THE EFFECTS OF DIETARY SUPPLEMENTATION OF NEEM (*Azadirachta indica*), MORINGA (*Moringa oleifera*) AND JUTE (*Corchorus olitorious*) LEAF POWDER ON THE GROWTH PERFORMANCE AND HEALTH STATUS OF BROILER CHICKEN

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This is to certify that the thesis entitled ***"THE EFFECTS OF DIETARY SUPPLEMENTATION OF NEEM (Azadirachta indica), MORINGA (Moringa oleifera) AND JUTE (Corchorus olitorius) LEAF POWDER ON THE GROWTH PERFORMANCE AND HEALTH STATUS OF BROILER CHICKEN"*** *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* ***RABIUL ISLAM****, Registration No.* ***17-08294*** *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.

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TO

MY PARENTS AND TEACHERS

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The Author

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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
BBS	=	Bangladesh Bureau of Statistics
BLRI		Bangladesh Livestock Research Institute
Ca	=	Calcium
CAT	=	Catalase
CBC	=	Complete Blood Count
CF	=	Crude Fibre
CFU	=	Colony Forming Units
Cm	=	Centimeter
cm^2	=	Squre Centimeter
CONTD.	=	Continued
CP	=	Crude Protein
CRD	=	Complete Randomized Design
DMD	=	Dry Matter Digestibility
Dr.	=	Doctor
DSP	=	Dried S*pirulina* Powder
e.g.	=	For Example
EDTA	=	Ethylene Diethyle Tetraacitic Acid
et al.	=	And others/Associates
FC	=	Feed Consumption
FCR	=	Feed Conversion Ratio
FOS	=	Fructo-oligosaccharides
gGSH		gGram Glutathi one
Hb	=	Haemoglobin
HETE	=	Hydroxy Eicosatetraenoic Acid
HPA	=	Hypothalamus Pituitary Axis
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines

ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
Kcal	=	Kilo-calorie
Kg	=	Kilogram
M.S.	=	Master of Science
MDA	=	Malondialdehyde
ME MOS	=	Metabolizable Energy Mannan-oligosaccharides
Ml	=	Mililitre
MCHC	=	Mean Corpuscular Hemoglobin Concentration
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
SOD	=	Superoxide dismutase
SPSS	=	Statistical Package for Social Sciences
UK	=	United Kingdom
USA	=	United States of America
viz.	=	Such as
Vs	=	Versus
WBCWHO		White Blood Cell World Health Organization
WPSA	=	World‟s Poultry Science Association

ACRONYMS AND ABBREVIATIONS

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

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ABSTRACT

The study was planned to determine the comparative efficacy of Neem (*Azadirachta indica*), Moringa (*Moringa oleifera*) and Jute (*Corchorus olitorios*) leaf powder on the productive performance, haematology and health status of commercial broilers. A total of 200 day-old Cobb 500 broiler chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. Chicks were divided randomly into 5 experimental groups of 4 replications and each replication contains 10 chicks. These groups were allotted to five treatment designated as T_0, T_1, T_2, T_3 and T_4 Group. T_0 was offered basal feed without any supplementation and served as a control. Whereas, group T_1, T_2, T_3 and T_4 were offered basal feed supplemented with Neem Leaf Powder (NLP) 2%, Moringa Leaf Powder (MLP) 2%, Jute Leaf Powder (JLP) 2% and Doxivet (1g/L) respectively. The results showed that the weekly body weight gain (g/bird) in 4^{th} week was significantly ($P<0.05$) higher in T_2 group (718.5±2.50) than T_0 group (659.65±1.135). Final live weight (g/bird) was significantly higher T_2 (1664.30±6.29) than T_0 group (1610.80±3.31). Weekly feed consumption (FC) was insignificant in different group but total FC was significantly ($P<0.05$) lower in T_2 (2288.35±10.14g) than T_4 group (2337.50±2.39g). Weekly FCR was significantly ($P<0.05$) lower in T_2 group (1.38±0.01) than T_3, T_4, and T_0 group in 4^{th} week .The overall FCR significantly was lower in T_2 (1.38±0.01) than T_0, T_3 and T_4 group. Dressing percentage (DP) and survivability were non-significanty ($P>0.05$) affected by the dietary inclusion of NLP, MLP and JLP compared to control fed broilers. However, higher DP had found in the T_2 group (70.80±.610) and lower survivability rate in T_0 group than others. There was no significant ($P>0.05$) difference in relative weight of spleen (2.13±0.12) and bursa (1.64±0.09) among the dietary groups. In addition, the present study showed that feeding dietary NLP, MLP, JLP and antibiotic had no significant ($P>0.05$) effects on liver, gizzard and heart weight except intestines which were significantly higher ($p<0.05$) in T_3 group (102.13±3.28) compared with T_0 and T_4 group. Dietary supplementation of NLP, MLP, JLP and Antibiotic had no no significant on the concentration of blood glucose, Cholesterol and hemoglobin. However slightly higher hemoglobin was found in T_2 (12.81±.26) group and lower cholesterol found in T_1 group (183.67±8.21) compared to T_0 and T_4 group. In conclusion, it can be said that 2% MLP can positively affect the productive and health status of broiler.

CHAPTER I

INTRODUCTION

The most important sources of animal protein in the world is poultry meat and therefore, contributing significantly in maintaining the health status of the people, especially in developing countries like Bangladesh. Poultry meat alone contributes 37% of the total meat production in Bangladesh (Hamid *et al.*, 2017*)*. Overall poultry contributes about 22-27% of the total animal protein supply in the country (DLS., 2015). However, fast augment in human population of the country is demanding more efforts to increase meat production for food security. Besides the risk of ever increasing population, expand of diseases, high feed price and non-availability of quality ingredients for balanced feed formulation are some of the factors, which limit the production performance of broilers. According to our socio-economic situation, the knowledge of our farmer is very little because most of them are not properly trained for broilers production, but unemployed young generation is coming in this business for short return of value and profit. Pharmaceutical companies take this advantage. They are convincing farmers for using antibiotic as a growth promoter for chicken. As a result, each and every broiler is a depot of antibiotic. When these broilers are consumed by human this antibiotic residue enters into human body and causing serious human health hazards with drug residues. Due to the prohibition of most of antimicrobial growth promoters (AGP), plant extracts have gained interest in animal feed strategies (Charis, 2000). The risk of the presence of antibiotic residues in milk and meat and their harmful effects on human health have led to their prohibition for use in animal feed in the European Union (Cardozo *et al.*, 2004). The poultry industry is currently moving towards a reduction in use of synthetic antibiotics due to this reason (Barton, 1998). As an alternative to antibiotic growth promoters, medicinal plants are the most popular options (Durrani *et al.*, 2008).

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). 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). It is conceivable that herbal agents could serve as safer alternatives as growth promoters due to their suitability and preference, lower cost of production, reduced risks toxicity and minimum health hazards. Interestingly recent biological trials of certain herbal formulations as growth have shown encouraging results and some of the reports have demonstrated improvement with respect to weight gain, feed efficiency, lowered mortality, increased immunity and increased livability in poultry birds (Kumar, 1991). Also these herbal growth promoters have shown to exert therapeutic effects against liver damage due to feed contaminants like aflatoxin (Ghosh, 1992).

