davies, 2000; Turnidge, 2004).

Concerns were raised that the use of antibiotics as therapeutics and for growth promotion could lead to a problem of increasing resistance in bacteria of human and animal origin (Jensen, 1998), particularly regarding resistance in gram-negative bacteria (*Salmonella* spp.

and *Escherichia coli*). In addition they also will have effect on gut flora composition, specifically in regard to increased excretion of food-borne pathogens (Neu, 1992; Williams and Tucker, 1975). The poultry industry is currently moving towards a reduction in use of synthetic antibiotics due to this reason (Barton, 1998).

Because of the growing concern over the transmission and proliferation of resistant bacteria via the food chain, the European Union (EU) banned antibiotic growth promoters to be used as additives in animal nutrition (Cardozo *et al.*, 2004). Alternative feed additives for farm animals are referred to as Natural Growth Promoters (NGP) or non-antibiotic growth promoters (Steiner, 2006) which include acidifiers, probiotics, prebiotics, phytobiotics, feed enzymes, immune stimulants and antioxidants are gaining the attention. The NGPs, particularly some natural herbs have been used for medical treatment since prehistoric time (Dragland *et al.*, 2003). There are some important bioactive components such as alkaloids, bitters, flavonoids, glycosides, mucilage, saponins, tannins (Vandergrift, 1998) phenols, phenolic acids, guinones, coumarins, terpenoids, essential oils, lectins and polypeptides (Cowan, 1999) in the structures of nearly all the plants. The use of various plant materials as dietary supplements may positively affect poultry health and productivity.

The large number of active compounds in these supplements may therefore present a more acceptable defense against bacterial attack than synthetic antimicrobials. There is evidence to suggest that herbs, spices and various plant extracts have appetizing and digestion-stimulating properties and antimicrobial effects (Madrid *et al.*, 2003, Alçiçek *et al.*, 2004, Zhang *et al.*, 2005) which stimulate the growth of beneficial bacteria and minimize pathogenic bacterial activity in the gastrointestinal tract of poultry (Wenk, 2000). On the other hand, supplementing the diet with plant material that is rich in active substances with beneficial effects for the immune system can be used as an alternative to antibiotic growth promoters.

Beneficial effects of herbal extracts or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response, antibacterial, anti-viral, antioxdant and antihelminthic actions.

Generally plant extracts have no problem of resistance (Tipu *et al.*, 2006) and broilers fed on herbal feed additives were accepted well by the consumers (Hernandez *et al.*, 2004). Neem, a tropical ever green tree is native to the asian sub-continent. Neem dry leaves fed to broilers have been reported to significantly enhance the antibody titres against new castle diseases

virus (NDV) antigen and also potentiated the inflammatory Reactions. Biologically active ingredients isolated from different parts of the plants include; azadirachtin, meliacin, gedunin,salanin, nimbin, valassin etc (Chari, 1996). Neem has attracted worldwide prominence due to its vast range of medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective and various other properties without showing any adverse affects (Kale *et.al*., 2003). Also, neem promotes growth and feed efficiency of birds because of its antibacterial and hepatoprotective properties (Padalwar, 1994).

Neem (*A. indica*) is one of those trees in the world and which is currently under discussion on a large scale has been found that different parts of the Neem tree contain chemicals such as azadiractin, nimbin, nimbindin and quercetin and others. The rapid growth of the tree which is evergreen and has medicinal and nutritional effectiveness of chicken meat. Neem in the water led to an increase in the effectiveness of the feed conversion and an increase in weight. So present study aims to investigate the determination of impact of Neem powder added to the diet in broiler chickens to evaluating growth performance & immune response of comercial broiler. With this background, the work was planned to explore the possibilities of Neem Leaf in broiler chicken feeds as a replacement for the antibiotic growth promoters, with the following specific objectives:

1. To evaluate the growth performance and hematological properties of broiler fed DNLP based diet comparison with antibiotic and basal diet.
2. To find out the effect of DNLP on microbial load.
3. To determine the inclusion level of DNLP in broiler ration as a supplement of antibiotics.

CHAPTER 2

REVIEW OF LITERATURE

Sources of literature

(i) Book and journal in different libraries as mentioned below-

a. Sher-e-Bangla Agricultural University (SAU) Library, Dhaka.

b. Bangladesh Agricultural Research Council (BARC) Library, Farmgate, Dhaka.

c. Bangladesh National Scientific And Technical Documentation centre .

. (BANSDOC) Library, Agargaon, Dhaka

d. Bangladesh Livestock Research Institute (BLRI) library, Savar, Dhaka.

(ii) Abstract searching at BARC, Farmgate, Dhaka and BANSDOC, Dhaka.

(iii) Internet browsing.

A total about 100 literature were reviewed to identify the background, drawbacks and prospects of research, understand previous findings and to answer the research status. Among them 22 were full article and 60 abstracts, 18 were only titles and some were miscellaneous. A brief account is given below depending on five main headlines viz, antibiotic impacts on poultry, Antibiotic growth promoters (AGPs), Antimicrobial resistance, Alternatives to antibiotic growth promoters and Neem Leaf.

Mentioning the references in a traditional way or sequence is avoided. A very critical enquires was made of each article and significant information was collected and arranged according to specific title. It is expected to be pioneering efforts in Bangladesh for higher research review attempts.

Poultry farming has emerged as one of the fastest growing agribusiness industries in the world. Research on meat production globally indicates poultry as the fastest growing livestock sector especially in developing countries. It has triggered the discovery and widespread use of a number of “feed additives”. Further, disease surveillance, monitoring and control will also decide the fate of this sector.

Unlike livestock farming, poultry farming is always intensive and hence the birds are more subjected to stressful conditions. Stress is an important factor that renders the birds vulnerable to potentially pathogenic microorganisms. These pathogenic microflora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat soluble

vitamins due to deconjugating effects of bile acids (Engberg *et al.*, 2000). This ultimately leads depressed growth performance and increase incidence of disease.

2. 1 Antibiotic impacts on poultry

The discovery of antibiotics was a success in controlling infectious pathologies and increasing feed efficiencies (Engberg *et al.*, 2000). Antibiotics, either of natural or synthetic origin are used to both prevent proliferation and destroy bacteria. Antibiotics are produced by lower fungi or certain bacteria. They are routinely used to treat and prevent infections in humans and animals. The poultry industry uses antibiotics to improve meat production through increased feed conversion, growth rate promotion and disease prevention. Antibiotics can be used successfully at sub-therapeutic doses in poultry production to promote growth (Chattopadhyay, 2014; Engberg *et al.*, 2000) and protect the health of birds by modifying the immune status of broiler chickens (Lee *et al.*, 2012). This is mainly due to the control of gastrointestinal infections due to microbiota modification and increase in the intestine (Singh *et al.*, 2013; Torok *et al.*, 2011). The mechanism remains unclear, but antibiotics are likely to act by remodelling microbial diversity and relative abundance in the intestine to provide an optimal microbiota for growth (Dibner and Richards, 2005). For example, meta-genome sequencingapproaches have demonstrated that diet with salinomycin (60 ppm) has an impact on microbiome dynamics in chicken ceca (Fung *et al.*, 2013).

Similarly, the use of virginiamycin (100 ppm) as a growth promoter has been associated with an increased abundance of *Lactobacillus species* in broiler duodenaln loop at proximal ileum. This indicates that virginiamycin alters the composition of chicken gut microbiota (Dumonceaux *et al.*, 2006). In addition, populations of *Lactobacillus* spp. in the ileum of chickens receiving feed containing tylosin, a bacteriostatic, are significantly lower than in chickens receiving no tylosin (Lin *et al.*, 2013). This decrease in *Lactobacilli species* following the use of antibiotics has been demonstrated in other studies (Lee *et al.*, 2012). For reminder, *Lactobacillus* are the primary commensal bacteria for the production of bile hydrolase salt. The decrease in the lactobacillus population in antibiotic-treated animals probably reduces the intestinal activity of the bile hydrolase salts, which would increase the relative abundance of conjugated bile salts, thus promotes lipid metabolism and energy harvesting and increases animal weight gain (Lin *et al.*, 2013).

A change in the intestinal microbiota of chickens can influence their immunity and their health. However, changes in the intestinal microbiota of chickens can be influenced by several factors. These factors include housing conditions, exposure to pathogens, diet composition and the presence of antibiotics in feed (Lee *et al.*, 2012).

2.2 Antibiotic growth promoters (AGPs)

Feed antibiotics were first applied in animal nutrition in 1946. The term ''''antibiotic growth promoter"""" is used to describe any medicine that destroys or inhibit bacteria and is administered at a low, sub therapeutic dose for the purpose of performance enhancement (Hughes and Heritage, 2002). Antibacterial growth promoters are used to help the animals to digest their food more efficiently, get maximum benefit from it and allow them to develop in to strong and healthy individuals (Ellin, 2001). They may produce improved growth rate because of thinning of mucous membrane of the gut, facilitating better absorption, altering gut motility to enhance better assimilation, producing favorable conditions to beneficial microbes in the gut of animal by destroying harmful bacteria and partitioning proteins to muscle accretion by suppressing monokines (Prescott and Baggot, 1993). When used at sub-therapeutic levels, these antimicrobials improve overall performance (Falcao-e-Cunha *et al.*, 2007*)* through reduced normal intestinal flora (which compete with the host for nutrients) and harmful gut bacteria (which may reduce performance by causing sub clinical-diseases) (Jensen, 1998).

But the antibiotics are specific to their spectrum of activity only in the active multiplying stage of bacteria and it will not provide overall protection. Large numbers of antimicrobials were banned due to residual effects on human health and cross-resistance to antimicrobial drugs used in human medicine (WHO, 1997).

Some antimicrobial agents (Virginiamanycin, Zn bacitracin, etc.), which are not absorbed in the systemic circulation and exert their action locally in the gut are still used as growth promoters (Ian phillips, 1999). Administration of drugs to food-producing animals requires not only consideration of effects on the animal but also the effects on humans who ingest food from these animals. In short, after food-producing animals have been exposed to drugs in order to cure or prevent disease or to promote growth, the effects of the residues of such treatment on humans should be known.

In view of the above the use of antibiotic growth promoters (AGPs) in poultry industry is under serious criticism by governmental policy makers and consumers because of the development of microbial resistance to these products and the potential harmful effects on human health. At present, only four AGPs are permitted for use in poultry nutrition. Thus, there is increasing public and government pressure in several countries to search for natural alternative to antibiotics (Botsoglou and Fletouris, 2001; Williams and Losa, 2001; McCartney, 2002).

2.3 Antimicrobial resistance

Bacterial resistance to antimicrobial drugs has become an issue of increased public concern and scientific interest during the last decade. This resulted from a growing concern that the use of antimicrobial drugs in veterinary medicine and animal husbandry may compromise human health if resistant bacteria develop in animals and are transferred to humans via the food chain or the environment. While there is still no consensus on the degree to which usage of antibiotics in animals contributes to the development and dissemination of antimicrobial resistance in human bacteria, experiential evidence and epidemiological and molecular studies point to a relationship between antimicrobial use and the emergence of resistant bacterial strains in animals and their spread to humans, especially via the food chain (Moritz, 2001).

Bacitracin, chlortetracycline, tylosin, avoparcin, neomycin, oxytetracycline, virginiamycin, trimethoprim, lincosamides, cephalosporins etc are the commonly used antibiotics in poultry and some of which are of direct importance in human medicine. However, imprudent use of antibiotics in poultry production can lead to increased antibiotic resistant bacteria in poultry products. In general, when an antibiotic is applied in poultry farming, the drug eliminates the susceptible bacterial strains, particularly at a therapeutic dose, leaving behind or selecting those variants with unusual traits that can resist it. These resistant bacteria thus become the predominant micro-organism in the population and they transmit their genetically defined resistance characteristics to subsequent progeny of the strains and to other bacterial species via mutation or plasmid-mediated (Gould, 2008).

According to WHO, the resistance to antibiotics is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents (Catry *et al.*, 2003).

For example, the use of fluoroquinolone antibiotics in broiler chickens has caused an emergence of resistant *Campylobacter* in poultry (Randall *et al.*, 2003). Administration of avilamycin as a growth promoter resulted in an occurrence of avilamycin-resistant *Enterococcus faecium* in broiler farms (Aarestrup *et al.*, 2000).

Potential transfer of resistant bacteria from poultry products to human population may occur through consumption of inadequently cooked meat or handling meat contaminated with the pathogens (Van den Bogaard and Stobberingh, 2000). In turkeys fed vancomycin, there were concerns of glycopeptides resistance due to *enterococci* found in turkeys and humans (Stobbering *et al.*, 1999), which is an example of cross-resistance. Studies have shown that animal *enterococci* are mostly different from human colonizers, although concerns for transient transfers of resistance remain (Apata, 2009).

2.4 Alternatives to antibiotic growth promoters

In view of the concerns regarding the potential for selection of antibiotic resistant bacteria, residues and environmental effects attributed to the use of antimicrobial growth promoters, a host of non-antibiotic alternatives are available or under investigation.

2.4.1 Probiotics

Probiotics are individual microorganisms or groups of microorganisms, which have favourable effect on host by improving the characteristics of intestinal microflora (Fuller, 1989). Certain species of bacteria, fungi and yeasts belong to the group of probiotics. Existing probiotics can be classified into colonizing species (*Lactobacillus sp.*, *Enterococcus sp.* and *Streptococcus sp*.) and free, non-colonizing species (*Bacillus sp* and *Saccharomyces cerevisiae*s) (Zikic *et al.*, 2006).

Probiotics acts by inhibiting bacterial growth by secretion of products, which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide. The other way by which probiotics act is competitive exclusion, which represents competition for locations to adhere to the intestinal mucous membranes and in this way pathogen microorganisms are prevented from inhabiting the digestive tract and the third way is competition for nutritious substances (Patterson and Burkholder, 2003).

In this way, they create conditions in intestines, which favour growth of useful bacteria and inhibit the development of pathogenic bacteria (Line *et al.*, 1998). They improve the function of the immune system (Zulkifli *et al.*, 2000; Kabir *et al.*, 2004) and exhibit significant influence on morpho-functional characteristics of intestines (Yang *et al.*, 2009). These effects lead to growth of broiler chickens (Jin *et al.*, 1997; Li *et al.*, 2008), improvement of feed conversion (Li *et al.*, 2008; Zulkifli *et al.*, 2000; Kabir *et al.*, 2004) and reduced mortality (Mohan *et al.*, 1996).

Majority of authors concluded that the effect of probiotics depended on the combination of bacterial strains contained in the probiotic preparation, level of its inclusion in the mixture, composition of mixture, quality of chickens and conditions of the environment in the production facility (Jin *et al.*, 1997; Patterson and Burkholder, 2003).

Nutrition plays a key role in maintaining the prooxidant-antioxidant balance (Cowey, 1986). Under physiological conditions the reactive species figure a crucial role in primary immune defense (Diplock *et al.*, 1998). But prolonged excess of reactive species is highly damaging for the host biomolecules and cells, resulting in imbalance of the functional antioxidative network of the organism (Petrof *et al.*, 2004).

Several studies reported the antioxidant activity of probiotic bacteria using assays *in vitro* (Shen *et al.*, 2011). Lactic acid bacteria are evaluated as beneficial bacteria by their product of acids (lactic acid), bacteriocin-like substances or bacteriocins (Strus *et al.*, 2001). Widely accepted probiotics contain different lactic acid producing bacteria: *bifidobacteria*, *lactobacilli* or *enterococci* (Mikelsaar and Zilmer, 2009).

Their efficiency was demonstrated for the treatment of gastrointestinal disorders, respiratory infections and allergic symptoms. In most cases, evidence for a beneficial effect was obtained by studies using animal models (Travers *et al.*, 2011).