Scientists are again concentrating on the use of our ancient medicinal system to find beneficial herbs and plants, which can be safely used to increase the production. Many plants also produce secondary metabolites such as phenolic compounds, essential oils and sarasaponins (Chesson *et al.,* 1982; Wallace *et al.,* 1994; Kamel, 2001). Herbs normally used are picorhiza, garlic, cloves, slippery elm, neem fruit and leaves, sophora flavescens, nutmeg, cinnamon, ginger, peppermint, sage, thyme, mustard and fenugreek. These plants are used as digestive stimulants, antidiarrhoic, antiseptic, anti-inflammatory, antiparasitic and appetite stimulants in human beings as

well as animals. It is conceivable that herbal agents could serve as safer alternatives as growth promoters due to their suitability and preference, lower cost of production, reduced risks toxicity and minimum health hazards. One of such plants, neem (Azadirachta indica) is an indigenous plant of Asian subcontinent known for its useful medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective, immunomodulator and various other properties without showing any adverse affects (Kale *et al*., 2003; Sadekar *et al.*, 1998). Neem promotes growth and feed efficiency of birds because of its antibacterial and hepatoprotective properties (Padalwar, 1994). Neem preparations fed to laying hens have been reported by Sadre *et al.*, (1984) and Gowda *et al*., (1998) to significantly reduce the content of hemoglobin, erythrocyte count and packed cell volume. Low dose of neem leaves powder have an inhibitory action on wide spectrum of microorganisms (Talwar *et al.*, 1997) and immuomodulator actions that induce cellular immune reaction (Devakumar and Sukтt, 1993).

Moringa oleifera is one of the plants whose leaves are used in poultry diets because; it contains good sources of nutrients (Makkar and Becker, 1997). Moringa oleifera leaves are good sources of proteins, vitamins A, B and C and minerals such as calcium and iron (Deschepper, 1995). The protein content of Moringa oleifera leaf ranged between 20 to 23% on dry weight basis and is of high quality (Foidle and Paul, 2008). Moringa plant known as "Miracle tree" has been reported to have many medicinal uses as it possesses hypo-cholesterolemic properties (Olugbemi, *et al*., 2010) and impaction of carotenoid compound into the poultry muscles and could as such substitute conventional feed stuffs (Sarwalt, 2002).

Jute mallow like other traditional leafy vegetables represents a cheap but quality nutrition for large segments of the population in urban and rural areas (Freiberger *et al*., 1998; Kinabo *et al*., 2006; van Rensburg *et al.*, 2007; Lewu and Mavengahama 2010; Anbukkarasi and Sadasakthi, 2016). Apart from food value, Corchorus species are medicinal plants widely used for treatment of various diseases. The commonly used species include C. *olitorius,* C. *capsularis* and C. *aestuans*. These are used to treat general diseases and are also remedies for heart disease, enemas, parturition and febrifuges (Burkill, 2004). Other diseases include chronic cystitis, gonorrhea, dysuria, and toothache (Hillocks, 1998).

Considering the biological and pharmacological activities of Neem, Moringa and Jute leaf powder this experiment was designed to use these products in broiler chicken feeds as a replacement for the antibiotic growth promoters, with the following specific objectives:

1. To compare the production performance and dressing characteristics of broiler fed NLP, MLP and JLP diet.
2. To study the effect of these herbal leaves meal on haematological properties of broiler chicken.

CHAPTER 2

REVIEW OF LITERATURE

Sources of literature

a. Book and journal in different libraries as mentioned below-
    i. Sher-E-Bangla Agricultural University (SAU) Library, Dhaka
    ii. Bangladesh Agricultural Research Council (BARC) Library, Farmgate Dhaka
    iii. Bangladesh National Scientific And Technical Documentation centre (BANSDOC) Library, Agargaon, Dhaka
    iv. Bangladesh Livestock Research Institute (BLRI) library, Savar, Dhaka
b. Abstract searching at BARC, Farmgate, Dhaka, BANSDOC, Agargoan, and Dhaka.
c. Internet browsing.

A total about one hundred literature were reviewed to make out the background, drawbacks and prospects of research, understand previous findings and to answer the research status of this field. Among them twenty five were full article and sixty abstracts and some were miscellaneous. A brief account is given below depending on seven main headlines viz, antibiotic impacts on poultry, Antibiotic growth promoters (AGPs), Antimicrobial resistance, Alternatives to antibiotic growth promoters such as Neem, Moringa and Jute Leaf.

In Bangladesh, the demand for broiler meat is increased rapidly, propelled by increased income and population growth and urbanization. Feed cost accounts for up to 80% of the total cost of production and is a very important component in determining the extent of poultry survival and profitability (Olugbemi *et al.,* 2010). Feed is a major component affecting net return from the poultry enterprise. Various strategies like feed supplements and additives are being used to ensure more net return and to minimize expenditure on feed. Economical broiler production largely depends on optimum utilization of feed, improved body weight, prevention of

diseases and reduced mortality rate. Use of chemical feed additives as growth promoters has criticism due to adverse effects on consumers health and there is increasing demand for organic meat and eggs. In view of this, herbal and plant derivatives would be a valuable alternative to promote growth and health in poultry as there is no residual toxicity (Agashe *et al.*, 2017). Specifically, these are raised for meat production under intensive production system using commercial feed ration. However, broiler production cost has gone up substantially in recent years due to the increase in price of feed ingredients. The search for cheap, locally available and equally nutritive feed sources to partially substitute commercial poultry diet has never been more pressing. Plant proteins are good sources of dietary fiber and essential amino acids in the diet. 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 like E.coli, salmonella, clostridium, camphylobacter etc. 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 de-conjugating effects of bile acids (Engberg *et al.*, 2000). This ultimately leads depressed growth performance and increase incidence of disease.

2.1 Antibiotic growth promoters (AGPs) impacts on poultry

Antibiotics have been used in the poultry industry in the United States and other countries, for more than five decades. Supplementation of antibiotics as sub-therapeutics improves bird feed efficiency and maintain the gut health, growth and development (Rosen, 1995 and Danzeisenet *et al.*, 2011). In North America, antibiotic growth promoters (AGPs), commonly used in the poultry industry include: Avilamycin, Enramycin, Monensin, Penicillin, Virginamycin and Bacitracin methylene disalicylate (BMD) (Danzeisen *et al.*, 2015). BMD is commonly used in the broiler diet for the prevention and control of necrotic enteritis, as well as improvement of weight gain and feed efficiency (Singh, 2008 and Waldroup *et al.*, 1986). Inclusion of antibiotics in poultry diet can also reduce the prevalence of enteric pathogens. Regardless of successful use of AGPs, the definitive mechanism underlying their growth promoting effect is still unresolved. With increasing concern over agricultural use of antibiotics as growth promoters (AGPs) and the emergence and dissemination of antibiotic resistance in foodborne pathogens, there is consumer

pressure to eliminate the use of AGPs as feed additives in the U. S. Therefore, search for alternative strategies to replace antibiotics as a feed additive has gained interest in animal agriculture. Avian gastrointestinal tract is much shorter compared to the mammalian gastrointestinal tract and the average transit time is less than 3.5 h (Hughes, 2008). This short transit time selects for the bacterial community with better adherence property and faster growth in the ileum and other proximal part of gut. On the other hand, passage time in the ceca is slow and thus, represents an ideal habitat for the bacterial community (Pan, 2014).

The gut microbial community is diverse and their interactions significantly affect the physiological, immunological and nutritional status of the host (Zhao *et al.,* 2013). This complex interaction can have either beneficial or detrimental effect on the bird performance and health, depending on the structure and function of the gut microbial community. For instance, pathogen infection affects gut integrity and function (Droleskey, 1994) and poses a threat to the immune system (Neish, 2002). Antimicrobial peptides (β-defensins) in the avian gut are important part of innate immune system that can destroy various enteric pathogens by disrupting their cell membranes. These initial interactions between gut microbial community and host innate immune system can lead to subsequent adaptive immune response, which can either be B-cell dependent or T-cell dependent (Pan, 2014).