2.4.2 Prebiotics

Prebiotics are defined as non-digestible food components, which have positive effect on host in their selective growth and activation of certain number of bacterial strains present in intestines (Gibson and Roberfroid, 1995).

The most significant compounds, which belong to group of prebiotics, are fructo-oligosaccharides (FOS), gluco-oligosaccharides and mannan-oligosaccharides (MOS).

Their advantage, compared to probiotics is that they promote growth of useful bacteria, which are already present in the host organism and are adapted to all conditions of the environment (Yang *et al.*, 2009). Similar to probiotics, results of the effects of prebiotics on broiler performance are contradictory. A study was conducted to analyze the effects of incorporation of FOS on broiler performances and the results showed improvement in body weight gain by 5-8% and improvement of feed conversion by 2-6% (Li *et al.*, 2008; Yang *et al.*, 2009). But, Biggs *et al.* (2007) obtained results showing decrease of body weight gain by 2% in-group fed FOS in diet.

Application of MOS to fattening chicks resulted in improvement of body weight gain and feed conversion in fattening chickens by up to 6% (Roch, 1998; Newman, 1999). This proves that effect of application of prebiotics depends on the condition of animals, environment conditions, composition of food and level and type of prebiotic included in the mixtures.

2.4.3 Synbiotics

This is relatively recent term among additives used in poultry nutrition. Synbiotics are combination primarily of probiotics and prebiotics, as well as other promoting substances which together exhibit joint effect with regard to health of digestive tract, digestibility and performances of broilers. Investigations showed that combinations used in synbiotics are often more efficient in relation to individual additives (Uščebrka *et al.*, 2005; Li *et al.*, 2008). Maiorka *et al.* (2001) suggest that the substitution of antibiotics by symbiotics in broiler diets

is an alternative to poultry industry, since no negative effect was found on performance. According to Cristina *et al.* (2012) the usage of probiotic-prebiotic-ficofytic compounds as feed additive generated better results related to hens performance, feed valorization, eggs yield and their quality.

The administration of symbiotic to broiler chickens early in life increased significantly ($p<0.05$) the phagocytic activity, lysozyme activity and nitric oxide levels in a dose dependent manner and improved the oxidative state by increasing glutathione (GSH) and decreasing malondialdehyde (MDA).

High concentration of symbiotic improves the antibody response to Newcastle Disease Vaccine (NDV) and Infectious Bronchitis Vaccines (IBV) (El-Sissi and Mohamed, 2011).

2.4.4 Enzymes

Supplementation of broiler feed with enzymes is applied in order to increase the efficiency of production of poultry meat. This is especially interesting if enzymes, which enable utilization of feeds of poorer nutritive value, are used. Numerous authors have reported that administration of enzymes can improve the production performances by 10% (Cowieson *et al.*, 2000, Cmiljanic *et al.*, 2001), whereas in some studies no positive effect has been reported (Peric *et al.*, 2002). It is obvious that the positive effect of application of additives depends on the quantity and quality of feeds included in the mixture, type of enzyme, as well as fattening conditions (Acamovic, 2001; Lukic *et al.*, 2002). Obtained results in some researches indicate that better effect is realized with utilization of two or more enzymes in food (Silversides and Bedford, 1999; Chesson, 2001). Therefore, new enzyme combinations are constantly analyzed, as well as their optimum doses, in order to realize positive financial effect through improved utilization of feeds. The main reasons for supplementing wheat- and barley-based poultry diets with enzymes is to increase the available energy content of the diet. Increased availability of carbohydrates for energy utilization is associated with increased energy digestibility (Partridge and Wyatt, 1995; Van der Klis *et al.*, 1995).

Enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals, such as barley and wheat (Friesen *et al.*, 1992; Marquardt *et al.*, 1994), maize (Saleh *et al.*, 2003), oats and rye (Friesen *et al.*, 1991, 1992; Bedford and Classen 1992; Marquardt *et al.*, 1994) and to those containing pulses, such as lupins (Brenes *et al.*, 1993). The effect of enzyme supplementation on dry matter digestibilities (DMD) in pigs and poultry depends on the type of diet and the type of animal: increases in DMD range from 0.9 (Schutte *et al.*, 1995) to 17% (Annison and Choct, 1993) in poultry.

Morgan and Bedford (1995) reported that coccidiosis problems could be prevented by using enzymes. According to Bharathidhasan *et al.* (2009) when Broilers were supplemented with enzyme level at 0, 250, 500, 750 and 1000 g/ton of feed there was no significant difference in carcass yield, dressing percentage, giblet weight, carcass weight, intestinal length and organoleptic characteristics of the meat.

2.4.5 Acidifiers

Acidifiers have been used in poultry nutrition for long time, in different forms and combinations, which are constantly changing. Organic acids reduce pH value of food and act as conserving agents and prevent microbial contamination of food in digestive tract of poultry (Freitag *et al.*, 1999). As a result of this there will be improved consumption of food, better-feed conversion and increased gain. Favourable effect of supplementation of individual organic acids to mixtures was established relatively long time ago for formic acid (Kirchgessner *et al.*, 1991) .n research published by Ao *et al.* (2009) it was established that citric acid in combination with α –galactosidase increased the effect of enzyme action, but also had negative effect on feed consumption and weight gain.

2.4.6 Antioxidants

Antioxidants are the agents, which donate free electron to reactive oxygen species (ROS) and reactive nitrogen species (RNS) and convert them to harmless substances and break the chain reaction (Dekkers *et al.*, 1996). After donating an electron, an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive.

Antioxidants are synthesized within the body and can also be extracted from the food that humans and animals eat, such as fruits, vegetables, seeds, nuts, meat, oil, leaves and grass (natural antioxidants). There are two lines of antioxidant defense within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, *beta*-carotene and coenzyme-Q (Kaczmarski, 1999). Of these, vitamin E is considered to be the most potent chain-breaking antioxidant within the membrane of the cell. The second line, inside the cell consists of water soluble antioxidant scavengers that include vitamin C, glutathione peroxidase, superoxide dismutase (SOD) and catalase (CAT) (Dekkers *et al.*, 1996). To maximize the oxidative stability of meat, antioxidants, mostly α-tocopheryl acetate (ATA), are added to feeds.

The beneficial effect of dietary ATA supplementation for the enhanced stability of lipids in muscle foods has been extensively reported for poultry, beef cattle, veal calves and pigs

(Gray *et al.*, 1996; Jensen *et al.*, 1998). Selenium is component of enzyme glutathione peroxidase, which prevents formation of free radicals, which are very harmful to cells as they disrupt their integrity (Kanacki *et al.*, 2008). Therefore, selenium and other antioxidants have favourable effect on quality of broiler meat (Surai, 2002; Tomovic *et al.*, 2006; Peric *et al.*, 2007a). Protective effect of selenium and vitamin E is also stated by Roch *et al.* (2000). One of the most accepted approaches for preservation of sensory properties of the meat is addition of antioxidants, such as selenium or vitamin E, directly to livestock food or during technological procedure of processing (Surai, 2002, Peric *et al.*, 2007b). Beside positive effect on quality of meat, Edens *et al.* (2000) and Peric *et al.* (2006) established better feathering and body mass of chickens fed organic forms of selenium. Peric *et al.* (2008b) also stated that addition of organically bound selenium into feed for broiler parents significantly increases quality of one-day-old chickens. Lower plasma concentrations of antioxidant vitamins such as vitamin C, E and folic acid and minerals like zinc and chromium have been inversely correlated to increased oxidative damage in stressed poultry (Cheng *et al.*, 1990; Sahin *et al.*, 2002).

Super oxide dismutase (SOD), is a class of closely related enzymes that catalyze the breakdown of the highly reactive superoxide anion into oxygen and hydrogen peroxide. SOD proteins are present in almost all aerobic cells and in extra cellular fluids. Each molecule of superoxide dismutase contains atoms of copper, zinc, manganese or iron. SOD that is formed in the mitochondria contains manganese (Mn-SOD) and synthesized in the matrix of the mitochondria. SOD that is formed in the cytoplasm of the cell contains copper and zinc (Cu/Zn-SOD). The SOD is a specific catalyst of the reaction and decreases concentration of O_2^- (Izumi *et al.*, 2002).

2.4.7 Herbal adaptogens

An adaptogen is a substance that shows some nonspecific effect, such as increasing body resistance to physical, chemical, or biological noxious agents and have a normalizing influence on pathological state, independent of the nature of that state .

A vast number of plants have been recognized as valuable sources of natural antimicrobial compounds (Mahady, 2005). A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al.*, 2005).

Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone and methanol are often used to extract bioactive compounds (Eloff, 1998).

In terms of active ingredients, adaptogenic preparations can be divided into three groups.

a. Those that contain phenolic compounds such as phenylpropanoids, phenylethane derivatives and lignans, which structurally resemble catecholamines that activates sympatho-adrenal system and possibly imply Those that contain tetracyclic triterpenes, such as cucurbitacin R diglucoside, an effect in the early stages of the stress response (Kochetkov *et al.,* 1962; Wagner, 1995).

b. which structurally resemble the specific corticosteroids that inactivate the stress system to protect against overreaction to stressors (Munck, 1984; Panossian *et al.*, 1999).

c. Those that contain unsaturated trihydroxy or epoxy fatty acids such as oxylipins structurally similar to leukotrienes and lipoxines (Panossian *et al.,* 1999).

Mechanism of action of these additives is not completely clear. Some plant extracts influence digestion and secretion of digestive enzymes and besides, they exhibit antibacterial, antiviral and antioxidant action (Ertas *et al.,* 2005; Cross *et al.,* 2007).

There is extensive evidence that single-dose administration of adaptogens activates corticosteroid formation and repeated dosage with adaptogens normalizes the levels of stress hormones, such as adrenocorticotropic hormone (ACTH) (Panossian, 1999). The effects of adaptogens become somewhat more clear when it is recalled the stress is a defensive response to external factors and that it stimulates the formation of endogenous messenger substances such as catecholamines, prostaglandins, cytokines, NO and platelet-activating factor, which inturn activate other factors that may either counteract stress or conversely, induce or facilitate disease. According to this concept, the "stress-executing" or „"switch-on"" mechanism activates the sympathoadrenal system (SAS) and over the longer term also activates the HPA, together with various regulators of cell and organ function (Panossian, 1999).

Results of research of application of phytobiotics in nutrition of broiler chickens are not completely consistent. Some authors state significant positive effects on broiler performance (Ertas *et al.,* 2005; Cross *et al.,* 2007, Peric *et al.,* 2008a), whereas another group of authors established no influence on weight gain and consumption or conversion of food (Cross *et al.,* 2007; Ocak *et al.,* 2008).

The differences in results are consequences of numerous factors, of which Yang *et al.* (2009) pointed out four:

1) type and part of plant used and their physical properties, 2) time of harvest, 3) preparation method of phytogenic additive and 4) compatibility with other food components.

Tipakom, (2002) found that feeding of *Andrographis paniculatis* to broiler chickens resulted in improved feed conversion ratio, increased live weight and decreased mortality rate and opined that the plant feeding could be an alternative to chlortetracycline in the broiler diet.

In the past two decades a number of ayurvedic preparations have been extensively used in poultry industry in India. Preparations like Livol® and Zeestress® have been found to possess hepatoprotective and immunopotentiative actions in vaccinated birds and reduced the stress in intensively housed chickens during summer (Parida *et al.,* 1995; Rao *et al.*, 1995).

2.5 Neem

2.5.1 Chemical composition of neem leaves:

Neem leaves are chemically composed of proteins, fibers, ether, ash and other compounds, (Biswas et al, 2002) showed that neem leaves contain Crude protein 15.8%, Crude fiber 14.6%, Ether extract 8.5%, Ash 4.5%, Moisture 13.0% and NFE 56.6%, These percentages vary from one place to another due to variations in nutrient composition of the soil where the neem plant is grown.

2.5.2 Mechanism of action on neem:

Neem (*Azadirachta indica*), a member of the Meliaceae family, has therapeutics implication in the diseases prevention and treatment. But the exact molecular mechanism in the prevention of pathogenesis is not understood entirely. It is considered that*Azadirachta indica* shows therapeutic role due to the rich source of antioxidant and other valuable active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin. Possible mechanism of action of *Azadirachta indica* is presented as follows:

Neem (*Azadirachta indica*) plants parts shows antimicrobial role through inhibitory effect on microbial growth/potentiality of cell wall breakdown.

Azadirachtin, a complex tetranortriterpenoid limonoid present in seeds, is the key constituent responsible for both antifeedant and toxic effects in insects (Mordue Luntz, 2000) Results suggest that the ethanol extract of neem leaves showed *in vitro* antibacterial activity

against both *Staphylococcus aureus* and MRSA with greatest zones of inhibition noted at 100% concentration. (Sarmiento, 2011)

1. Neem plays role as free radical scavenging properties due to rich source of antioxidant. Azadirachtin and nimbolide showed concentration-dependent antiradical scavenging activity and reductive potential in the following order: nimbolide > azadirachtin > ascorbate. (Hossain M. A. 2013)
2. Neem ingredient shows effective role in the management of cancer through the regulation of cell signaling pathways. Neem modulates the activity of various tumour suppressor genes (e.g., p53, pTEN), angiogenesis (VEGF), transcription factors (e.g., NF-κB), and apoptosis (e.g., bcl2, bax).
3. Neem also plays role as anti-inflammatory via regulation of proinflammatory enzyme activities including cyclooxygenase (COX), and lipoxygenase (LOX) enzyme.

2.5.3 Antioxidant properties of neem:

Antioxidants are the chemicals that reduce the rate of particular oxidation reaction. They help to protect the body from damage of cell by free radicals. Free radicals are chemical species possessing an unpaired electron that can be considered as fragment of molecules and which are generally very reactive. There is a report that the more the toxic metals in our body, the higher the free radical activity. Thus toxic metals are a cause of free radicals. They cause to oxidative damage of protein, DNA and other essential molecules and cause cancer, cardiovascular diseases and heart disease, and oxidative stress. Free radical or reactive oxygen species are one of the main culprits in the genesis of various diseases. However, neutralization of free radical activity is one of the important steps in the diseases prevention. Antioxidants stabilize/deactivate free radicals, often before they attack targets in biological cells (Nunes P. X. 2012) and also play role in the activation of antioxidative enzyme that plays role in the control of damage caused by free radicals/reactive oxygen species. Medicinal plants have been reported to have antioxidant activit (Rahmani, 2015). Plants fruits, seeds, oil, leaves, bark, and roots show an important role in diseases prevention due to the rich source of antioxidant. Leaf and bark extracts of *A. indica* have been studied for their antioxidant activity and results of the study clearly indicated that all the tested leaf and bark extracts/fractions of neem grown in the foothills have significant antioxidant properties (Ghimeray A. K. 2009). Another

important study was performed based on leaves, fruits, flowers, and stem bark extracts from the Siamese neem tree to assess the antioxidant activity and results suggest that extracts from leaf, flower, and stem bark have strong antioxidant potential (Sithisarn, 2005). A valuable study was carried out to evaluate *in vitro* antioxidant activity in different crude extracts of the leaves of *Azadirachta indica* (neem) and antioxidant capacity of different crude extracts was as follows: chloroform > butanol > ethyl acetate extract > hexane extract > methanol extract. Result of the current finding suggested that the chloroform crude extracts of neem could be used as a natural antioxidant (Hossain, 2013). Other results revealed that azadirachtin and nimbolide showed concentration-dependent antiradical scavenging activity and reductive potential in the following order: nimbolide > azadirachtin > ascorbate. Furthermore, administration of azadirachtin and nimbolide inhibited the development of DMBA-induced HBP carcinomas through prevention of procarcinogen activation and oxidative DNA damage and upregulation of antioxidant and carcinogen detoxification enzymes (Priyadarsini, 2009).