Therefore, gut community helps in supporting proper development and homeostasis of immune system (Oakley *et al.,* 2014). Bird age also has a significant effect on the microbial community, and greater diversity occurs at species level (Ballou, 2016; Danzeisen *et al*., 2013).

Development of antibiotic resistance in foodborne pathogens, Salmonella spp. and Campylobacter, is a public health concern. Public demand to reduce the use of sub-therapeutic antibiotic growth promoters (AGP) in poultry feeding has resulted in greater adoption of antibiotic-free poultry production systems. There is a need to understand the effects of AGP removal from poultry feed on gut microbiota and its impact on prevalence of foodborne pathogens. The effect of antibiotic withdrawal from poultry feed on gut microbial community, host performance and immunity, and prevalence of Salmonella and Campylobacter was evaluated (Kumar *at al.,* 2018).

2.2 Antimicrobial Residues and resistance in poultry

The discovery of antibiotics in the early 20th Century was a breakthrough for human health. Before that, even minor injuries could be deadly if an infection set in. But the more we use antibiotics, the faster bacteria adapt and become resistant to the drugs' effects. The Centers for Disease Control and Prevention estimate that 2 million people get sick and 23,000 die each year in the United States from antimicrobial resistant infections. Globally, the number could be more than 700,000 people (O'Neill, 2014). Drug resistance is now spreading so rapidly that there is talk of a nightmarish post-antibiotic future where minor cuts could again become lethal and surgery and cancer treatment would be far riskier. Antibiotic resistant bacteria are a threat to all of us. But the greatest danger is in poor countries where respiratory infections and diarrheal diseases remain leading causes of death, especially for children. The second- and third-line drugs to which doctors turn when initial treatments fail are also generally more expensive. Having to use them strains the resources of already weak public health systems in developing countries and leaves the poor with few options (Center for Global Development, 2010). The O'Neill review on antimicrobial resistance (2014), commissioned by UK Prime Minister David Cameron, projects that, if current trends continue, 10 million more people would die prematurely each year from drug resistant infections. The global economy would also be $60 trillion to $100 trillion smaller by 2050 and developing countries in Africa and Asia would bear the brunt of these burdens. Several years ago, a CGD working group examined the large human and economic costs associated with drug resistance, particularly for developing countries (Nugent *et al.*, 2010). Livestock producers in some countries use large amounts of antibiotics in low doses for extended periods to promote growth in their animals. That is a recipe for accelerating resistance. And many of the drugs used in animals are the same as those used in human health, or are in chemically related classes of drugs. Intensive, high density livestock operations, which are expanding rapidly, also routinely use antibiotics to prevent disease. By 2005, large, intensive livestock operations "account[ed] for three-quarters of the world's poultry supply, 40% of its pork, and over two-thirds of all eggs" (Naylor *et al*., 2005). Unfortunately, the failure to systematically monitor antibiotic use and resistance in humans and animals remains a key barrier to sound analysis and well-informed policy (WHO, 2014). In 2015, the World Health Organization will also launch a global action plan to

combat antimicrobial resistance. A 2014 review commissioned by the United Kingdom government estimated that antimicrobial resistance (AMR) could cause 10 million deaths a year by 2050. The report on "Antimicrobial resistance: Tackling a crisis for the health and wealth of nations" was prepared by Lord Jim O'Neill and his team. With increasing public concerns about bacterial resistance to antibiotics, the use of antibiotics in therapeutic or subtherapeutic doses in poultry feed has been severely limited or eliminated in many countries. European Union has preventively banned the use of antibiotics as growth promoters since 1st January 2006 (Catala-Gregori *et al.*, 2008).

At the Ministerial Conference on Antibiotic Resistance that took place in the Netherlands in June 2014, a global call was made to take action on antimicrobial resistance, acknowledging it as a global threat to effective prevention and treatment of infections (WHO, 2014). Antibiotics have been used in livestock in sub-therapeutic concentrations (for growth promotion and disease prevention) and in therapeutic concentrations (to treat sick animals). Since many antibiotics commonly used in sub-therapeutic concentrations are the same as or similar to antibiotics used in human medicine, there is global concern that drug-resistant organisms may pass from animals to humans and present a serious threat to public health. The European Commission's Impact Assessment, which accompanied the proposal on veterinary medicinal products on 10 September 2014, stated that "Indications exist that antimicrobial resistance in animals is transmitted to humans.

A wide range of antimicrobials is used in livestock worldwide. Twenty-seven different antimicrobial classes are used in animals, most of which have human antimicrobial counterparts. Nine of these classes are exclusively used in animals (Page and Gautier, 2012). The top three antimicrobial classes by sales for animal use in 2009 were: macrolides (USD 0.6 billion), penicillins (USD 0.6 billion) and tetracyclines (USD 0.5 billion), three classes of antimicrobials considered as critically important in human medicine by the WHO (WHO, 2011).

The act of feeding antibiotics to livestock has been practiced for over fifty years (Choe *et al.,* 2013). The mode of action of antibiotics is that they alter microbial metabolism thereby suppressing the growth of pathogenic microbes in the gut (Gadd *et al.,* 1997). The use of antibiotics has been criticized for having negative impacts on

animal production and health as it could have residual effects on tissues long after withdrawal. The usage of antibiotics as feed additives for long periods in poultry diets lead to antibiotic resistance (Shazali et al., 2014) and high residue levels in poultry products such as meat and egg (Olatoye *et al.,* 2010).

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).

2.3 Alternatives to antibiotic growth promoters

Various herbal products are being used as growth promoters in the poultry rations like garlic (Ahmad, 2005). Medicinal plants are cheap and renewable sources of pharmacologically active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile *et al.,* 1999).

Broiler production is the quickest way to produce high quality protein for human consumption. Many feed additives, antibiotics, phytogenics or phytobiotics, acidifier, prebiotics and probiotics, have been used not only to improve feed efficiency but also to improve the health and productive performance of birds (Park and Kim, 2014 and Gadde *et al*,2017). Use of antibiotics in broiler diets as growth promoters has become unwanted because of the residues in meat products and development of antibiotic- resistant bacteria populations in human. So, in recent years, use of antibiotics as growth promoters in poultry feed has been banned or restricted and the use of other feed additives as alternative compounds has been included in poultry feed. Replacement of antibiotic growth promoters with other safe additives and natural alternatives may be an important goal of the poultry production (Krishan and Narang, 2014).

Antibiotic growth promoters and antibiotic resistance are closed related. The increased concern about the potential for antibiotic resistant strains of bacteria has compelled the researchers to utility of other non therapeutic alternatives like enzymes, probiotics, prebiotics, herbs, essential oils, immunostimulants and organic acids as

feed additives in animal production. The focus of alternative strategies has been to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved (Ravindran, 2006).

The use of sub therapeutic levels of antibiotics in poultry feed improves performance and morbidity in poultry. However, the growing concern over then transmission and the proliferation of resistant bacteria in human via the food chain has led to a ban of Antibiotic Growth Promoters (AGP) in livestock feed within the European Union since, 2006. As a result, new commercial additives derived from nature have been examined as part of alternative feed strategies for the future. Such products have several advantages over commonly used commercial antibiotics and recognized as safe items in the food industry. AGPs have an antibacterial action that favors performance of broilers in different ways (Botlhoko, 2009). A good AGP alternative should be capable of reducing the incidence and severity of subclinical intestinal infections of broilers by reducing the microbial use of nutrients (Bray, 2008) and improving absorption because of thinning of the intestinal wall (Mroz, 2005).

2.4 Phytogenics

Phytogenic feed additives (PFAs), also referred as phytobiotics or botanicals, are natural bioactive compounds that are derived from plants and incorporated into animal feed to enhance productivity (Windisch *et al.,* 2008). Phytogenic additives influence improvement of consumption and conversion of food, digestibility and gain of broiler chickens (Peric *et al*., 2009). The addition of herbs, oils, botanicals and spices in feed additives increases the secretion of digestive fluids and improves the immune system of broilers (Tollba, 2010). Despite the improved health, a better nutrient digestibility, reduced frequency of digestive disorders and also increased performance of broilers is ensured (Botlhoko, 2009). A wide range of plants and their products fall under this category and, based on their origin (part of the plant), they can be broadly classified as herbs (flowering, non-woody, non-persistent plants from which leaves and flowers are used) or spices (non-leaf parts of plants, including seeds, fruits, bark or root with intensive taste or smell) (Windisch *et al.,* 2008; Van Der Klis and Vinyeta-Punti, 2014). Phytogenic feed additives include medicinal plants/herbs, which are non-woody flowering plants known to have medicinal properties; spices, which are herbs with intensive smell or taste, commonly added to human food;

essential oils, which are aromatic oily liquids derived from plant materials such as flowers, leaves, fruits and roots; and oleoresins, which are extracts derived by non-aqueous solvents from plant material (Jacela *et al.,* 2010). Phytogenic feed additives include medicinal plants/herbs, which are non-woody flowering plants known to have medicinal properties; spices, which are herbs with intensive smell or taste, commonly added to human food; essential oils, which are aromatic oily liquids derived from plant materials such as flowers, leaves, fruits and roots; and oleoresins, which are extracts derived by non-aqueous solvents from plant material (Jacela *et al.,* 2010).

A wide variety of herbs and spices (e.g., thyme, oregano, rosemary, marjoram, yarrow, garlic, ginger, green tea, black cumin, coriander, and cinnamon) have been used in poultry for their potential application as AGP alternatives. Guo *et al.* (2004) showed a significant increase in body weight gain and improvement in feed efficiency when broilers were given diets supplemented with a mixture of 14 herbs. Similar results were shown with the addition of oregano (Florou-Paneri *et al*., 2006), dried ground leaves of stevia (Atteh *et al*., 2008), black cumin seeds (Khalaji *et al*., 2011), fermented Ginkgo biloba leaves (Cao *et al*., 2012), and dried and ground Scrophularia striata and Ferulago angulata (Rostami *et al.,* 2015) to poultry feed. Various plant extracts used as PFAs were also shown to improve the performance of broilers. Research trials conducted with the inclusion of sugar cane extract (El-Abasy *et al*., 2002), aniseed extract (Durrani *et al.*, 2007), chestnut wood extract (Schiavone et al., 2008), Forsythia suspensa extract (Wang *et al.*, 2008), and Portulaca oleracea extract (Zhao *et al.,* 2013b) showed a significant increase in body weight gain and a lower FCR. In contrast, several other PFAs such as grape pomace, cranberry fruit extract, Macleaya cordata extract, garlic powder, grape seed extract, and yucca extract tested as growth promoters did not show any effects on performance parameters (Goñi *et al*., 2007; Brenes *et al.*, 2008; Leusink *et al*., 2010; Juskiewicz *et al*., 2011; Viveros *et al*., 2011; Issa and Omar, 2012; Chamorro *et al*., 2013). Nevertheless, one commercial blend of phytonutrients (containing carvacrol, cinnamaldehyde, and capsicum oleoresin) was approved in the EU as the first botanical feed additive for improving performance in broilers. Several research trials performed with this commercial blend demonstrated consistent improvement in growth and feed efficiency (Bravo *et al.,* 2014; Karadas *et al*., 2014; Pirgozliev *et al.,* 2015). A meta-analysis of 13 broiler studies involving the use of this commercial blend showed that its inclusion in diets

increased body weight gain and decreased FCR and mortality (Bravo and Ionescu, 2008).

The mechanism of action of PFAs is not clearly understood and depends greatly upon the composition of the active ingredients in the product being used. In general, the beneficial effects of PFAs are attributed to their antimicrobial and antioxidant properties. The inclusion of PFAs in the diets was shown to alter and stabilize intestinal microflora and reduce microbial toxic metabolites in the gut owing to their direct antimicrobial properties on various pathogenic bacteria, which results in relief from intestinal challenge and immune stress, thus improving performance (Tiihonen *et al.,* 2010; Viveros *et al*., 2011; Zhang *et al*., 2013; Zhao *et al*., 2013b; Liu *et al.*, 2014). Another important beneficial effect of dietary inclusion of PFAs is reduction in oxidative stress and increase in antioxidant activity in various tissues and thus improved health (Basmacioğlu *et al*., 2004; Brenes *et al*., 2008; Wang *et al.,* 2008; Cao et al., 2012; Mueller *et al*., 2012; Zhang *et al*., 2013; Liu *et al*., 2014; Settle et al., 2014). PFAs also exert their action through immunomodulatory effects such as increased proliferation of immune cells, elevated expression of cytokines, and increased antibody titers (Kim *et al*., 2010; Lee *et al.,* 2010b; Park *et al.,* 2011; Pourhossein *et al*., 2015). The addition of PFAs to the diet was also shown to increase intestinal and pancreatic enzyme production and activity and increase bile flow (Lee *et al.*, 2003; Jang *et al*., 2007; Malayoğlu *et al.,* 2010; Hashemipour *et al.,* 2013, 2014). PFAs also help maintain and improve gut histology, increase villi height and thus expand absorptive surface of the intestine (Ghazanfari *et al.,* 2015; Murugesan *et al.,* 2015). Increase in digestive enzyme secretion and absorption results in improved apparent nutrient digestibility and thus improves performance (Jamroz *et al.,* 2003; Hernández *et al*., 2004; Jørgensen *et al.,* 2008; Wang *et al*., 2008; Amad *et al*., 2011; Amerah *et al*., 2011; Issa and Omar, 2012). They also might play a role in maintaining the intestinal barrier function as evidenced by the increase in the transepithelial electrical resistance of duodenal mucosa of broilers that included thymol in their diets (Placha *et al.,* 2014).

In 1943, Osborn reported more than 60 genera of plants that exhibit inhibitory properties toward the growth of either E. coli or Staphylococcus aureus or both. Guo *et al.* (2000) have demonstrated that herbs and herbal products have a positive effect on broiler growth performance. Mottaghitalab (2000) have reported that garlic may be

used as a natural herbal growth promoter for broilers without side effects, neither for chicken performance nor consumers, and meat was not tainted with flavour or smell of garlic. Wezyk *et al*. (2000) reported that replacing antibiotic growth promoters with herbs resulted in decreased body weights, increased feed conversion per kg of weight gain and insignificant effects on carcass yield and carcass fatness. The results of some experiments with broiler chicks indicate that herb supplements have a positive effect on performance and the colour of skin (Zglobica *et al.,* 1994). Results from chick performance experiments show that feeding dietary garlic powder for 21 d significantly reduced plasma cholesterol level of broiler without altering growth of the chickens or feed efficiency (Konjufca et al., 1997). Gebert *et al.* (1999) reported that replacing antibiotic growth promoter (Zinc Bacitracin) by Rhubarb (Rheum rhaponticum willd.) as a herb did not significantly affect body weight, body weight gain, feed intake, feed efficiency and dry matter content of excreta.