2.5.4 Neem as nutritionl and therapeutic supplement in poultry

Active constitutes play role in the diseases cure via activation of antioxidative enzyme, rupture the cell wall of bacteria and play role as chemopreventive through the regulation of cellular pathways. Pharmacological activities of neem are discussed in detail (Figure 1).

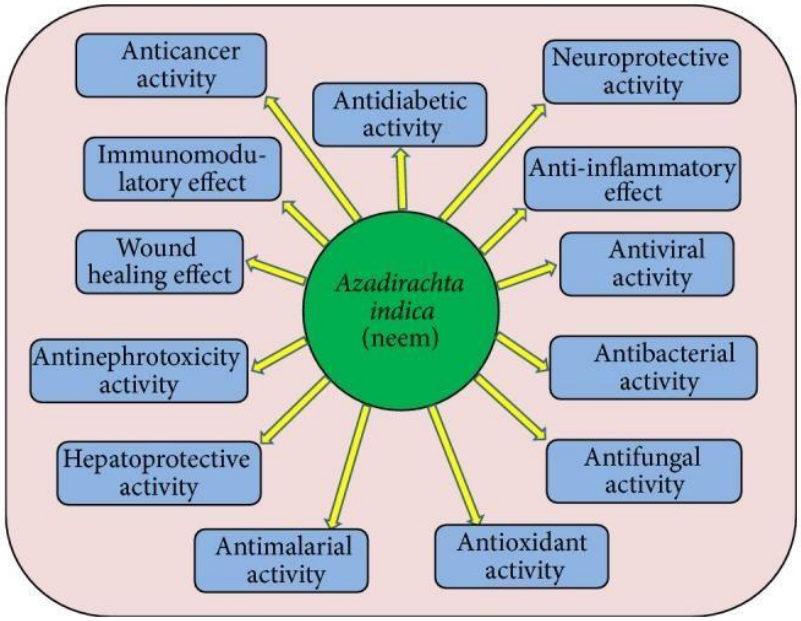

Figure 1. Pharmacological activities of *Azadirachta indica* in diseases management through the modulation of various activities.

2.5.5 Effect of neem on internal organs

Medicinal plants and their ingredients play a pivotal role as hepatoprotective without any adverse complications. A study was performed to investigate the hepatoprotective role of azadirachtin-A in carbon tetrachloride (CCl_4) induced hepatotoxicity in rats and histology and ultrastructure results confirmed that pretreatment with azadirachtin-A dose-dependently reduced hepatocellular necrosis (Baligar, 2014). Furthermore results of the study show that pretreatment with azadirachtin-A at the higher dose levels moderately restores the rat liver to normal.

Another study was carried out to evaluate the protective effect of active constituent of neem such as nimbolide against carbon tetrachloride (CCl_4) induced liver toxicity in rats and results suggest that nimbolide possesses hepatoprotective effect against CCl_4 induced liver damage with efficiency similar to that of silymarin standard (Baligar, 2014) and another study finding revealed that leaf extract was found to have protection against paracetamol-induced liver necrosis in rats (Bhanwra, 2000).

A study assesses the hepatoprotective activity of *Azadirachta indica* leaf extract on antitubercular drugs-induced hepatotoxicity and results confirmed aqueous leaf extract significantly prevented changes in the serum levels of bilirubin, protein, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase and significantly prevented the histological changes as compared to the group receiving antitubercular drugs (Kale, 2003). Additionally, other results showed that ethanolic and aqueous leaf extracts of *A. indica* exhibited moderate activity over carbon tetrachloride treated animals (Kalaivani, 2009). Hepatoprotective effect of methanolic and aqueous extracts of *Azadirachta indica* leaves was evaluated in rats and study result established that the plant has good potential to act as hepatoprotective agent (Devmurari, 2010).

An experiment was made to investigate the protective effect of neem extract on ethanol-induced gastric mucosal lesions in rats and results showed that pretreatment with neem extract showed protection against ethanol-induced gastric mucosal damage (Ofusori, 2010). A study was performed to investigate the neuroprotective effects of *Azadirachta indica*leaves against cisplatin- (CP-) induced neurotoxicity and results showed that morphological findings of neem before and after CP injection implied a well-preserved brain tissue. No changes, in biochemical parameters, were observed with neem treated groups.

2.5.6 Effect of neem on immune organs

Plants or their isolated derivatives are in the practice to treat/act as anti-inflammatory agents. A study result has confirmed that extract of *A. indica* leaves at a dose of 200 mg/kg, p.o., showed significant anti-inflammatory activity in cotton pellet granuloma assay in rats (Chattopadhyay, 1998). Other study results revealed that neem leaf extract showed significant anti-inflammatory effect but it is less efficacious than that of dexamethasone (Mosaddek, 2008) and study results suggest that nimbidin suppresses the functions of macrophages and neutrophils relevant to inflammation (Kaur, 2004).

Earlier finding showed immunomodulator and anti-inflammatory effect of bark and leave extracts and antipyretic and anti-inflammatory activities of oil seeds. Experimentation was made to evaluate the analgesic activity of neem seed oil (Arora, 2011; Biswas, 2002) on albino rats and results of the study showed that neem seed oil showed significant analgesic effect in the dose of 1 and 2 mL/kg and oil has dose-dependent analgesic activity (Kumar, 2012).

Another study was made to investigate the anti-inflammatory effect of neem seed oil (NSO) on albino rats using carrageenan-induced hind paw edema and results revealed that NSO showed increased inhibition of paw edema with the progressive increase in dose from 0.25 mL to 2 mL/kg body weight. At the dose of 2 mL/kg body weight, NSO showed maximum (53.14%) inhibition of edema at 4th hour of carrageenan injection (Naik M. R 2014). Results of the study concluded that the treated animals with 100 mg kg^{-1} dose of carbon tetrachloride extract (CTCE) of *Azadirachta indica* fruit skin and isolated ingredient azadiradione showed significant antinociceptive and anti-inflammatory activities (Ilango, 2013).

2.5.7 Effect of neem on microbial activity

Neem and its ingredients play role in the inhibition of growth of numerous microbes such as viruses, bacteria, and pathogenic fungi. The role of neem in the prevention of microbial growth is described individually as follows.

2.5.7.1 Antibacterial activity

A study was performed to evaluate antimicrobial efficacy of herbal alternatives as endodontic irrigants and compared with the standard irrigant sodium hypochlorite and finding confirmed

that leaf extracts and grape seed extracts showed zones of inhibition suggesting that they had antimicrobial properties. Furthermore, leaf extracts showed significantly greater zones of inhibition than 3% sodium hypochlorite (Ghonmode, 2013).

The antibacterial activity of guava and neem extracts against 21 strains of foodborne pathogens was evaluated and result of the study suggested that guava and neem extracts possess compounds containing antibacterial properties that can potentially be useful to control foodborne pathogens and spoilage organisms (Hoque, 2007).

Another experiment was made to evaluate the antibacterial activity of the bark, leaf, seed, and fruit extracts of *Azadirachta indica* (neem) on bacteria isolated from adult mouth and results revealed that bark and leaf extracts showed antibacterial activity against all the test bacteria used. Furthermore, seed and fruit extracts showed antibacterial activity only at higher concentrations (Yerima, 2012).

2.5.7.2 Antiviral activity

Results showed that neem bark (NBE) extract significantly blocked HSV-1 entry into cells at concentrations ranging from 50 to 100 μg/mL. Furthermore, blocking activity of NBE was noticed when the extract was preincubated with the virus but not with the target cells suggesting a direct anti-HSV-1 property of the neem bark (Tiwari, 2010). Leaves extract of neem has shown virucidal activity against coxsackievirus virus B-4 as suggested via virus inactivation and yield reduction assay besides interfering at an early event of its replication cycle (Badam, 1999).

2.5.7.3 Antifungal activity

Experiment was made to evaluate the efficacy of various extracts of neem leaf on seed borne fungi *Aspergillus* and *Rhizopus* and results confirmed that growth of both the fungal species was significantly inhibited and controlled with both alcoholic and water extract. Furthermore, alcoholic extract of neem leaf was most effective as compared to aqueous extract for retarding the growth of both fungal species (Mondali, 2009). Another finding showed the antimicrobial role of aqueous extracts of neem cake in the inhibition of spore germination against three sporulating fungi such as *C. lunata*, *H. pennisetti*, and *C. gloeosporioides* f. sp. *mangiferae* (Anjali, 2013) and results of the study revealed that methanol and ethanol

extract of *Azadirachta indica* showed growth inhibition against *Aspergillus flavus*, *Alternaria solani*, and *Cladosporium* (Shrivastava, 2014).

2.5.8 Effect of neem on biochemical (safety, toxicity, LD50 value) properties

The measurement of toxicities of natural compound is crucial before their application in health management. Various studies based on animal model and clinical trials confirmed the neem is safe at certain dose and on the other side neem and its ingredients showed toxic/adverse effect. Several studies reported, in children, neem oil poisoning causing vomiting, hepatic toxicity, metabolic acidosis, and encephalopathy (Sundaravalli, 1982) and another study based on rat model showed that administration of leaf sap caused an antianxiety effect at low doses, whereas high doses did not show such types of effect (Jaiswal, 1994). An important study based on rats model showed that azadirachtin did not show toxicity even at 5 g/kg bw (Raizada, 2001). A study based on rabbit was performed to check the toxicological analysis and results of the study showed there was progressive increase in body weight in both the test and control animals, and during the entire duration of the administration of the neem extract, there was no observed sign of toxicity in both groups (Boadu, 2011).

A study result showed that, in the acute toxicity test, the LD_{50} values of neem oil were found to be 31.95 g/kg (Deng, 2013). Another study was performed to evaluate the toxicity in chicken and finding showed that acute toxicity study of neem leaf aqueous extract revealed an intraperitoneal LD_{50} of 4800 mg/kg, and clinical signs were dose dependent (Biu, 2011).
A study reported that lethal median doses (LD_{50}) recorded for neem leaf and stem bark extracts were 31.62 and 489.90 mg/kg body weight, respectively (Akin-Osanaiya, 2013). The LD_{50} of water extract of *A. indica* leaves and seeds were 6.2, 9.4 mL kg^{-1}, respectively (Bakr, 2013). Lethal dose values were calculated with probit analysis and LD_{50} and LD_{90} values were found to be 8.4 and 169.8 μg/fly of neem extract, respectively (Khan, 2013). A test for acute oral toxicity in mice revealed that LD_{50} value of approximately 13 g/kg body weight (Okpanyi, 2011).

2.5.9 Effect of neem on blood parameter

Angiogenesis is complex process that supplies blood to the tissue and that is essential for growth and metastasis of tumour. Angiogenesis is regulated by activators as well as inhibitors. The development of antiangiogenic agents to block new blood vessel growth is crucial step in the inhibition/prevention of tumour growth. Medicinal plants and their ingredients play role in prevention of tumour growth due to their antiangiogenic activity.

An important study revealed that ethanolic fraction of neem leaf (EFNL) treatment effectively inhibited the expression of proangiogenic genes, vascular endothelial growth factor A, and angiopoietin, indicating the antiangiogenic potential of EFNL. Furthermore, inhibition of angiogenesis by ethanolic fraction of neem leaf (EFNL) could be a reason for reduction in mammary tumour volume and for blocked development of new tumours as observed in current studies (Arumugam , 2014).

CHAPTER 3

MATERIALS AND METHODS

3.1 Statement of the experiment

The research work was conducted at SAU poultry farm, with 180-day-old straight run (Cobb 500) commercial broilers for a period of 28 days from 11th May to 7th June, 2017 to assess the feasibility of using Dried Neem Leaf Powder (DNLP) in commercial broiler diet on growth performance, meat yield characteristics and immune status of broilers. This research helps to make a conclusion about DNLP as the alternative of antibiotic.

3.2 Collection of experimental broilers

A total of 180 day-old Cobb 500 broiler chicks were collected from a reputated hatchery in Dhaka.

3.3 Experimental materials

The collected chicks were carried to the university poultry farm early in the morning. They were kept in electric brooders equally for 5 days by maintaining standard brooding protocol. During brooding period 1% DNLP was used in four treatment except the teatment of antibiotic and control group. After two days 120 chicks were selected from brooders and distributed randomly in six (6) dietary treatments group where DLNP used, another 60 chicks were distributed randomly in one treatment for antibiotic and another treatment for control. Each treatment had three (3) replications with 10 birds per replication. The total numbers of treatments were six (6) and their replications were eighteen (18).

3.4 Experimental treatments

Control: Basal Diets

Antibiotics: Basal Diets + Antibiotic (Oxytetracycline)

N_1: 1% of Dried Neem Leaf Powder (2 kg DNLP / 100 kg of the feed)

N_2: 1.5% of Dried Neem Leaf Powder (2.5 kg DNLP / 100 kg of the feed)

N_3: 2% of Dried Neem Leaf Powder (2 kg DNLP / 100 kg of the feed)

N_4: 2.5% of Dried Neem Leaf Powder (2 kg DNLP / 100 kg of the feed)

Table 1. Layout of the experiment

Treatment groups	No. of Replications			Total
	R_1	R_2	R_3	
Control	10	10	10	30
Antibiotic	10	10	10	30
N_1	10	10	10	30
N_2	10	10	10	30
N_3	10	10	10	30
N_4	10	10	10	30
Total	60	60	60	180

3.5 Preparation of experimental house

The experimental room was properly cleaned and washed by using tap water. Ceiling walls and floor were thoroughly cleaned and disinfected by spraying diluted Iodophor disinfectant solution (3 ml/liter water). After proper drying, the house was divided into 18 pens of equal size where using wood materials and wire net. The height of wire net was 36 cm. A group of 10 birds were randomly allocated to each pen (replication) of the 6 (six) treatments. The stocking density was $1m^2$/10 birds.

3.6 Experimental diets

Starter and grower feed were purchased from a reputated feed company from the market. Minimum nutrients present in starter and finisher ration are presented in table 2.

Table 2. Name and minimum percentage of nutrients present in starter ration:

Name of nutrients in starter ration	Minimum percentage present
Protein	21.0%
Fat	6.0%
Fiber	5.0%
Ash	8.0%
Lysine	1.20%
Methionine	0.49%
Cystine	0.40%
Tryptophan	0.19%
Threonine	0.79%
Arginine	1.26%

Table 2. Name and minimum percentage of nutrients present in finisher ration:

Name of nutrients in finisher ration	Minimum percentage present
Protein	19.0%
Fat	6.0%
Fiber	5.0%
Ash	8.0%
Lysine	1.10%
Methionine	0.47%
Cystine	0.39%
Tryptophan	0.18%
Threonine	0.75%
Arginine	1.18%

Feed were supplied 4 times daily by following Cobb-500 Manual and *ad libitum* drinking water 2 times daily. Appendix 1 and 2.

3.6.1 Collection of neem leaf

Dried Neem Leaf powder (DNLP) was used in commercial basal diets. This Neem Leaf was collected from the several Neem plants in SAU. This Neem leaf was dried by sun heat and fragmented by hand and ensure traceability by sieve.

Table 3. Nutrient composition of DNLP

Nutrient Component	Amount
1. Dry matter	90.24%
2. Crude protein	23.40%
3. Ether extract	3.36%
4. Ash	9.90%
5. Caude fiber	7.81%
6. Calcium(g)	1.40
7. Phosphorus(g)	0.25

Source: Ilango *et al.* (2013)

3.7 Management procedures

Body weight and feed intake were recorded every week and survivability was recorded for each replication up to 28 days of age. The following management procedures were followed during the whole experiment period.