2.4.1 Neem (*Azadirachta indica*)

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.

2.4.1.1 Antioxidant Properties of Neem (*Azadirachta indica)*

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, 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 activity (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, 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).

2.4.1.2 Therapeutic and Antimicrobial Properties of Neem

Neem (*Azadirachta indica*) 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, 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). 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, N.S. 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, N.S. 2014)* and another study finding revealed that leaf extract was found to have protection against paracetamol-induced liver necrosis in rats (Bhanwra, 2000*)*.

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).

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

2.4.1.3 The Effect of Neem leaf powder on Performance in Broiler Chickens

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).

2.4.2 Moringa (*Moringa oleifer*)

Moringa oleifera is a well-known cultivated species in the genus Moringa, (family Moringaceae) under the order Brassicales. The common names of *Moringa oleifera* include moringa, drumstick tree, horseradish tree, and ben oil tree or benzoil tree or miracle tree (Arora, *et al.,* 2013). The moringa seed and leaves have a broad use in the food industry and therapeutic issues (Fahey, 2005). It is popular for its seeds, flowers and leaves inhuman food and as herbal medicine (Oyeyinka, 2018). The different parts of the M. oleifera tree are used as a good source of human nutrition and in traditional diets in different countries of the world Olugbemi *et al*., 2010; Onunkwo & George, 2015). *Moringa oleifera* leaves have antimicrobial roles and are rich with fats, proteins, vitamins, and minerals (Abbas, 2013). The extracts from leaves of *Moringa oleifera* contain low amounts of polyphenols, which might have effects on blood lipid metabolism (Leone *et al.,* 2015). *Moringa oleifera* can be used as a source of micronutrient and as a dietary supplemention in poultry (Makkar, 2007; Mahajan, 2007).

2.4.2.1 Antioxidant Properties of Moringa (*Moringa oleifera*)

Moringa oleifera leaves are reported to have potential prebiotic effects and potentially antioxidant phytochemicals, such as chlorogenic acid and caffeic acid (Siddhuraju and Becker, 2003). *Moringa oleifera* leaf meal, widely available in many tropical countries, is also a good source of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Teixeira *et al.,* 2014). M. oleifera tree leaves possess various phytochemicals that have antioxidant properties and roles in controlling a wide range of diseases, like diarrhea, asthma, and various cancers. The leaves of M. oleifera have also been reported to hold extensive amounts of total phenols, proteins, calcium, potassium, magnesium, iron, manganese, and copper. They also contain rich sources of different phytonutrients, such as carotenoids, tocopherols, and ascorbic acid, which are good sources of dietary antioxidants. The leaves of the tree have been reported to have an antioxidant activity due to the higher amount of polyphenols (Moyo *et al*., 2012; Sreelatha and Padma, 2009). The HPLC

analysis indicated the presence of phenolic acids (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (kaempferol, quercetin and rutin) in moringa. Moringa oleifera leaf meal may be a promising source of natural antioxidants for broiler meat. The leaves of moringa tree have been reported to have an antioxidant activity due to the higher amount of polyphenols (Moyo *et al*., 2012; Sreelatha and Padma, 2009).

2.4.2.2 Therapeutic and Antimicrobial Properties of Moringa

Moringa oleifera is very useful as a feed supplement for animals, as its leaves are highly nutritious. The leaves of M. *oleifera* are the most nutritious part, being a significant source of vitaminB complex, vitamin C, pro-vitamin A as beta-carotene, vitamin K, manganese, and protein among other essential nutrients. The leaves, flowers and pods are used as good sources of vitamins A, B and C, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and alpha-tocopherol (Dahot, 1988). The pods are considered as an important source of the essential amino acids. A compound, pterygospermin found in the flowers and roots of the Moringa has powerful antibiotic and fungicidal effects (Das *et al.,* 1957).

Aqueous leaf extracts are being used to treat hyperthyroidism as they help regulating thyroid hormone (Tahiliani & Kar, 2000). Leaf extracts are also used to treat ulcer (Pal *et al*., 1995). It has been reported that Moringa leaves and pods also have a positive effect in reducing blood cholesterol (Ghasi et al., 2000), and anti-tumor promoting activity (Guevara *et al*., 1999). Nevertheless, it is an important source of the glucosinolate precursors of the isothiocyanate group of chemopreventives (Daxenbichler *et al*., 1991) that can inhibit carcinogenesis. Moringa is a potential plant that could be used to enhance immune responses and to improve intestinal health of broiler chicken. Yang, *et al*. (2006), reported that the dehydrated leaves of M. oleifera in the diets of broiler chicken significantly enhanced immune responses and reduced E. coli and increased Lactobacillus counts in ileum.

M. *oleifera* leaf extracts have been distinguished as having anticancer, cytotoxic, anti-proliferative, anti-leukemia, anti-hepatocarcinoma, and chemo-protective properties Khalafalla *et al.,* 2010; Pamok *et al*., 2012; Berkovich *et al.,* 2013). The antitumor functionof leaf extracts of M. oleifera is associated with the antioxidant and apoptosis inducing properties (Jung, 2014 and Tiloke, 2013). The antimicrobial properties of M.*oleifera* are well established. The extracts derived from M. *oleifera* tree leaves

have been reported to be potential antibacterial and antifungal functions against various bacterial and fungal species Chuang *et al*., 2007; Oluduro , 2012). *Moringa oleifera* is one of the plants that can be utilized in the preparation of poultry feeds. The plant apart from being a good source of vitamins and amino acids, it has medicinal uses (Makkar and Bekker 1999; Francis *et al*., 2005). *Moringa oleifera*, otherwise regarded as a "miracle tree" has been used in the treatment of numerous diseases (Pal *et al*, 1995; Makomen *et al*, 1997; Gbasi et al, 2000 and Matthew *et al*, 2001) including heart disease and obesity due to its hypocholesterolemic property (Gbasi *et al*, 2001; Olugbemi *et al* 2010) also reported this quality. Moringa oleifera leaves have the calcium equivalent of 4 glasses of milk, 3 times the iron of spinach, 4 times the amount of vit A in carrot, and 2 times protein in milk (Loren, 2007). The leaves of Moringa are good source of protein, vitamins A, B and C and minerals such as calcium and iron (Dahot, 1988). The leaves of Moringa has high protein content which is between 20 – 33% on a dry weight basis, the protein is of high quality having significant qualities of all the essential amino acid as reported by Foidl and Paull (2008). Murro et al (2003) reported that the leaves contain a high level of vitamins A, B, C and calciuim. *Moringa oleifera* can be used as a source of micronutrient and as a dietary supplement in poultry (Mahajan *et al*., 2007).In most of the feeding experiments in poultry, the fresh, green, and undamaged mature M.*oleifera* leaves were properly air-dried, and then the dried leaves were ground to a fine powder in a hammer mill and considered as moringa leaf powder or leaf meal. Similarly, fresh mature moringa seeds were air-dried and ground and considered as moringa seed meal.

In addition, Briones *et al.* stated that moringa leaves can be applied as a dietary supplement in layers and broilers due to high production performance and improved eggs quality. However, still there are many debates on the chicken's performance with different dosesof M.*oleifera* in the previous studies. There are also many variables on doses and part of plant used, such as leaves, extract, sods, or seeds. Finally, many scientists agreed that M. *oleifera* plant might have a positive role in improving the production performance and health status in chickens. Further studies are still needed to detect the actual doses of application for optimum performance in chickens. Similarly, feeding with moringa leaf meal in broilers led to a lower feed intake with higher FCR, as reported by Gakuya *et al.* Olugbemi *et al*. (2010) stated that average

daily growth rate was lower with Moringa oleifera leaf meal at the inclusion level below 5% in diets, and the authors suggested to use maximum level of 5% without any harmful effects on growth performance and FCR in broilers. Abdulsalam *et al*. conducted an experiment with moringa leaf meal in broilers and found that supplemented diets could enhance the growth performance at finisher period.