3.7.1 Brooding of baby chicks

The experiment was conducted during **11th May to 7th June, 2017**. The average temperature was 35°C and the RH was 60% in the poultry house. Common brooding was done for one week. After one week the chicks were distributed in the pen randomly. There were 10 chicks in each pen and the pen space was $1m^2$. Due to hot climate brooding temperature was maintained as per requirement. Brooding temperature was adjusted (below 35°C) with house temperature. So when the environmental temperature was above the recommendation, then no extra heat was provided. At day time only an electric bulb was used to stimulate the chicks to eat and drink. In brooding extra heat was not provided at day time except mid night to morning. Electric fans were used as per necessity to save the birds from the heat stress.

3.7.2 Room temperature and relative humidity

Daily room temperature (°C)and humidity were recorded every six hours with a thermometer and a wet and dry bulb thermometer. Average room temperature and percent relative humidity for the experimental period were recorded and presented in Appendix 3 & 4.

3.7.3 Litter management

Rice husk was used as litter at a depth of 6cm. At the end of each day, litter was stirred to prevent accumulation of harmful gases and to reduce parasite infestation. At 3 weeks of age, droppings on the upper part of the litter were cleaned and for necessity fresh litter was added.

3.7.4 Feeding and watering

Feed and clean fresh water was offered to the birds *ad libitum.* One feeder and one round drinker were provided in each pen for 4 birds. Feeders were cleaned at the end of each week and drinkers were washed daily. Fed to all birds *ad libitum* throughout the experimental period.

3.7.5 Lighting

At night there was provision of light in the broiler farm for easy feed intake and body growth. For first 2 weeks 24 hours light was used. Thereafter 22 hours light and 2 hours dark was scheduled up to 28 days.

3.7.6 Bio-security measures

To keep disease away from the broiler farm recommended vaccination, sanitation program was undertaken in the farm and its premises. All groups of broiler chicks were supplied Vitamin B-Complex, Vitamin-ADEK, Vitamin-C, Ca and Vitamin-D enriched medicine and electrolytes.

3.7.7 Vaccination

The vaccines collected from medicine shop (Ceva Company) and applied to the experimental birds according to the vaccination schedule. The vaccination schedule is shown in Table 4.

Table 4. Vaccination schedule

Age of birds	Name of Disease	Name of vaccine	Route of administration
3 days	IB + ND	MA-5 + Clone-30	One drop in each eye
9 days	Gumboro	G-228E (inactivated)	Drinking Water
17days	Gumboro	G-228E (inactivated) booster dose	Drinking Water
21 days	IB + ND	MA-5 + Clone-30	Drinking Water

3.7.8 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation it was easy to remove polluted gases from the farm. Besides ventilation was regulated as per requirement by folding polythene screen.

3.7.9 Sanitation

Strict sanitary measures were taken during the experimental period. Disinfectant (Virkon) was used to disinfect the feeders and waterers and the house also.

3.8 Study parameters

3.8.1 Recorded parameters

Weekly body weight, feed consumption and death of chicks were recorded. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter gizzard, liver, spleen, intestine, hear and bursa were measured from each broiler chicken. Dressing yield was calculated for the bird of each replication to find out dressing percentage. Blood sample was analysis from each replication to measure, complete blood count (CBC), sugar and cholesterol level. Feces sample was collected to measure microbial load in the gut.

3.9 Data collection

3.9.1 Live weight: The initial day-old live weight and weekly live weight of each replication was kept to get final live weight record per bird.

3.9.2 Dressing yield = Live weight- (blood + feathers + head + shank+ digestive system+ Liver+Heart)

3.9.3 Feed consumption: Daily feed consumption record of each replication was kept to get weekly and total feed consumption record per bird.

3.9.4 Mortality of chicks: Daily death record for each replication was counted up to 28 days of age to calculate mortality.

3.9.5 Dressing procedures of broiler chicken:

Three birds were picked up at random from each replicate at the 28^{th} day of age and sacrificed to estimate dressing percent of broiler chicken. All birds to be slaughtered were weighed and fasted f by halal method or overnight (12 hours) but drinking water was provided *ad-libitum* during fasting to facilitate proper bleeding. All the live birds were weighed again prior to slaughter. Birds were slaughtered by severing jugular vein, carotid artery and the trachea by a single incision with a sharp knife and allowed to complete bleed out at least for 2 minutes. Outer skin was removed by sharp scissor and hand. Then the carcasses were washed manually to remove loose singed feathers and other foreign materials from the surface of the carcass. Afterward the carcasses were eviscerated and dissected according to the methods by

Jones (1982). Heart and liver were removed from the remaining viscera by cutting them loose and then the gall bladder was removed from the liver. Cutting it loose in front of the proventiculus and then cutting with both incoming and outgoing tracts removed the gizzard. Dressing yield was found by subtracting blood, feathers, head, shank, liver, heart and digestive system from live weight.

3.9.6 Blood sample analysis

Blood samples (1 ml/bird) were collected into ethylenediethyletetraacitic acid (EDTA) tubes from the wing veins. Samples were transferred to the laboratory for analysis within 1 hour of collection. All the haematological test was measured at DR. M A Wazed Miah Research Centre, SAU, maintaining standard protocol.

3.10 Calculations

3.10.1 Live weight gain

The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds. Body weight gain = Final weight – Initial weight

3.10.2 Feed intake

Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication. Feed intake (g/bird) =

$$\frac{\text{Feed intake in a replication}}{\text{No. of birds in a replication}}$$

3.10.3 Feed conversion ratio

Feed conversion ratio (FCR) was calculated as the total feed consumption divided by weight gain in each replication. FCR=

$$\frac{\text{Feed intake (kg)}}{\text{Weight gain (kg)}}$$

3.10.1 Statistical analysis

The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 16. Differences between means were tested using Duncan‟s multiple comparison test, LSD and significance was set at $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production performances of broiler chicken

4.1.1 Final live weight

Final live weight of broiler (g/bird) fed different level of dried neem leaf powder (DNLP) containing diets comparison to antibiotic based diet were presented to table-5. The live weight of broiler in the dietary group Control (C), Antibiotic (A) , N_1 (1%), N_2 (1.5%) N_3 (2%) and N_4 (2.5%) were 1717.67^{ab}±12.914, 1666.33^{b}±31.991, 1696.67^{ab}±6.009, 1732.00^{a}±1.155, 1707.67^{ab}±24.265 and 1711.67^{ab}±6.667 respectively. The highest body weight was found in N_2 group whereas lowest in (A) group of broiler. However, final live weight of broiler fed neem leaf based diets was significantly (P<0.05) higher compared with that of the antibiotic treated group.

Table 5: Production performance of broiler treated with neem leaf and antibiotic.

Parameters	Control	Antibiotic	N_2	N_2	N_3	N_4	Mean ±SE	LSD (0.05)
Final Live Weight (g/broiler)	1717.67^{ab} ±12.91	1666.33^{b} ±31.991	1696.67^{ab} ±6.01	1732.00^{a} ±1.16	1707.67^{ab} ±24.27	1711.67^{ab} ±6.67	1705.33 ±7.81	24.91^{*}
FC (g)	2228.10^{a} ±39.295	2154.43^{ab} ±3.40	2206.60^{a} ±27.8	2215.90^{a} ±21.78	2101.70^{b} ±45.81	2103.90^{b} ±6.23	2168.44 ±15.96	40.58^{*}
FCR	1.27±0.01	1.26±0.02	1.27±0.02	1.25±0.01	1.22±0.04	1.22±0.01	1.25±0.01	0.03^{NS}
DP% (Skinless)	72.29 ±1.11	71..80 ±2.84	72.41 ±1.18	71.65 ±2.23	70.42 ±1.08	71.92 ±2.81	71.74 ±0.78	2.48^{NS}

Here, N_1 = (1% DNLP Supplementation), N_2 = (1.5% DNLP Supplementation), N_3 = (2% DNLP Supplementation), N_4 = (2.5% DNLP Supplementation).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don‟t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ *means significant at 5% level of significance (p<0.05)
- ✓ NS= Non-significant

Alam *et al.* (2015) and Ansari (2012) found significantly higher live weight in neem leaf treated groups compared to control group. However, birds supplemented with neem leaf powder had higher body weight and feed efficiency. These results may be due to antimicrobial and

antiprotozoal properties of neem leaves, which help to reduce the microbial load of birds and improved the feed consumption and feed efficiency of the birds (Kale *et al.* 2003). Similar observation was found in the study of Manwar *et al.* (2005) who supplemented neem leaf powder in feed and reported significant increase body weight of broilers in the neem fed groups when compared with antibiotic group.

4.1.2 Weekly body weight gain

The mean body weight gain (g) of broiler at the end of 4th week in different dietary groups Control (C), Antibiotic (A) , N_1 (1%), N_2 (1.5%) N_3 (2%) and N_4 (2.5%) were 673.00±14.74, 631.67±22.73, 673.67±8.84, 678.00±7.77, 665.33±15.62 and 654.67±29.98 respectively. The overall mean body weight gain of different groups showed that there were no significant differences (P>0.05) among the groups but better live weight were found in DNLP supplemented and control group than that of antibiotic group. (table 6 and figure 2).

Adeyeri (2012) recommended that the neem leaf meal inclusion in the diets of the broiler chickens can be used as growth promoters during the chick phase or growth (Bonsu 2012).

Table 6. Effects of feeding different level of Neem leaf and antibiotic on body weight gain (g/bird) of broiler at different week.

Treatments	1st week	2nd week	3rd week	4th week
Control	172.00^{ab}±3.786	329.67±7.424	498.00±9.504	673.00±14.742
Antibiotic	180.00^{a}±2.517	310.67±12.333	499.00±10.599	631.67±22.733
N_1	182.33^{a}±1.764	326.67±2.963	467.67±16.697	673.67±8.838
N_2	179.33^{ab}±3.283	312.33±9.333	517.33±6.009	678.00±7.767
N_3	169.00^{b}±4.726	324.67±8.647	504.00±17.349	665.33±15.624
N_4	173.00^{ab}±1.732	330.33±9.528	508.67±26.934	654.67±29.980
Mean±SE	175.94±1.600	322.39±3.591	499.11±6.661	662.72±7.360
$LSD_{(0.05)}$	4.468*	12.499^{NS}	22.686^{NS}	25.919^{NS}

Here, N_1 = (1% DNLP Supplementation), N_2 = (1.5% DNLP Supplementation), N_3 = (2% DNLP Supplementation), N_4 = (2.5% DNLP Supplementation).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don"t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ *means significant at 5% level of significance (p<0.05)
- ✓ NS= Non-significant

4.1.3 Feed consumption (FC)

Different treatment groups (table 5) showed significant (P<0.05) differences in FC of broiler chicken. Control (C) group consumed higher amount of feed (2228.10^{a}±39.295) and 2% (N_3) dried Neem leaf powder treated group consumed lower amount of feed (2101.70^{b}±45.805). Antibiotic treated group (A) (2154.43^{ab}±3.398) showed no significant (P>0.05) difference in feed consumption the other treatmented groups**.** The observation was that of decreasing feed consumption as the level of supplementation is increased. This might be attributed to the bitter nature of the neem leaf which reduced the palatability of the feed. This is in consonance with the observations of Bawa *et al.* (2007) who fed broiler with DNLP and reported reduced feed consumption among the broiler on the test diets. Edens *et al*. (2000) has also established the presence of bitter triterpenoids in the neem leaf.

4.1.4 Weekly feed consumption

The mean feed consumption (FC) of broiler (g/birds) at the end of 4th week in different dietary groups Control (C), Antibiotic (A) , N_1 (1%), N_2 (1.5%) N_3 (2%) and N_4 (2.5%) were 968.03^{a}±36.679, 914.23^{ab}±4.201, 950.33^{a}±25.536, 952.93^{a}±18.495, 858.17^{b}±28.486 and 850.63^{b}±1.450 correspondingly (table-7). The overall mean FC of different groups showed that there was significant (P<0.05) increase in groups C, N_1, and N_2 compared to N_3 and N_4 (Table 7 and Figure 3). However, highest FC was observed in the bird of control group which is statically similar to the other groups but dissimilar to the N_3 and N_4 groups.

Table 7. Effects of feeding different level of Neem leaf and antibiotic on feed consumption (g/bird) of broiler at different week.

Treatments	1st week	2nd week	3rd week	4th week
Control	186.97±1.506	368.43±7.414	704.67±3.833	968.03^{a}±36.679
Antibiotic	184.50±1.607	358.70±5.866	697.00±0.00	914.23^{ab}±4.201
N_1	185.73±0.897	365.87±4.493	704.67±3.833	950.33^{a}±25.536
N_2	184.47±1.444	377.67±3.686	700.83±3.833	952.93^{a}±18.495
N_3	185.10±0.802	357.60±13.551	700.83±3.833	858.17^{b}±28.486
N_4	184.10±1.069	372.17±7.452	697.00±0.00	850.63^{b}±1.450
Mean±SE	185.14±0.492	366.74±3.170	700.83±1.315	915.72±13.724
LSD$_{(0.05)}$	1.782^{NS}	10.990^{NS}	4.426^{NS}	32.510*

Here, N_1 = (1% DNLP Supplementation), N_2 = (1.5% DNLP Supplementation), N_3 = (2% DNLP Supplementation), N_4 = (2.5% DNLP Supplementation).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don"t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ *means significant at 5% level of significance (p<0.05)
- ✓ NS= Non-significant

Here also observed that of decreasing feed consumption as the level of supplementation is increased. However, debitterization through water washing, alkali soaking and urea ammoniation to improve palatability has been recommended (Katiyar *et al*., 1991). Also the decrease in feed intake observed could be as a result of antinutritional factors present in the test ingredients. This findings agrees with the previous observation of Nworgu, (2002) who incorporated different leaf meals noted for their antinutritional factors in the diets of poultry, and observed decreases in feed intake.

4.1.5 Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was non significant (P>0.05) and the FCR of different groups Control (C), Antibiotic (A) , N_1 (1%), N_2 (1.5%) N_3 (2%) and N_4 (2.5%) showed 1.27±0.007, 1.26±0.015, 1.27±0.017, 1.25±0.007, 1.22±0.038 and 1.22±0.006 respectively (Table 5).

No significant (P>0.05) difference were found in FCR of broiler among different treatment groups but better FCR were found in most of the DNLP supplemented groups than antibiotic and control groups. Alam *et al.* (2015) found identical non-significant FCR in all Neem treated groups compared to that of control group of broilers. Zanu *et al.* (2011) also got no significant effect of Neem decoctions on feed conversion efficiency. But Ansari *et al.* (2012) found contrary result and reported that at 28 days birds fed diets supplemented with 2.5 g/kg of leaf meal had significantly greater better FCR than those fed diets with 1.25, 5.0 g/kg of *Neem* leaf meal and controls.

4.1.6 Weekly feed conversion ratio

The mean FCR of broiler at the end of 4^{th} week in different dietary groups Control (C), Antibiotic (A) , N_1 (1%), N_2 (1.5%) N_3 (2%) and N_4 (2.5%) were 1.44±0.037, 1.45±0.052, 1.41±0.052, 1.41±0.041, 1.29±0.062 and 1.30±0.065 respectively. The overall mean FCR of different groups showed that there was no significant (P>0.05) differences in the groups N_1, N_2, N_3 and N_4 compared to control and antibiotic (Table 8 and Figure 4). But Ansari *et al.* (2012) found contrary result and reported that at 28 days age birds fed diets supplemented with 2.5 g/kg of leaf meal had significantly better FCR than those fed diets with 1.25, 5.0 g/kg of Neem leaf meal and controls.

Table 8. Effects of feeding different level of Neem leaf and antibiotic on FCR of broiler at different week.

Treatments	1st week	2nd week	3rd week	4th week
Control	1.09^{a}±0.032	1.12^{b}±0.006	1.42±0.022	1.44±0.037
Antibiotic	1.03^{ab}±0.012	1.16^{ab}±0.027	1.40±0.032	1.45±0.052
N_1	1.02^{b}±0.006	1.12^{b}±0.010	1.51±0.052	1.41±0.052
N_2	1.03^{ab}±0.010	1.21^{a}±0.032	1.36±0.009	1.41±0.041
N_3	1.09^{a}±0.027	1.11^{b}±0.035	1.39±0.059	1.29±0.062
N_4	1.06^{ab}±0.015	1.13^{b}±0.022	1.38±0.076	1.30±0.065
Mean±SE	1.05±0.010	1.14±0.012	1.41±0.020	1.38±0.024
$LSD_{(0.05)}$	0.027^{*}	0.034^{*}	0.067^{NS}	0.074^{NS}

Here, N_1 = (1% DNLP Supplementation), N_2 = (1.5% DNLP Supplementation), N_3 = (2% DNLP Supplementation), N_4 = (2.5% DNLP Supplementation).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don‟t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ *means significant at 5% level of significance (p<0.05)
- ✓ NS= Non-significant

4.1.7 Dressing percentage (DP)

The dressing percent (Table 5) data of broiler were affected by DNLP and antibiotic. The treatment groups C (72.41%), A (71.65%), N_1 (70.42%), N_2 (71.92%), N_3 (71.81%) and N_4 (72.29%) showed no significance (P>0.05) difference in dressing percent of broiler chicken. Alam *et al.* (2015) also found that polyherbal (including neem) extrat did not exhibit any effect on the dressing percentage values of broiler.

4.1.8 Immune organs

Effect of dried Neem leaf powder supplementation on immune organs of Cobb 500 strain broiler during the period from 0 to 28 days of age are summarized in Table 9 and Figure 5. The liver weight of broiler fed different level of DNLP were statically higher (P<0.05) compared with that of antibiotic group but no effects on control group. However, higher liver weight was in in N_1 (1% DNLP) group and lowest in control (C) group. Significantly (P<0.05) higher liver weight was found in DNLP treated groups than antibiotic group. The weight of liver was higher in N_1 group (46.67^{a}±4.410) significantly (P<0.05) highest liver weight; whereas antibiotic treated group (31.00^{b}±2.082) was lowest. Control (37.67^{ab}±2.333), N_2 (44.00^{a}±1.528), N_3 (43.67^{a}±1.764) and N_4 (45.00^{a}±3.215) correspondingly. Increased liver weight at DNLP treated birds indicates better detoxification of blood, better health and meat quality. Similar findings noted by (Steel *et al.* 1980)

and stated that liver weights significantly increased with the inclusion of Neem decoction in broiler diets. But, (Talwari *et al.* 2010) found no significant (P>0.05) difference in liver weight fed by the DNLP.

The comparative weight of spleen (g) of broiler in the dietary group Control, Antibiotic, N_1, N_2, N_3 and N_4 were 1.67±0.167, 1.50±0.029, 2.33±0.333, 2.33±0.333, 1.83±0.167, ±0.167 respectively. The highest value was recorded in N_1 and N_2 (2.33±0.333) and lowest value was Antibiotic treated group (1.50±0.029). However, the relative weight of spleen of different groups showed non significant (P>0.05) difference among the groups.

Supplementation of DNLP in broiler diets did not exert any effect on the mean relative values of spleen weights of the broilers used in this study. But relatively higher spleen weight was found in DNLP treated groups than antibiotic and control groups. Larger size spleen produces more antibodies which results stronger immune system. The results of the study is consistent with previous findings by (Alam et al.2015; Ahmad, 2005 and Landy, 2011) also stated that internal organs weight was not influenced by the dietary treatments of Neem.

Table 9. Effect of dietary supplementation of Neem leaf to broiler diets on some immune organs.

Parameters	Control	Antibiotic	N_1	N_2	N_3	N_4	Mean ±SE	LSD (0.05)
Liver Weight (gm)	37.67^{ab} ±2.333	31.00^{b} ±2.082	46.67^{a} ±4.410	44.00^{a} ±1.528	43.67^{a} ±1.764	45.00^{a} ±3.215	41.33 ±1.611	3.873^{*}
Spleen Weight (gm)	1.67 ±0.167	1.50 ±0.029	2.33 ±0.333	2.33 ±0.333	1.83 ±0.167	1.83 ±0.167	1.92 ±0.116	0.360^{NS}

Here, N_1 = (1% DNLP Supplementation), N_2 = (1.5% DNLP Supplementation), N_3 = (2% DNLP Supplementation), N_4 = (2.5% DNLP Supplementation).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don"t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ *means significant at 5% level of significance (p<0.05)
- ✓ NS= Non-significant

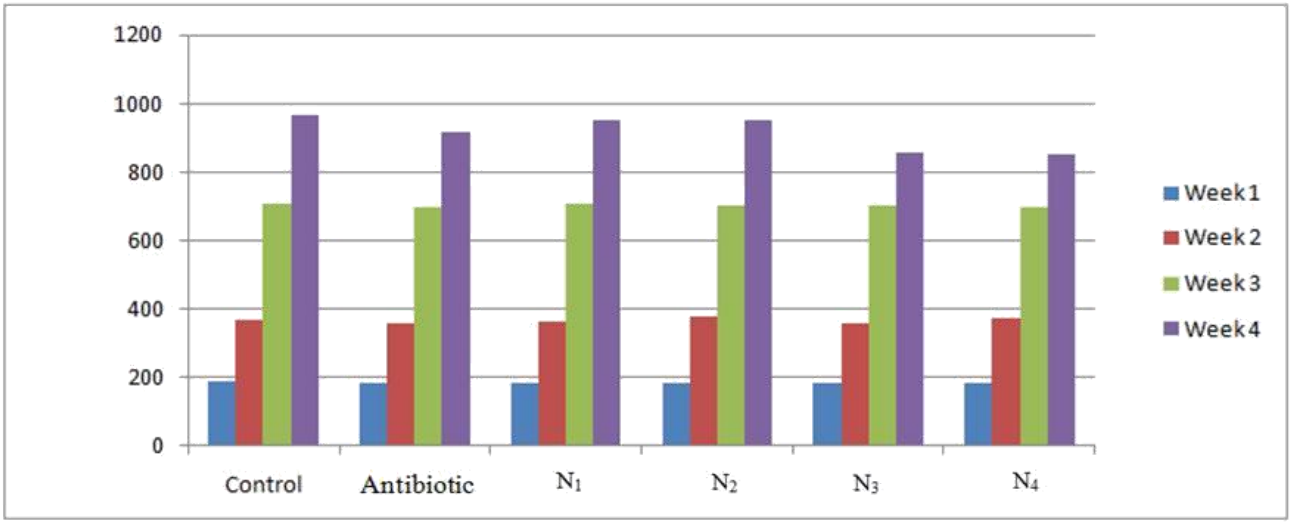

Figure 2. Effects of feeding different level of Neem leaf and antibiotic on Feed Consumption (FC) (g/bird) of broiler chickens at different weeks.

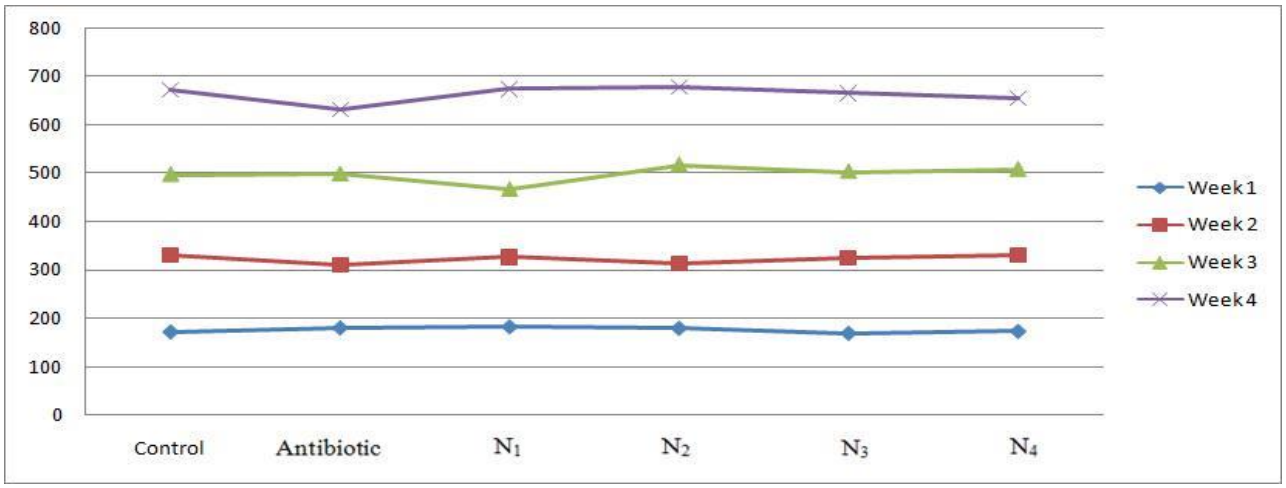

Figure 3. Effects of feeding different level of Neem leaf and antibiotic on body weight gain (BWG) (g/bird) of broiler chickens at different weeks.

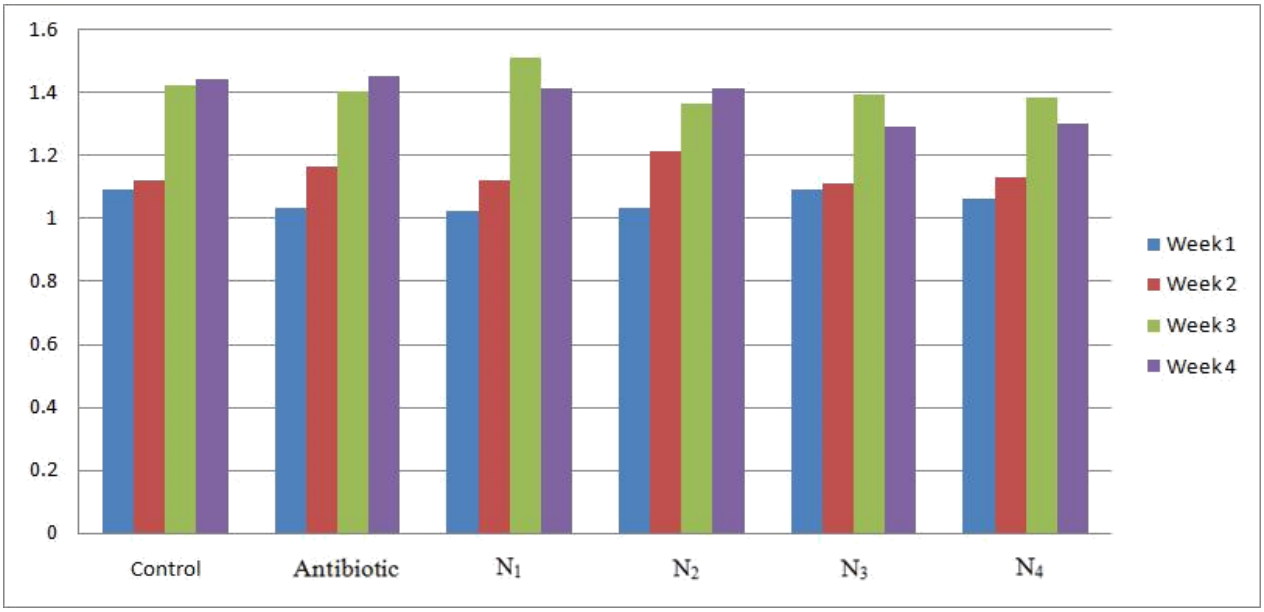

Figure 4. Effects of feeding different level of Neem leaf and antibiotic on FCR of broiler chickens at different week.

4.2 Haematological parameters

Tables 10. show the effect of dietary levels of dried neem leaf powder (1%, 1.5%, 2% and 2.5%) in feed, and their impact on some blood parameters. Concerning the treatment effect on blood constituents, the results indicated no significant differences due to supplementation of dried Neem leaf powder, except, Hemoglobin and Uric acid which were significantly affected.

Table10. Effect of supplementation of Dried Neem Leaf Powder (DNLP) to broiler diets on blood parameters.

Parameters	Control	Antibiotic	N_1	N_2	N_3	N_4	Mean ±SE	LSD (0.05)
Sugar (mg/dL)	10.50 ±0.13	10.83 ± 0.8	10.55 ±0.10	10.71 ±0.28	10.33 ± 0.12	10.40 ±0.13	10.72 ±0.50	3.01^{NS}
Cholesterol (mg/dL)	219.3 ±24.88	219.00 ±11.15	225.00 ±20.075	197.33 ±2.73	207.00 ±10.44	205.67 ±19.46	212.22 ±6.13	23.393 NS
Hemoglobin (g/dL)	13.17^{b} ±0.054	14.23^{b} ±0.874	13.60^{b} ±0.173	13.17^{b} ±0.318	16.33^{a} ±0.731	14.50^{b} ±0.416	14.17 ±0.327	0.791^{*}
Uric Acid (mg/dL)	5.20^{ab} ±0.321	5.70^{a} ±0.265	5.10^{ab} ±0.115	4.80^{b} ±0.265	5.03^{ab} ±0.120	5.07^{ab} ±0.120	5.15 ±0.100	0.309^{*}
Neutrophil (%)	32.00 ±1.528	29.33 ±1.856	29.00 ±2.309	31.67 ±1.764	33.67 ±3.756	28.67 ±0.882	30.72 ±0.887	3.115^{NS}
Lymphocyte (%)	58.67 ±1.453	62.00 ±2.309	58.33 ±0.882	56.33 ±2.333	56.67 ±2.333	59.67 ±1.202	58.61 ±0.784	2.618^{NS}
Monocyte (%)	7.33 ±1.856	6.67 ±0.882	8.33 ±1.202	8.33 ±0.882	8.33 ±1.202	8.00 ±1.000	7.83 ±0.445	1.721^{NS}
Eosinophil (%)	2.00 ±0.577	2.00 ±0.000	4.33 ±0.333	3.67 ±2.082	3.00 ±0.577	3.67 ±0.667	3.11^{a} ±0.312	0.943^{NS}

Here, N_1 = (1% DNLP Supplementation), N_2 = (1.5% DNLP Supplementation), N_3 = (2% DNLP Supplementation), N_4 = (2.5% DNLP Supplementation).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don‟t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ *means significant at 5% level of significance (p<0.05)
- ✓ NS= Non-significant

4.2.1 Sugar

Effects of dietary dried neem *leaf* powder supplementation on concentration of sugar of broiler chickens are presented in Table 10. Different treatment groups of broiler chicken treated with DNLP and antibiotic showed no significance (P>0.05) difference in blood glucose in broiler chicken. The blood glucose data in different treatment groups are C (10.50±0.13 mmol/L), A (10.83±0.8 mmol/L), N_1 (10.55±0.10 mmol/L), N_2 (10.71±0.28 mmol/L), N_3 (10.33±0.12 mmol/L), N_4 (10.40±0.13 mmol/L). Here the broiler chicken of antibiotic treated group 'A' showed the tendency of increasing the blood glucose level. (Obikaonu et al. 2012) reported that blood sugar was significantly (P<0.05) increased by supplementing DNLP to broiler diets.