2.4.2.3 The Effect of Moringa (*Moringa oleifera)* on Performance in Broiler Chickens

Moringa oleifera can be used as a source of micronutrient and as a dietary supplement in poultry (Mahajan *et al*., 2007).In most of the feeding experiments in poultry, the fresh, green, and undamaged mature M.*oleifera* leaves were properly air-dried, and then the dried leaves were ground to a fine powder in a hammer mill and considered as moringa leaf powder or leaf meal. Similarly, fresh mature moringa seeds were air-dried and ground and considered as moringa seed meal. In some experiments, the ground particles were then soaked into distilled water for 24 h, and the filtered aqueous solution was considered as moringa extract. Due to the rich nutrient content, especially the high amount of crude protein (CP), vitamins, and minerals, M. *oleifera* leaves can be used as a useful resource of dietary supplementation for livestock as well as poultry (Nouman *et al.*, 2014; Moreki *et al.*, 2014; Sekken, 2015). In addition, Briones *et al.* stated that moringa leaves can be applied as a dietary supplement in layers and broilers due to high production performance and improved eggs quality. However, still there are many debates on the chicken's performance with different doses of M.*oleifera* in the previous studies. There are also many variables on doses and part of plant used, such as leaves, extract, sods, or seeds. Finally, many scientists agreed that M. *oleifera* plant might have a positive role in improving the production performance and health status in chickens. Further studies are still needed to detect the actual doses of application for optimum performance in chickens. Similarly, feeding with moringa leaf meal in broilers led to a lower feed intake with higher FCR, as reported by Gakuya *et al.* Olugbemi *et al.* (2010) stated that average daily growth rate was lower with *Moringa oleifera* leaf meal at the inclusion level below 5% in diets, and the authors suggested to use maximum level of 5% without any harmful effects on growth performance and FCR in broilers. Abdulsalam *et al*. conducted an experiment with moringa leaf meal in broilers and found that supplemented diets could enhance the growth performance at finisher period. Analyzing blood parameters

is very important in detecting the health status of birds. According to Voemesse *et al.*, serum albumin level was higher in laying hens fed with 3% level of moringa leaf meal than the control group, but the number of white blood cells (WBCs), red blood cells (RBCs), lymphocytes, and the packed cell volume were lower in moringa-fed groups than the control diets.

2.4.3 Jute (*Corchorus olitorius*)

Jute (*Corchorus olitorius)* commonly known as jute and locally known as "Tossa Patpata" is a popular vegetable in the Bangladesh. It grows on rice-paddy banks, in fallow paddies, in and near settlements throughout the Bangladesh. Jute (*Corchorus olitorius* L.): Annual or biennial herb, erect, stout, branched, to 1.5 m high; rootstock woody (Leung, Busson & Jardin, 1968).

2.4.3.1 Antioxidant Properties of Jute (*Corchorus olitorius*)

The leaves of *C. olitorius* were reported to exhibit antioxidant, antitumor, gastroprotective, antibacterial and antifungal, anti-inflammatory and analgesic activities (Oboh *et al.*, 2009). The free radical scavenging properties of some plants found in Malaysia such as, *Corchorus olitorius* was studied. The air-dried leaves of the plant were soaked in distilled water (1:20; w/v) for 72 h at room temperature. The collected supernatants were tested for the free radical scavenging activity against the DPPH and superoxide anion radical scavenging assays. The extract showed remarkable antioxidant activity in both assays with the percentage of inhibition nearly 90% (Zakaria, 2007). The crude methanolic extract of *Corchorus olitorius* (leaves) and its fructions (5-25 μg/μl), were tested for the free radical scavenging activity against the DPPH and superoxide anion radical scavenging assays. Extracts were found to show remarkable antioxidant activity in both assays with the percentage of inhibition. Hexan extract caused 65.44-97.43% inhibition and appeared the most potent antioxidant extract, followed by butanol, methanol and ethyl acetate extracts *(*Rume, 2010*).* The leaves of Corchorus are rich in betacarotene, iron, calcium, and vitamin C. The plant has an antioxidant activity with a significant α-tocopherol equivalent vitamin E. Jute leaf as vegetable contains an abundance of antioxidants that have been associated with protection from chronic diseases such as heart disease, cancer, diabetes, and hypertension as well as other medical conditions. The leaves of C. *olitorius* were reported to exhibit antioxidant (Obohet *et al.*, 2009).

2.4.3.2 Therapeutic and Antimicrobial Properties of Jute (*Corchorus olitorius*)

Pharmacologically jute (C. *olitorius*) possesses a diverse biological activities which includes, antioxidant, anti-tumor, hypoglycemic, antimicrobial, anti-inflammatory, analgesic, antiobesity, gastroprotective and wound healing effects (Oboh *et al.*, 2009 and Das *et al*. 2010). The leaves are rich in betacarotene, iron, calcium, and Vitamin C. The plant has an antioxidant activity with a significant α-tocopherol equivalent Vitamin E (http://en.wikipedia.org/wiki/Jute). Vitamins A, C and E present in jute leaf/Saluyot "spongeup" free radicals, scooping them up before they can commit cellular sabotage. Jute grows under wide variation of climatic conditions and stress of tropic and subtropics. Jute is as old as civilization and has been used in almost as many applications as one can imagine. This paper reviews history, chemical constituents, plant morphology and the most interesting studies on the various biological activities of jute (*Corchorus* spp) (Duke, 1979). Furthermore, the different parts of C. olitorius were found to exhibit diverse biological activities. The leaves of C. olitorius were reported to exhibit antioxidant (Obohet *et al.,* 2009), antitumor (Furumoto *et al*., 2002), gastroprotective (Al Batran *et al.,* 2013), antibacterial and antifungal (İlhan *et al*., 2007), anti-inflammatory and analgesic (Das *et al.*, 2010 activities. In addition, the leaves are used as demulcent and febrifuge (Nishiumi *et al*., 2016). It exhibited antiinflammatory, hepatoprotective, gastroprotective, immunoregulatory and anti-ulcer activities (Valchalkova *et al*., 2004), and gastroprotective effect on experimentally induced gastric lesions in rats and mice (Astudillo *et al.,* 2002). It has been reported to lower plasma cholesterol levels, inhibit intestinal cholesterol and plant sterol absorption, and suppress hepatic cholesterol and classic bile acid synthesis in Winstar and WKY rats (Batta *et al*., 2006). In other studies, stigmasterol showed cytostatic activity against Hep-2 and McCoy cells, markedly inhibited tumour promotion in two stage carcinogenesis experiments, and exhibited antimutagenic, topical antiinflammatory, antiosteoarthritic and antioxidant activities (Gómez *et al.,* 2001; Kasahara *et al.,* 1994; *Lim et al.,* 2005; García *et al*., 1999; Gabay *et al*., 2010; Panda *et al*., 2009). The antinociceptive and anti-inflammatory properties of jute leaves chloroform extract were investigated in experimental animal models. The antinociceptive activity was measured using the writhing, hot plate and formalin tests, while the anti-inflammatory activity was measured using the carrageen an induced paw edema test. The extract was used in the