4.2.2 Total cholesterol

Total cholesterol concentration (mg/dL) in the blood of different groups ranged from (197.33±2.73) to (225.00±20.075). Statistical analysis revealed a nonsignificant (P>0.05) deference among the group. The cholesterol level of different treatments were Control (C) (219.3±24.88), Antibiotic (A) (219.00±11.15), N_1 (225.00±20.075), N_2 (197.33±2.73), N_3 (207.00±10.44) and N_4 (205.67±19.46) correspondingly. (Table 10)

Ansari et al. (2012) found contrary results by investigating the serum cholesterol. They reported that serum cholesterol progressively decreased if dietary levels of *Azadirachta indica* leaf meal are increased. Alam *et al*. (2015) also reported that Cholesterol was significantly (P<0.05) decreased by neem leaf meal.

4.2.3 Hemoglobin

The hemoglobin data (Table 10) of broiler chicken were affected significantly (P<0.05) treated by DNLP and antibiotic. The DNLP treated groups N_3 showed the highest hemoglobin level than other treatment groups of N_1, N_2, N_4, Antibiotic and Control respectively, but no significant (P>0.05) difference was found among most of the *Neem* treatment with control and antibiotic groups except 2% DNLP treatment. The current results supported by (Bonsu *et al.* 2012) who found that hemoglobin were not significantly influenced by DNLP. Similarly (Odo and Bratte,2015) also found that DNLP had no significant (P>0.05) effect on Haemoglobin of layer chicken.

4.2.4 Uric acid

The blood uric acid data (Table 10) of broiler chicken treated with DNLP and antibiotic showed significant (P<0.05) differences among the treatment groups of N_1, N_2, N_3, N_4, Antibiotic and Control. The antibiotic treated group T5 (5.70±0.265 mg/dl) showed the significantly (P<0.05) highest uric acid level and N_2 (4.80±0.265 mg/dl) showed the lowest uric acid level in comparison with other treatment groups. It can be concluded from the table that blood uric acid level showed a decreasing trend in Neem treated groups than antibiotic and control group. Here the DNLP acted as hepatoprotecter in broiler physiology. Lower uric acid level in blood is a sign of good renal function and good health, so Neem leaf meal can be used in broiler ration instead of antibiotic. "Serum uric acid values showed a decreasing trend with increased level of Neem Leaf Meal'' as reported by Jawad *et al.* (2014).

4.2.5 Number of leukocytes of broiler

The Neutrophils percent of broiler chicken presented in Table 10 ranges from 28.67±0.882 to 33.67±3.756 showed non significant (P>0.05) difference among the different treatment groups. Bonsu et al. (2012) stated dissimilar result at DNLP produced significant (P<0.05) differences in the neutrophils between different treatment groups of layer chicken.

The Lymphocytes percent of broiler chicken presented in Tables 10. ranges from 56.33±2.333 to 62.00±2.309 were not affected significantly (P>0.05) by DNLP and antibiotic. Contrary findings published by (Odo and Bratte, 2015) and they found DNLP produced significant (P<0.05) differences between treatment means in the lymphocytes of layer chicken. Similarly, Zanu *et al.* (2011) reported that Lymphocytes which were significantly (P<0.05) influenced by Neem decoction in broiler chickens.

The Monocytes cell percent of broiler chicken presented in Table10 ranges from 6.66 to 8.33 did not show any significantly (P>0.05) difference among the different treatment groups. This finding which is also in agreement with the findings of (Alam *et al*, 2015.) who observed no significant difference in monocytes cell of broiler chicken. But, (Odo and Bratte, 2015) found significant (P<0.05) differences between treatment means in the monocytes counts by treated DNLP in layer chicken.

The Eosinophils cell percent of broiler chicken presented in Table 10 ranges from 2.00 towere not affected significantly (P>0.05) by DNLP and antibiotic. This result is in line

with the findings of Obikaonu *et al.* (2012) who observed no significant effect on mean values of eosinophils of broiler chicken. Similarly, Odo and Bratte (2015) noted that DNLP produced no significant (P>0.05) differences between treatment means in the eosinophils of layer chicken.

4.3 Intestinal microflora

Total viable count of bacteria from caecal faeces of broiler chicken treated with Neem and antibiotic presented in Table 11. Different treatment groups showed significant (P<0.05) difference among treatments. The cfu/gram in Neem treated groups ranges from 20 x 10^4 to 44 x 10^4 . The highest (P<0.05) viable bacteria was found in control group (163 x 10^{4a}) than antibiotic (33 x 10^{4b}) and Neem treated groups. But, Neem and antibiotic treated groups showed non significant (P>0.05) difference among them. This findings confirmed by Adams (2001).

Table 11. Total viable count of bacteria from caecal faeces of broiler chicken treated with Neem and antibiotic (Using dilution factor 10^{-4})

Treatments	Colony forming unit of bacteria (cfu)/gram
Control	163 x 10^{4a}
Antibiotic	33 x 10^{4b}
N_1	20 x 10^{4b}
N_2	20 x 10^{4b}
N_3	23 x 10^{4b}
N_4	24 x 10^{4b}
Mean±SE	33.83 x 10^4±11.68X10^4
$LSD_{(0.05)}$	36.81 x10^{4*}

Here, N_1 = (1% DNLP Supplementation), N_2 = (1.5% DNLP Supplementation), N_3 = (2% DNLP Supplementation), N_4 = (2.5% DNLP Supplementation).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don"t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ NS= Non-significant
- ✓ *means significant at 5% level of significance (p<0.05)

CHAPTER 5

SUMMARY AND CONCLUSION

Antibiotics at low doses are commonly used as growth promoters in broiler feed. However, antibiotics use is under severe criticism due to the development of antibiotic resistance and residual effects on human. In the present study, Dried Neem Leaf Powder (DNLP) for potentiality as an alternative to antibiotics. The experiment was conducted at SAU poultry farm.

The effects of supplementation of DNLP and antibiotic were measured. Diet and fresh drinking water were supplied ad. libitum to broiler chicks. The performance traits *viz.* body weight, weight gain, feed consumption, FCR, dressed bird weight of broiler on different replication of the treatments was recorded and compared in each group at 28 days of age, 18 broilers were dissected to compare meat yield characteristics among different treatments.

The group N_2 (1.5% DNLP) showed higher body weight compared to any others groups whereas N_4 (2.5% DNLP) group consume lowest amount of feed among all treated groups. On the other hand the FCR showed greater value in N_3 (2% DNLP) and N_4 (2.5% DNLP) groups against control and antibiotic treated groups. The relative weight of spleen did not show any difference between any of the treatment groups or the control but the relative weight of liver were better in neem leaf treated groups compared to antibiotic treated groups. The serum biochemistry parameters *viz.* sugar, total cholesterol, hemoglobin and uric acid were studied to evaluate the functional status body.

The sugar and cholesterol level of different treatments were similar in all treatments. The results indicated no alterations in biochemical parameters, except that a lower amount was observed in uric acid levels in neem leaf supplemented groups. Concerning the treatment effect on blood constituents, the results indicated no significant differences due to supplementation of dried neem leaf powder. The numbers of intestinal microflora were significantly higher in control group compared to other groups.

Analyzing the above research findings the production performance, FCR, carcass traits hematological parameter, weight of lymphatic organ and microbial load in feaces sample 2.5% Neem leaf meal was very effective. So, neem leaf meal could be used as an alternative of antibiotic in broiler ration. The study therefore recommends conducting field trial on commercial poultry farm to fix up up inclusion level of neem leaf meal.

REFERENCES

Abalaka M, Oyewole O, Kolawole AR (2012). Antibacterial activities of *Azadirachta indica* against some bacterial pathogens. *Advances in Life Sciences*, 42(2):5-8.

Abdel Moneim A. E. (2014). *Azadirachta indica* attenuates cisplatin-induced neurotoxicity in rats. *Indian Journal of Pharmacology*, 46(3):316–321.

Acamovic, T. (2001). Enzymes for poultry. *World's Poultry Sci J.* 57: 225-242.

Adams, H.R. (2001). Veterinary pharmacology and therapeutics. 18th ed. Iowa state press. USA. pp. 310-311.

Adeyeri OP. (2012). The effect of neem (Azadirachta indica) leaf meal on the growth performance of broiler chickens. Undergraduate and Postgraduate Thesis. Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria.

Ahmad S. (2005). Comparative efficiency of turmeric, garlic, turmeric and kalongi as growth promoter in broiler. M.Sc. (Hons.) Thesis, Department of Poultry Sciences, University of Agriculture, Faisalabad, Pakistan.

Alam M, ATM, Rakib FK, Al-Hasan MA, Hasan MS, Ali MA (2015). Effects of neem leave powder as a growth promoter in broilers. *International Journal of Natural and Social Sciences*, 2:22-27.

Alam MN, Uddin J, Akter MTD, Moni MIZ, Alom F, Rahman A, Al Noman MA. Broiler production by using polyherbal medication (Neem, Nishyinda, Tulsi and Turmeric Extract) 2014. *International Journal of Innovation and Applied Studies*, 9(3):1161-1175.

Amadioha A. C., Obi V. I. (1998). Fungitoxic activity of extracts from *Azadirachta indica* and *Xylopia aethiopica* on *Colletotrichum lindemuthianum* in cowpea. *Journal of Herbs, Spices and Medicinal Plants*, 6(2):33–40.

Anjali K., Ritesh K., Sudarshan M., Jaipal S. C., Kumar S. (2013). Antifungal efficacy of aqueous extracts of neem cake, karanj cake and vermicompost against some phytopathogenic fungi. *The Bioscan*, 8:671–674.

Annison G and Choct M (1993). Enzymes in poultry diets and Enzymes in Animal Nutrition. Proceedings Ist Symposium, Switzerland. Oct 13-16. pp. 61-63.

Ansari J, Khan SH, Haq A, Yousuf M. (2012). Effect of the levels of *Azadirachta indica* dried leaf meal as phytogenic feed additive on the growth performance and haematobiochemical parameters in broiler chicks. *J. of Applied Animal Research*, 40(4): 456-468.

Ao T, Cantor, A.H., Pescatore, A.J., Ford, M.J., Pierce, J.L. and Dawson, K.A. (2009). Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *J. Poult. Sci*. 88: 111-117.

Apata, D.F. (2009). Antibiotic resistance in poultry. *Int. J. Poult. Sci*., 8: 404-408

Arora N., Koul A., Bansal M. P. (2011). Chemopreventive activity of *Azadirachta indica* on two-stage skin carcinogenesis in murine model. *Phytotherapy Research*, 25(3):408–416.

Arumugam A., Agullo P., Boopalan T. (2014). Neem leaf extract inhibits mammary carcinogenesis by altering cell proliferation, apoptosis, and angiogenesis. *Cancer Biology and Therapy*, 15(1):26–34.

Badam L., Joshi S. P., Bedekar S. S. (1999). 'In vitro' antiviral activity of neem (*Azadirachta indica*. A. Juss) leaf extract against group B coxsackieviruses. *Journal of Communicable Diseases*, 31(2):79–90.

Baligar NS, Aladakatti RH, Ahmed M, Hiremath MB Can J Physiol Pharmacol. 2014 Apr; 92(4):267-77.

Barton, M.D. (1998). Does the use of antibiotics in animals affect human health? *Australian Vet. J.* 76:177–180.

Bawa, G.S., Orunmuyi, M., Agbaji, A.S., Ladan, Z. and Okekeifi, U.O. (2007). Effect of different methods of processing neem (*Azadirachta indica*) seeds on performance of young rabbits. *Pakistan Journal of Nutrition 6 (3): 212-216.*

Bharathidhasan, A., Chandrasekaran, D., Natarajan, A., Ravi, R. and Ezhilvalavan, S . (2009). Effect of enzyme supplementation on carcass quality, intestinal viscosity and ileal digestibilities of broilers to nutrient reduced diet. *Tamilnadu J. Vet. Animal Sci.* 5 (6):

Biggs, P., Parsons, C.M. and Fahey, G.C. (2007). Effects of several Oligosaccharides on growth performance, nutrient digestibilities, and caecal microbial populations in young chicks. *Poult. Sci*. 86: 2327-2336.

Biswas K., Chattopadhyay I., Banerjee R. K., Bandyopadhyay U. (2002). Biological activities and medicinal properties of Neem (*Azadirachta indica*) *Current Science*, 82(11)

Biu A. A., Yusufu S. D., Rabo J. S. (2010). Acute toxicity study on neem (*Azadirachta indica*, Juss) leaf aqueous extract in chicken (*Gallus gallus domesticus*) *African Scientist*, 11(4):241–244.

Boadu K. O., Tulashie S. K., Anang M. A., Kpan J. D. (2011). Toxicological analysis of the effect of neem tree extract in an organism. *European Journal of Experimental Biology*, 1:160–171.

Bonsu FRK, Kagya-Agyemang JK, Kwenin WKJ, Zanu HK. (2012). Medicinal response of broiler chickens to diets.

Botsoglu, N.A. and Fletouris, D.J. (2001). Drug resistant in foods. Pharmacology, Food safety and analysis; New York, Marcel Dekker, Inc., pp: 541-548.

Brenes, A., Guenter, W., Marquardt, R.R. and Rotter, B.A. (1993). Effect of glucanase pentosanase enzyme supplementation on the performance of chickens and laying hens fed wheat, barley, naked oats and rye diets. *Canadian J. of Ani. Sci*. 73: 941–951.

Cardozo, P.W., Calsamiglia, S., Ferret, A. and Kamel, C. (2004). Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. *J.of Ani. Sci*. 82: 3230–3236.

Catry, B., Laevens, H., Devriese, L.A., Opsomer, G. and De Kruif, A. (2003). Antimcrobial resistance in livestock. *J. of Vet. Phar.* Therapy. 26:81-93.

Chakravarty A and Prasad J (1991). Study on the effect of neem leaf extract and neem cake extract on the performance of broiler chicks. *Indian Journal of Poultry Science*, 24(9): 37-38.

Chari, M.S (1996). Neem and transfer of technology. In: Neem and environment, Sinh, R.P., Chari, M.S., Raheja, A.K and Kraus (eds). Vol. I. Oxford and IBH publishing Co. Pvt. Ltd: New Delhi, India, pp: 27-38.

Chattopadhyay R. R. (1998) Possible biochemical mode of anti-inflammatory action of *Azadirachta indica* A. Juss. in rats. *Indian Journal of Experimental Biology*, 36(4):418–420.

Chattopadhyay, M.K. (2014). Use of Antibiotics as Feed Additives: A Burning Question Front Microbiol. 5 : p. 334

Cheng, T.K., Coon, C.N. and Hamare, M.L. (1990). Effect of environmental stress on the ascorbic acid requirement of laying hens. *Poult Sci*. 69:774-80.

Chesson, A. (2001). Non-starch poly-saccharide degrading enzymes in poultry diets and influence of ingredients on selection of activities. *World's Poult. Sci. J.* 57: 3, 251-263.

Cmiljanić, R., Sretenović, L.J., Trenkovski, S. and Marinkov, G. (2001). Systems of poultry nutrition and their effect on production traits and quality of product. Biotechnology in Animal Husbandry. 17: 5-6, 179-185. containing neem (*Azadirachta indica*) leaf meal, haematology and meat sensory analysis. *World Applied Sciences Journal*. 2012;19(6).

Cowey, C.B. (1986). The role of nutritional factors in the prevention of peroxidative damage to tissues. In *Fish Physiology and Biochemistry,* vol. 2: pp. 171-178.

Cowieson, A.J., Acamovic, T. and Bedford, M.R. (2000). Enzyme supplementation of diets containing Camelina sativa meal for poultry. *Br. Poult. Sci.* 41: 689-690.

Cristiana, G.R., Pop, I.M. and Simeanu, D. (2012). Effect of a Symbiotic Feed Additive Supplementation on Laying Hens Performance and Eggs Quality. Lucrări Ştiinţifice - vol. 53: Seria Zootehnie.

Dekkers, J.C., Van Doornen, L.J.P., and Han, C.G.K. (1996). The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Medicine. 21: 213.

Dibner, J.J. and Richards, J.D. (2005). Antibiotic growth promoters in agriculture: History and mode of action. *Poult. Sci*.84: 634–643.

Diplock, A.T., Charleux, J.L., Crozier-Willi, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., Stahl, W. and Vina-Ribes, J. (1998). Functional food science and defense against reactive oxidative species. In *British J. of Nutrition*, vol. 80, pp. 77-112.

Divya J, Lakshmi J, Venkatraya PM, Gopalkrishna K. (2013). Antibacterial effect of neem (*Azadirachta indica*) oil on multidrug resistant bacteria isolated from human infections. *International Journal of Biological and Medical Research*, 4(4):3544-3546.

Dragland, S.H., Senoo, K., Wake, K., Holte, R. and Blomhoff. (2003). Several Culinary and Medicinal Herbs are Important Sources of Dietary Antioxidants. *J. Nutr*., 133: 1286-1290.

Dumonceaux, T.J., Hill, J.E., Hemmingsen, S.M.,Van Kessel, A.G. (2006). Characterization of Intestinal Microbiota and Response to Dietary Virginiamycin Supplementation in the Broiler Chicken. Appl Environ Microbiol, 72 : pp. 2815-2823.

Durrani F. R., Chand N., Jan M., Sultan A., Durrani Z., Akhtar S. (2008). Immunomodulatory and growth promoting effects of neem leaves infusion in broiler chicks. *Sarhad Journal of Agriculture*, 24:655–659.

Edens, F.W., Carter, T. A., Parkhurst, C. R. and Sefton, A. E. (2000). Effect of selenium source and litter type on broiler feathering. *Journal of Applied Poultry Research*, 9, 407-413.

Egbewande O.O., Olorede, B.R and Onwuchekwa. C. (2004). Performance of broiler chicks fed mistletoe (*Tapinanthus bangwensis*) Leaf meal. *Proceedings of the* 29th. *Annual Conference of the Nigerian Society for Animal production. pp. 279-281.*

Ellin Doyle, M. (2001). Alternative to antibiotic use for growth promotion in animal Husbandary.

El-Sissi, A. Fand Samah, Mohamed, H. (2011). Impact of Symbiotic on the Immune Response of Broiler Chickens Against NDV and IBV Vaccines. *Global J. of Bio. & Biochem.* 6 (4).

Engberg, R.M., Hedemann, M. S., Lese,r T.D. and Jensen, B.B. (2000). Effect of Zinc bacitracin and Salinomycin on Intestinal Microflora and Performance of Broilers. *Poult. Sci.,* vol. 79: pp. 1311-1319.

Ertas, O.N., Güler, T., Çiftçi, M., Dalkiliç, B. and Simsek, Ü.G. (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. *International J. of Poult. Sci*. 4: 879-884.

Falcao-e-Cunha, L., Castro-Solla, L., Maertens, L., Marounek, M., Pinheiro, V., Freire, J. and Mourao, J. L. (2007). Alternatives to antibiotic growth promoters in rabbit feeding. A review: *World Rabbit Sci.* 15: 127-140.

Freitag, M., Hensche, H.U., Schulte-Sienbeck, H. and Reichelt, B. (1999). Biologische Effekte konventioneller and alternativer Leistungs förderer. Kraftfutter. 49-57.

Friesen, O.D., Guenter, W., Marquardt, R.R. and Rotter, B.A. (1992). The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats, and rye for the young broiler chick. *Poultr. Sci*. 71: 1710–1721.

Ghimeray A. K., Jin C. W., Ghimire B. K., Cho D. H. (2009) Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica* A. Juss grown in foothills of Nepal. *African Journal of Biotechnology*, 8(13):3084–3091.

Ghonmode W. N., Balsaraf O. D., Tambe V. H., Saujanya K. P., Patil A. K., Kakde D. D. (2013). Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against *E. feacalis*—an in vitro study. *Journal of International Oral Health*, 5(6):61–66.

Gibson, G.R. and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. of Nut.* 125: 1401-1412.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Megias, M.D. (2004). Influence of two plant extracts onn broiler performance, digestability and digestive organ size. *Poult. Sci*. 83.

Hussein, A. Kaoud. (2015). Effect of spirulina platensis as a dietary supplement on broiler performance in comparison with prebiotics. *Specialty Sci J App Res*. Vol. 1 (2):44-48, 2012.

Ian Philips, I. (1999). Assessing the evidence that antibiotic growth promoters influence human infections. *J. of Hospital Infections.* 43: 173-178.

Ilango K., Maharajan G., Narasimhan S. (2013). Anti-nociceptive and anti-inflammatory activities of *Azadirachta indica* fruit skin extract and its isolated constituent azadiradione. *Natural Product Research*, 27(16):1463–1467.

Jabeen K., Hanif S., Naz S., Iqbal S. (2013). Antifungal activity of *Azadirachta indica* against *Alternaria solani*. *Journal of Life Sciences and Technologies*, 1(1):89–93.

Jaiswal A. K., Bhattacharya S. K., Acharya S. B. (1994). Anxiolytic activity of *Azadirachta indica* leaf extract in rats. *Indian Journal of Experimental Biology*, 32(7):489–491.

Jawad Z, Younus M, Rehman MU, Munir R, Maqbool A, Shahzad W, Masood S, Muhammad K. (2014). Effect of *Azadirachta indica* on the hepato-renal functions in broilers chickens. *The Journal of Animal & Plant Sciences*, 24(4):1012-1018.

Jensen, C., Lauridsen, C. and Bertelsen, G. (1998). Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends in Food Science and Technology* 9: 62-72.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. (1997). Probiotics in poultry-Mode of action. *Worlds Poult. Sci. J*. 53: 351-368.

Kabir, S.M.L., Rahman, M.M., Rahman, M.B., Ahmed, S.U. (2004). The dynamics of probiotics on growth performance and immune response in broilers. *Int. J. Poult. Sci*. 3 (5): 361-364.

Kaczmarski, M., Wojicicki, J., Samochowiee, L., Dutkiewicz, T. and Sych, Z. 1(999). The influence of exogenous antioxidants and physical exercise on some parameters associated with production and removal of free radicals. *Die Pharmaize* 54: 303-306.

Kale BP, Kothekar MA, Tayade HP, Jaju JB and Mateeddin M (2003). Indian Journal of Pharmacology, 35:177.

Katiyar, R.C., Sastry, V.R.B. and Agrawal, D.K. (1991). Urea and alkali treatment of neem cake as livestock feed. Annual Report of Animal Nutrition Division. IVRI. Izatanagar, India.

Kaur G., Sarwar Alam M., Athar M. (2004). Nimbidin suppresses functions of macrophages and neutrophils: relevance to its anti-inflammatory mechanisms. *Phytotherapy Research*. 18(5):419–424.

Kiranmai M., Kumar M., Ibrahim M. (2011). Free radical scavenging activity of neem tree (*Azadirachta indica* A. Juss Var., Meliaceae) root barks extract. *Asian Journal of Pharmaceutical and Clinical Research*, 4:134–136.

Kumar S., Agrawal D., Patnaik J., Patnaik S. (2012). Analgesic effect of neem (*Azadirachta indica*) seed oil on albino rats. *International Journal of Pharma and Bio Sciences*, 3(2):P222–P225.

Landy N, Ghalamkari GH, Toghyani M. (2011). Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (*Azadirachta indica*) as alternative for an antibiotic growth promoter. Livestock Science, 142(1):305-309.

Lavanya Uppuluri L. P. B., Garge V. N., Kadam V. J. (2015). Evaluation of anti-angiogenesis activity of neem root using zebra fish model. *International Journal of Pharmaceutical Sciences and Research*, 6(6):2437–2440.

Lee, K.W., Hong, Y. Ho., Lee, S.H., Jang, S.I. , Park, M.S., Bautista, D.A. (2012). Effects of Anticoccidial and Antibiotic Growth Promoter Programs on Broiler Performance and Immune Status. *Res Vet Sci*, 93 : pp. 721-728.

Li, X. Qiang, L. and Liu, xu. C.H. (2008). Effects of supplementation of fructooligosaccharide and/or *Bacillus subtilis* to diets on performance and on intestinal microflora in broilers. Archiv fur Tierzucht 51: 1, 64-70.

Lin, J., Hunkapiller, A.A., Layton, A.C. Y.J., Chang, K.R. (2013). RobbinsResponse of Intestinal Microbiota to Antibiotic Growth Promoters in Chickens. *Foodborne Pathog Dis*, 10 : pp. 331-337.

Line, E.J., Bailey, S.J., Cox, N.A., Stern, N.J. and Tompkins, T. (1998). Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. *Poult. Sci.* 77: 405-410.

Lloyd C. A. C., Menon T., Umamaheshwari K. (2005). Anticandidal activity of *Azadirachta indica Indian Journal of Pharmacology*, 37(6):386–389.

Low, Dog. T. (2009). Smart talk on supplements and botanicals. Alternative and Complementary Therapies 15: 101-102.

Madrid, J., Hernandez, F., Garcia, V., Orengo, J., Magias, D. and Savilla, V. (2003). Effect of plant extracts on ileal apparent digestibility and carcass yield in broilers at level of farm 14th European Symp. Poultry Nutrition. August 10-14, Norway. pp. 187-188.

Mahady, G.B. (2005). Medicinal plants for the prevention and treatment of bacterial infections. Curr. Pharm. Des. 11: 2405-2427.

Mahapatra S., Young C. Y. F., Kohli M. (2012). Antiangiogenic effects and therapeutic targets of azadirachta indica leaf extract in endothelial cells. *Evidence-Based Complementary and Alternative Medicine*, 12:14-19.

Mahfuzul Hoque M. D., Bari M. L., Inatsu Y., Juneja V. K., Kawamoto S. (2007). Antibacterial activity of guava (*Psidium guajava* L.) and neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathogens and Disease*. 4(4):481–488.

Maiorka, A., Santin, E., Sugeta, S.M., Almeida, J.G. and Macari, M. (2001). Utilizacao de prebioticos, probioticos ou simbioticos em dietas para frangos. Revista Brasileira de Ciencia Avicola, v.**3**, pp. 75-82.

Manwar SJ, Thirumurgan P, Konwar D,Chidanandaiah and Karna DK (2005). *Indian Veterinary Journal*, 84: 159-162

McCartney, E. (2002). The natural empire strcks book. poult. Int. 41 (1): 36-42.

Mikelsaar, M. and Zilmer, M. (2009). *Lactobacillus fermentum* ME-3 - an antimicrobial and antioxidative probiotic. In *Microbial Ecology in Health and Disease*, vol. 21. 1-27.

Mohan, I.K., Khan, M., Shobha, J.C., Naidu, M.U.C., Prayag, A. and Kuppusamy, P. (2006). Protection against cisplatin-induced nephrotoxicity by *Spirulina* in rats. Cancer Chem. Pharm. **58**(6): 802–808.

Mondali N. K., Mojumdar A., Chatterje S. K., Banerjee A., Datta J. K., Gupta S. (2009). Antifungal activities and chemical characterization of Neem leaf extracts on the growth of some selected fungal species in vitro culture medium. *Journal of Applied Sciences and Environmental Management*, 13(1):49–53.

Mordue (Luntz) A. J., Nisbet A. J. (2000). Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brasil*, 29(4):615–632.

Mosaddek A. S. M., Rashid M. M. U. (2008). A comparative study of the anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone. *Bangladesh Journal of Pharmacology*, 3(1):44–47.

Munck, A., Guyre, P.M. and Holbrook N.J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Rev; 5: 25–44.

Nahak G., Sahu R. K. (2011). Evaluation of antioxidant activity of flower and seed oil of *Azadirachta indica A*. juss. *Journal of Applied and Natural Science*, 3(1):78–81.

Naik M. R., Bhattacharya A., Behera R., Agrawal D., Dehury S., Kumar S. (2014). Study of anti-inflammatory effect of neem seed oil (*Azadirachta indica*) on infected albino rats. *Journal of Health Research and Reviews*, 1(3):66–69.

Natarajan V., Venugopal P. V., Menon T. (2003). Effect of Azadirachta indica (Neem) on the growth pattern of dermatophytes. *Indian Journal of Medical Microbiology*, 21(2):98–101.

Nemade PP and Kukde RJ (1993). Indian Poultry, 24(1): 30-32.

Neu, H.C. (1992). The crisis in antibiotic resistance. *Poult. Sci.* 257: 1064–1073.

Nunes P. X., Silva S. F., Guedes R. J., Almeida S. (2012) *Phytochemicals as Nutraceuticals—Global Approaches to Their Role in Nutrition and Health*. Biological oxidations and antioxidant activity of natural products.

Nworgu, F.C and Fapohunda, J.B. (2002). Performance of broiler chicks fed mimosa (*Mimosa invisa*) leaf meal supplements. *In proc. Of the 28th Annual conference. NSAP, Federal University of Technology, Akure, Nigeria, pp.128-131.*

Obikaonu HO, Opara MN, Okoli IC, Okoro VM, Ogbuewu IP, Etuk EB and Udedibie (2012). Haematological and serum biochemical indices of starter broilers fed leaf meal of neem (*Azadirachta indica*). *Journal of Agriculture Technology,* 8(1):71-79.

Odo LI, Bratte L. (2015). Effects of varying levels of neem (*Azadirachta indica*) leaf meal in layer diets on haematological and serological indices, and faecal bacterial counts of layers. *Journal of Natural Sciences Research,* ISSN 2224-3186.

Parida, R.N.S., Bisoi, P.C., Sahoo, P.K. and Mohapatra, M. (1995). Protective effect of Livol during experimental aflatoxicosis in chicks: a histomorphological approach. *Indian J Indg Med*. 17: 19-25.

Partridge, G. and Wyatt, C. (1995). More flexibility with new generation of enzymes. World Poultry. **11**: 17-21.

Patterson, J. A. and Burkholder, M. K. (2003). Application of prebiotics and probiotics in poultry production. *Poult. Sci.* 82**:** 627-631.

Perić, L., Nollet, L., Milošević, N. and Žikić, D. (2007b). Effect of Bioplex and Sel-Plex substituting inorganic trace mineral sources on performance of broilers. Archiv für Geflügelkunde. 71: 3, 122-129.

Petrof, E.O., Kojima, K., Ropeleski, M.J., Musch, M.W., Tao, Y. De Simone, C. and Chang, E. B. (2004). Probiotics inhibit nuclear factor kappa-B and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. In Gastroenterology. vol. **127**: 2004, p. 1474-1487.

Prescott, J.F. and Baggot, J.D. (1993). Antimicrobial Therapy in Veterinary Medicine. 2nd edition, pp 564-565: Iowa State University Press.

Priyadarsini R. V., Manikandan P., Kumar G. H., Nagini S. (2009). The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by modulating xenobiotic-metabolizing enzymes, DNA damage, antioxidants, invasion and angiogenesis. *Free Radical Research*, 43(5):492–504.

Rahmani A. H., Aly S. M. (2015). *Nigella sativa* and its active constituents thymoquinone shows pivotal role in the diseases prevention and treatment. *Asian Journal of Pharmaceutical and Clinical Research*, 8(1):48–53.

Raizada R. B., Srivastava M. K., Kaushal R. A., Singh R. P. (2001) Azadirachtin, a neem biopesticide: subchronic toxicity assessment in rats. *Food and Chemical Toxicology*, 39(5):477–483.

Randall, L.P., Ridley, A.M., Coole, S.W., Sharma, M., Sayers, A.R. and Pumbwe, L. (2003). Prevalence of multiple antibiotic resistance in 443 Campylobacter species Isolated from humans and animals. *J. Antimicrob Chemotherapy*. 52: 507-510.