doses of 20, 100 and 200 mg/kg. It was administered subcutaneously, 30 min prior to subjection to the respective assays. The extract was found to exhibit significant ($p<0.05$) antinociceptive and anti-inflammatory activities (Zakaria *et al.* 2007). The antinociceptive, anti-inflammatory and antipyretic properties of an aqueous extract of jute leaves were studied in experimental animals. The antinociceptive activity was measured using the abdominal constriction, hot plate and formalin tests, while, the anti-inflammatory and antipyretic activities were measured using the carrageenan-induced paw edema and brewer's yeast-induced pyrexia tests, respectively. The extract was used as 11.57, 57.85, and 115.7 mg/kg, it was administered subcutaneously, 30 min prior to subjection to the mentioned assays. The extract was found to exhibit significant antinociceptive, antiinflammatory and anti-pyretic activities in a dosage-independent manner (Zakaria *et al.*, 2009). Disc diffusion method was used to determine the antibacterial and antifungal activity of the crude methanolic extract of jute leaves and its fructions against Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus,* Beta hemolytic *streptococcus, Bacillus cereus* and *Streptococcus pyrpgen*), Gram negative bacteria (*Shigella boydii, Salmonella typhi E.coli, Klebsiella and Vibrio mimicus*), yeast and *fungi (Candida albicans, Saccharomyces cerevisiae* and *Bacillus megaterium*). Jute leaves extracts possessed antimicrobial antifrungal and anti-yeast activity. N-hexane fraction of methanolic extract of leaves of Jute leaves showed the highest acivities against gram positive, gram negative bacteria and fungi with a zone of inhibition 0.9-1.5mm, followed by hexane extract (Rume, 2010). *Corchorus olitorius* is usually recommended for pregnant women and nursing mother because it is believed to be rich in iron (Oyedele *et al*., 2006).

2.4.3.3 The Effect of Jute (*Corchorus olitorius*) on Performance in Broiler Chickens

White Jute (*Corchours capsularis* L.) and Tossa Jute (*Corchorus olitorius* L.) both the species have medicinal values. The dried material is there known as "nalita." Injections of olitoriside markedly improve cardiac insufficiencies and have no cumulative attributes; hence, it can serve as a substitute for strophanthin. Deobstruent, diuretic, lactagogue, purgative, and tonic, tussah jute is a folk remedy for aches and pains, dysentery, enteritis, fever, dysentery, pectoral pains, and tumors (Duke and Wain, 1981; List and Horhammer, 1969-1979). Ayurvedics use the leaves for ascites,

pain, piles, and tumors. Elsewhere the leaves are used for cystitis, dysuria, fever, and gonorrhea. The cold infusion is said to restore the appetite and strength (Duke, 1983).

Jute plant consists of considerable amount of Vitamin K which is helpful in reducing the threat of bleeding in the liver, poor nutrient absorption, jaundice or the combination of long term use of antibiotics or aspirin. Some of the problems related with the gastrointestinal system due to a decrease of this vitamin include colitis, obstructions, sprue and Crohn's disease. All these problems are due to a reduced content of Vitamin K. Regular consumption of Jute plant helps to get rid of this problem because Jute plant consists of 2.73 mg of Iron which is 34.13% of the daily recommended value. Muscle spasms are also one of the main symptoms of iron deficiency. The leaves of *C. olitorius* have been claimed to possess stimulant, demulcent, laxative, appetizer and stomachic effects. The infusion of the leaves is traditionally used to treat fever, constipation, dysentery, liver disorders and dyspepsia. In Japan, the young leaves were used as a substitute for coffee or tea and were regard as a health food (http://www.globinmed.com).

CHAPTER 3

MATERIALS AND METHODS

3.1 Statement of the experiment

The present study was conducted in the experimental poultry shed at the Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. About two hundred (200) number of day-old (42.7g) commercial broiler chicks (Cobb 500) was taken. The experiment was accomplished from 18th September to 16th October, 2018 to assess the feasibility of using NLP, MLP and JLP in commercial broiler diet on production performance, dressing characteristics, hematological and immune status of broilers. This research helps to make a conclusion that 2% MLP can positively affect the production performance and health status as the alternative of antibiotic. Birds were maintained following standard feeding and uniform managemental practices under deep litter system of rearing.

3.2 Collection of experimental broilers

A total of 200 day-old Cobb 500 broiler chicks were collected from Kazi hatchery, Savar, Dhaka.

3.3 Experimental materials

The collected chicks were carried to the university poultry farm early in the morning. They were kept under electric brooders for 2 days by maintaining standard brooding protocol. During brooding time only basal diet was given. After two days the healthy chicks were distributed randomly into treatments of NLP, MLP and JLP, antibiotic and control group and each treatment had four (4) replications with 10 birds.

3.4 Experimental treatments

T_0: Basal Diets/ Control

T_1: 2% of Neem leaf Powder (2.0 kg NLP/100 kg of the feeds)

T_2: 2% of Moringa Leaf Powder (2.0 kg MLP /100 kg of the feed)

T_3: 2 % of Jute Leaf Powder (2.0 kg JLP / 100 kg of the feed)

T_4: Basal Diets + Antibiotics (0.1 kg/100kg of the Doxivet)

Table 1. Layout of the experiment

Treatment groups	No. of replications				Total
	R_1	R_2	R3	R4	
T_0	10	10	10	10	40
T_1	10	10	10	10	40
T_2	10	10	10	10	40
T_3	10	10	10	10	40
T_4	10	10	10	10	40
Total	50	50	50	50	200

3.5 Preparation of experimental house

The experimental shed was properly cleaned and washed by using tap water. Ceiling, walls, floor, feeder and waterer were thoroughly cleaned and disinfected by spraying diluted Iodophor disinfectant solution (3 ml/liter water). After proper drying, the house was divided into 20 pens of equal size 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 5 (five) treatments. One feeder and one waterer were distributed each pen. The stocking density was $1m^2$/10 birds.

3.6 Experimental diets

Starter and grower commercial Kazi broiler feed were purchased from the market. Starter diet was enriched with minimum:-

Table 2. Name and minimum percentage of nutrients present in Starter and Grower 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%
Name of nutrients in grower 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%

3.6.1 Collection of Neem, Moringa and Jute Leaves and feeds

The experiment was carried out at the Sher-e-Bangla Agricultural University Poultry Farm in Dhaka .Feeds was purchased from Diamod feed Limited, Savar, Dhaka, while neem, moringa and jute leaves were harvested from SAU campus. The neen, moringa and jute leaves were harvested and air dried under shade for 4 days and milled, after which the leaf meal was added into the diets at 2 % level different treatment groups.

Table 3. Nutritional composition of Neem, Moringa and Jute leaves

Nutrient Component	Neem	Moringa	Jute
Dry matter	90.24%	93.78%	-
Moisture	_	-	79.98%
Crude protein	23.40%	22.60%	6.21 %
Ether extract	3.36%	-	-
Ash	9.90%	11.24%	0.64%
Crude fiber	7.81%	8.07%	0.33%
Carbohydrate	-	44.69%	6.25%
Crude fat	-	13.40%	5.07 %
Calcium(g)	1.40	-	-
Phosphorus(g)	0.25	-	-

Source: Iran Journal Veterinary Research (2015), Winter 16(1), Lesten and Emmanuel (2018) and Adeniy *et al.* (2012).

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 from 18th September to 16th October, 2018. The average temperature was 30.5^{0}C and the RH was 79% in the poultry house. Common brooding was done for one week. 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^{0}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

The room temperature (^{0}C) and humidity were recorded every six hours with a thermometer and a wet and dry bulb thermometer respectively. Averages of room temperature and percent relative humidity for the experimental period were recorded and presented in Appendix 1& 2.