Rao, A.T., Panda, S.K. and Sahu, R.K. (1995). Effect of zeetress on infectious bursal disease vaccinated & challenged birds. *Indian J Indg Med*. 17: 43-6.

Roch, G., Boulianne, M. and De Roth, L. (2000). Dietary antioxidants reduce ascites in broilers. World Poultry. 11, 16.

Saleh, F., Ohtsuka, A., Tanaka, T. and Hayashi, K. (2003). Effect of enzymes of microbial origin on *in vitro* digestibilities of dry matter and crude protein in maize. Journal of *Poult. Sci*. 40: 274-281.

Sarica, S., Ciftci, A., Demir, E., Kilinc, K., Yildirim, Y. (2005). Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets; *S. Afr. J. Anim. Sci*. 35:61–72.

Sarmiento W. C., Maramba C. C., Gonzales M. L. M. (2011) An in vitro study on the antibacterial effect of neem (*Azadirachta indica*) leaf extracts on methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* . *PIDSP Journal*, 12(1):40–45.

Schutte, J.B., de Jong, J. and Langhout, D.J. (1995). Effect of a xylanase enzyme supplementation to wheat-based diets in broiler chicks in relation to dietary factors. Proceedings of the Second European Symposium on Feed Enzymes, Noordwijkerhout, Netherlands. pp. 95–101.

Shen, Q., Shang, N. and Li, P. (2011). Invitro and invivo antioxidant activity of Bifidobacterium animals isolated from centenarians. In Current Microbiology, vol. 62: 2011, no. 4, p. 1097-1103.

Silversides, F.G. and Bedford, M.R. (1999). Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. *Poult. Sci*. 78: 1184-1190.

Singh, P., Karimi, A., Devendra K., Waldroup P.W., Cho K.K., Kwon, Y.M. (2013). Influence of Penicillin on Microbial Diversity of the Cecal Microbiota in Broiler Chickens. *Poult Sc.,* 92 : pp. 272-276.

Sinniah D. and Baskaran G. (1981) Margosa oil poisoning as a cause of Reye's syndrome. *The Lancet*, 317(8218):487–489.

Sithisarn P., Supabphol R., Gritsanapan W. (2005) Antioxidant activity of Siamese neem tree. *Journal of Ethnopharmacology*. 99(1):109–112.

Steel RGD, Torrie JH. (1980). Principles and procedures of statistics. New York, McGrae Hill, 137-269

Strus, M., Pakosz, K., Gościniak, H., Przondo-Mordarska, A., Rozynek, E., Pituch, H., Meisel-Mikolajczyk, F. and Heczko, P.B. (2001). vol. 53: p. 133-142.

Sundaravalli N., Bhaskar Raju B., Krishnamoorthy K. A. (1982). Neem oil poisoning. *The Indian Journal of Pediatrics*, 49(3):357–359.

Talwar GP, Raguvanshi P, Misra R, Mukherjee S, Shah S. (1997). Plant immunomodulators for termination of unwanted pregnancy and contraception and reproductive health. Immunology & Cell Biology, 75(2):190-192.

Tipu, M.A., Akhtar, M.S., Anjum, M.I. and Raja, M.L. (2006). New dimension of medicinal plants as animal feed. *Pakistan Vet. J.* 26 (3): 144-148.

Tiwari V., Darmani N. A., Yue B. Y. J. T., Shukla D. (2010). In vitro antiviral activity of neem (*Azardirachta indica* L.) bark extract against herpes simplex virus type-1 infection. *Phytotherapy Research*, 24(8):1132–1140.

Torok, G.E. Allison, N.J. Percy, K. Ophel-Keller, R.J.(2011). HughesInfluence of Antimicrobial Feed Additives on Broiler Commensal Posthatch Gut Microbiota Development and Performance. *Appl Environ Microbiol*. 77 : pp. 3380-3390.

Travers, M.A., Florent, I., Kohl, L. and Grellier, P. (2011). Probiotics for the control of parasites: an overview. In *J. of Para. Res.* in press.

Ušćebrka, G., Žikić, D., Perić, L., Jurcoane, S. and Milošević, N. (2005). Effect of probiotic supplementation on the small intestinal morphology of broiler chickens. Proceedings of the 34th International session of scientific communications of the faculty of animal science, Bucharest, Romania. 67-71.

Van den Bogaard, A.E. and Stobberingh, E.E. (2000) . Epidemiology of resistance to antibiotics Links between animals and humans. *Int. J. of Anti. Agents* 14: 327–335.

Van der Klis, J.D., Schelle, C. and Kwakernaak, C. (1995). Wheat characteristics related to its feeding value and to the response of enzymes. Proceedings 10th European Symposium on Poultry Nutrition, Antalya, Turkey. World's Poultry Science Association pp. 160–168.

Vandergrift, B. (1998). Biotechnology in the Feed Industry. In: Proc. Altech's 14th Annual Symposium Altech Technical Publications Nottingham University Press. Nicholasville. K.Y. pp: 293-300.

Wagner, H. (1995). Immunostimulants and adaptogens from plants. In Amason JT *et al.,* eds., Phytochemistry of Medicinal Plants. New York: Plenum Press. 1-18.

Wenk, C. (2000). Why all the discussion about herbs?. In: Proc. *Alltech's 16th Ann. Symp. Biotechnology in the Feed Industry*. (Ed. Lyons, T.P.), Alltech Tech. Publ., Nottingham, University Press, Nicholasville, KY. pp.79-96.

Williams, P. and Losa, R. (2001). The use of essential oils and their compounds in poultry nutrition. World Poultry-Elsevier. 17 (4): 14-15.

Williams, S.H. and Tucker, J.F. (1975). The effect of feeding diets containing permitted antibiotics on the faecal excretion of Salmonella typhimurium by experimentally infected chicks. J. Hyg. (Camb.) 75: 293–301.

Yerima M. B., Jodi S. M., Oyinbo K., Maishanu H. M., Farouq A. A., Junaidu A. U. (2012). Effect of neem extracts (*Azadirachta indica*) on bacteria isolated from adult mouth. *Journal of Basic and Applied Sciences*, 20:64–67.

Zanu HK, Kagya-Agyemang JK, Kwenin WKJ, Bonsu FRK, Antwi E, Ateni S. (2011) Physiological response of broiler chickens to neem (*Azadirachta indica*) and akakapenpen (*Rauvolfia vomitoria*) decoctions: Performance and carcass characteristics. *International Journal of Poult. Sci*, 10(9):730-733.

Zikic, D., Peric, L., Uscebrka, G., Milosevic, N. and Jotanovic, S. (2006). Probiotici prebiotici u ishrani brojlera: 1. Efekat na proizvodne rezultate. 11. Savetovanje biotehnologiji, Čačak. 11: 2, 471.

APPENDICES

Appendix 1. Recommended level of nutrients for broiler

Nutrients	Starter	Grower
ME (kcal/kg)	3000	3100
% CP	22	20
% Ca	1.0	0.85
% P (Available)	0.5	0.4
% Lysine	1.2	1.0
% Methionine	0.5	0.45
% Tryptophane	0.21	0.18

Source: Cobb500 Broiler Management Guide, 2016

Appendix 2. Nutrient composition of the ingredients used to formulate experimental diets

Ingredients	**DM (%)**	**ME (K. Cal/kg)**	**CP (%)**	**CF (%)**	**Ca (%)**	**P (%)**	**Lys (%)**	**Meth (%)**	**Tryp (%)**
Soybean meal	90	2710	44.50	7.5	0.26	0.23	2.57	0.76	0.57
Maize	89.5	3309	9.2	2.4	0.25	0.40	0.18	0.15	0.09
DCP					22	17.21			
Soybean oil	100	8800							
Protein concentrate (Jeso-prot)	91.64	2860	63.30	8.1	6.37	3.24	3.87	1.78	.53
Meat and Bone meal	95.5	1044	14.6	2.5	7.0	12.11	.66	0.24	0.12

Source: Cobb500 Broiler Management Guide, 2016

Appendix 3. Recorded temperature (°C)during experiment

Age in weeks	Room temperature (˚C)							
	Period	8 A.M	12P.M	4 P.M.	8 P.M.	12 A.M.	4 A.M	Average
1st	11.05.17 - 17.05.17	30.9	35.5	32.6	31.5	31.0	28.5	31.66
2nd	28.05.17 - 24.05.17	29.3	31.5	31.1	30.6	29.2	28.5	30.03
3rd	25.05.17 - 31.05.17	28.0	29.2	30.8	28.2	27.0	26.8	28.33
4th	01.06.17 - 07.06.17	27.8	28.5	28.8	28.0	27.4	27.2	27.95

Appendix 4. Relative humidity (%) during experiment

Age in		Relative humidity (%)						
weeks	Period (day)	8 A.M	12A.M	4 P.M.	8 P.M.	12 P.M.	4 A.M	Average
1st	11.05.17-17.05.17	77	72	73	74	76	78	75
2nd	28.05.17-24.05.17	76	71	68	70	73	75	72.16
3rd	25.05.17-31.05.17	76	72	67	72	74	75	72.66
4th	01.06.17-07.06.17	72	67	66	70	71	74	70

Appendix 5. Average Live weight, Eviscerated Weight and Dressing Percentage of different replication of broiler under different treatment.

Treatments	Replications	Live Weight (g)	Eviscerated Weight (g)	Dressing Percentage (%) (Skinless)
Control	R1	1698	1380	71.42
	R2	1742	1358	71.55
	R3	1713	1340	71.42
Antibiotic	R1	1730	1418	74.85
	R2	1640	1135	69.20
	R3	1629	1210	71.88
N_1	R1	1705	1398	71.42
	R2	1685	1454	71.55
	R3	1700	1400	71.42
N_2	R1	1732	1259	72.69
	R2	1730	1312	71.88
	R3	1734	1340	71.02
N_3	R1	1670	1250	70.85
	R2	1700	1222	71.88
	R3	1753	1245	71.02
N_4	R1	1705	1471	70.28
	R2	1725	1232	71.42
	R3	1705	1220	71.55

Appendix 6. Weight of internal organs of broiler under different treatment groups (g/bird).

Treatments	Replications	Liver weight (g)	Spleen weight (g)
Control	R1	42	1.5
	R2	34	1.5
	R3	37	2.0
Antibiotic	R1	27	1.0
	R2	34	2.0
	R3	32	1.5
N_1	R1	45	2.0
	R2	55	3.0
	R3	40	2.0
N_2	R1	41	2.0
	R2	45	3.0
	R3	46	2.0
N_3	R1	47	2.0
	R2	43	1.5
	R3	41	2.0
N_4	R1	51	2.0
	R2	44	1.5
	R3	40	2.0

Appendix 7. Biochemical data in different treatment groups.

Treatments	Replications	Glucose (mmol/L)	Cholesterol (mg/dL)
Control	R1	11.0	204
	R2	12.4	268
	R3	10.5	186
Antibiotic	R1	11.3	202
	R2	10.8	215
	R3	11.5	240
N_1	R1	10.5	202
	R2	11.0	265
	R3	11.3	208
N_2	R1	11.2	201
	R2	10.5	199
	R3	11.7	192
N_3	R1	10.7	226
	R2	11.3	190
	R3	11.1	205
N_4	R1	10.0	216
	R2	11.4	233
	R3	10.5	168

Appendix 8. Results of Complet blood count (CBC) under different treatment groups.

Treatments	Replications	Hb (gm/dL)	Uric Acid (mg/dL)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)
Control	R1	13.3	204	35	59	5	1
	R2	14.0	268	31	56	11	2
	R3	12.2	186	30	61	6	3
Antibiotic	R1	14.9	202	28	62	8	2
	R2	15.3	215	33	58	7	2
	R3	12.5	240	27	66	5	2
N_1	R1	13.3	202	25	60	10	5
	R2	13.9	265	33	57	6	4
	R3	13.6	208	29	58	9	4
N_2	R1	12.9	201	35	52	10	3
	R2	13.8	199	31	60	7	2
	R3	12.8	192	29	57	8	6
N_3	R1	15.2	226	40	52	6	2
	R2	17.7	190	27	59	10	4
	R3	16.1	205	34	59	9	3
N_4	R1	15.1	216	30	58	9	3
	R2	13.7	233	29	62	6	3
	R3	14.7	168	27	59	9	5

Appendix 9. Feed consumption (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments.

Treatments	Replications	1st Week Feed Consumption/ Bird (g)	2nd Week Feed Consumption/ Bird (g)	3rd Week Feed Consumption/ Bird (g)	4th Week Feed Consumption/ Bird (g)
Control	R1	188.9	370.2	708.5	899.3
	R2	188.0	380.3	708.5	1024.6
	R3	184.0	354.8	697.0	980.2
Antibiotic	R1	187.5	365.3	697.0	911.4
	R2	184.0	347.0	697.0	922.5
	R3	182.0	363.8	697.0	908.8
N1	R1	186.2	367.8	697.0	900.0
	R2	187.0	372.5	708.5	968.0
	R3	184.0	357.3	708.5	983.0
N2	R1	184.4	381.6	708.5	959.0
	R2	182.0	381.1	697.0	981.5
	R3	187.0	370.3	697.0	918.3
N3	R1	185.8	383.3	708.5	912.0
	R2	183.5	352.2	697.0	847.4
	R3	186.0	337.3	697.0	815.1
N4	R1	182.3	367.3	697.0	850.0
	R2	184.0	386.8	697.0	848.5
	R3	186.0	362.4	697.0	853.4

Appendix 10. Body weight (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments.

Treatments	Replications	DOC Body Weight/ DOC (g)	1st Week Body Weight/ Bird (g)	2nd Week Body Weight/ Bird (g)	3rd Week Body Weight/ Bird (g)	4th Week Body Weight/ Bird (g)
Control	R1	43	211	546	1053	1698
	R2	44	216	555	1063	1742
	R3	44	224	539	1018	1713
Antibiotic	R1	44	223	546	1053	1730
	R2	45	230	516	1028	1640
	R3	43	222	545	1023	1629
N1	R1	44	230	561	1023	1705
	R2	44	228	560	1002	1685
	R3	43	224	545	1044	1700
N2	R1	43	226	529	1058	1732
	R2	44	218	549	1063	1730
	R3	45	229	532	1041	1734
N3	R1	43	212	541	1011	1670
	R2	42	207	543	1058	1700
	R3	43	223	531	1058	1753
N4	R1	44	221	535	995	1705
	R2	43	218	565	1078	1725
	R3	43	215	545	1098	1705

Appendix 11.: Mortality of broilers under different treatments upto 4 weeks of age.

Day no.	Date	Mortality
Day 0	11.5.17	1 (N_1R_1)
Day 1	12.5.17	0
Day 2	13.5.17	0
Day 3	14.5.17	1 (N_3R_2)
Day 4	15.5.17	2 (N_3R_3 & N_4R_1)
Day 5	16.5.17	0
Day 6	17.5.17	0
Day 7	18.5.17	0
Day 8	19.5.17	0
Day 9	20.5.17	0
Day 10	21.5.17	0
Day 11	22.5.17	0
Day 12	23.5.17	1 (A_1R_1)
Day 13	24.5.17	0
Day 14	25.5.17	0
Day 15	26.5.17	0
Day 16	27.5.17	1 (C_1R_2)
Day 17	28.5.17	0
Day 18	29.4.17	0
Day 19	30.5.17	0
Day 20	31.5.17	1 (N_3R_1)
Day 21	1.6.17	0
Day 22	2.6.17	0
Day 23	3.6.17	0
Day 24	4.6.17	0
Day 25	5.6.17	0
Day 26	6.6.17	0
Day 27	7.7.17	1 (N_4R_1)