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 layer 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 10 birds. Feeders were cleaned at the end of each week and drinkers were washed daily.

3.7.5 Lighting

At night there was provision of light in the broiler farm to stimulate 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 Vaccination

Proper biosecurity measures were adopted during the experimental period. Chicks were vaccinated against Ranikhet Disease (RD), Infectious Bronchitis and Infectious Bursal Disease (IBD) as per standard schedule. 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.

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. The vaccination schedule of Broiler chicken

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
17 days	Gumboro	G-228E (inactivated) booster dose	Drinking Water
21 days	IB + ND	MA-5 + Clone-30	Drinking Water

3.7.7 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.

3.7.8 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

Weekly lives weight, weekly feed consumption and death of chicks were recorded to calculate mortality percent. FCR was calculated from final live weight and total feed consumption in each replication. After slaughter gizzard, liver, spleen, intestine, hear and bursa were measured from each broiler chicken. Dressing yield was calculated for each replication to find out dressing percentage. Blood sample was analysis from three birds each replication to measure, glucose, haemoglubin and cholesterol level.

3.9 Data collection

3.9.1 Live weight

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

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 fasted 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 ethylenediethy letetraacitic acid (EDTA) tubes from the wing veins. Samples were transferred to the laboratory for analysis within 1 hour of collection. Glucose, Cholesterol and haemoglubin was measured by easy test device using rapid test strip.

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.

3.10.3 Feed conversion ratio

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

3.11 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 and significance was set at $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production performance of broiler chicken

Broilers are among the most efficient feed converting livestock in the world. During the selection process, intensive selection pressures placed on broiler performance traits, such as increased body weight and growth rate.

4.1.1 Final Live weight

The effect of dietary inclusion of Neem Leaf Powder (NLP), Moringa Leaf Powder (MLP) and Jute Leaf Powder (JLP) on the production performances of broiler chickens was significant ($p<0.05$) and good fluctuation was observed among the different treatment groups (Table 5). Data presented in Table 5 showed that the effect of treatments on final live weight (gram per broiler chicken) was significant ($P<0.05$). The relative final live weight (g) of broiler chickens in the dietary group T_0, T_1, T_2, T_3, and T_4 were $1610.80^{c}\pm3.31$, $1633.55^{b}\pm4.45$, $1664.30^{a}\pm6.29$, $1633.55^{b}\pm7.28$ and $1648.55^{ab}\pm9.41$ respectively. The highest result was found in T_2 ($1664.30^{a}\pm6.29$) and lowest result was in T_0 ($1610.80^{c}\pm3.31$) group. Although the final live weight of broiler fed moringa leaf powder diets was higher than antibiotic treated group but the difference was non-significance. The present findings are in accordance with Banjo, O.S. (2012) who also observed significantly higher body weights on diets containing 2% level of M. *oleifera* leaf meal. The reason for the improved weight gain can be attributed to high amino acids, a highly potent antiinflammatory (Ezeamuzle *et al.*, 1996), and hepatoprotective properties (Pari and Kumar, 2002). The HPLC analysis indicated the presence of phenolic acids (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (kaempferol, quercetin and rutin) in moringa. *Moringa oleifera* leaf meal may be a promising source of natural antioxidants for broiler meat. It also possesses antimicrobial activity due to its principle component pterygospermin. The improvement in live body protein content of Moringa leaf meal as claimed by

Table 5: Production performance of broiler chicken treated with NLP, MLP, JLP and antibiotic.

Treatment	T_0	T_1	T_2	T_3	T_4	Mean± SE
Final live weight (g/bird)	1610.80^{c}±3.31	1633.55^{b}±4.45	1664.30^{a}±6.29	1633.55^{b}±7.28	1648.55^{ab}±9.41	1638.15*±4.83
FC(g)	2318.10^{bc}±5.71	2289.62^{c}±6.71	2288.35^{c}±10.14	2358.25^{a}±22.14	2337.50^{ab}±2.39	2318.37*±7.75
FCR	1.44^{a}±0.01	1.40^{bc}±0.02	1.38^{c}±0.01	1.44^{a}±0.02	1.42^{ab}±0.02	1.42*±0.02
DP% (Skinless)	67.60±.293	70.80±.610	70.30±1.071	69.38±.958	69.05±1.17	69.52^{NS}±.44
Survivability (%)	99.67.00±00	100.00±00	100.00±00	100.00±00	100.00±00	99.30^{NS}±07

Here, T_0 =(Control), T_1 =(2% NLP), T_2 =(2% MLP), T_3 =(2% JLP) and T_4 =(Antibiotic). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different ($P<0.05$)

- ✓ Mean within same superscripts don"t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ Means of sinnificant at level of significance (P>0.05)

(Danol, 1986); (Kakengi *et al* 2003) and (Olugbemi *et al*., 2010). *M. Oleifera* plant was reported to contain various

Weight of broilers observed due to the supplementation of M. oleifera leaf powder may also be attributed to the significant quantities of vitamins (A, B and C), calcium, iron and protein. Nkukwana *et al* (2012) also found that birds supplemented with M. *oleifera* leaf meal had higher body weight than the birds fed the control diets.
However, Eze *et al*. (2014) observed no significant differences in the body weight of broiler treated with 200mg/kg dose of *Moringa oleifera* extract than those of untreated groups. Gadzirayi *et al* (2012) also found that supplementation of *Moringa oleifera* leaf meal did not influence the final weights of broiler over the control group. These reports indicate that lower level of M. *Oleifera* did not exert significant changes in broiler performance. Divya *et al.* (2014) reported that addition of moringa leaf powder at 0.5%, 1.0%, 1.5% and 2.0% levels or antibiotic slightly decreased body weight. According to the Musa *et al*. (2017) was *Moringa oleifera* pods inclusion to broiler diet had decreased feed intake but improved live weight gains in broiler chickens. The final live weight of NLP and JPL was also significantly ($p<0.05$) higher comparaed to control group. Similar observation was found in the study of Manwar *et al.* (2005) who supplemented neem leaf powder @ 1-2 gm/kg feed and reported significant increase in the live body weight of broilers in the neem fed groups when compared with control group. Similarly, Nemade and Kukde (1993) reported increase in feed efficiency in neem fed groups.

4.1.2 Feed consumption (FC)

Different treatment groups (Table 5) showed significant ($P<0.05$) differences in feed consumption of broiler chicken. T_3 group consumed higher amount of feed ($2358.25^{a}\pm22.14$) and 2% (T_2) dried Moringa leaf powder treated group consumed

lower amount of feed ($2288.35^{c}\pm10.14$). The T_4 (2337.50^{ab} significantly ($P<0.05$) differed from the T_1 and T_2. The feed consumption of T_2 fed group was non-significant lower compared to control group. This result was in close agreement with Aderinola *et al.* (2013) revealed that control diet had significantly higher average daily feed intake in broiler chicks compared to MOLM diet (0.5%, 1%, 1.5 and 2%. Contrary to results of this study, Onu and Aniebo (2011) indicated that broilers chick fed MOLM starting from 7th day of age had significantly higher average feed intake compared to control birds. Moreover, Melesse *et al.* (2011) reported that Rhode Island Red chicks fed on 2%, 4% and 6% *Moringa Stenopetala* leaf meal had a significantly higher feed consumption than control ones. Furthermore, Banjo (2012) found that broilers supplemented with 1%, 2% and 3% MOLM from the 2nd week of age had a significantly higher feed intake when compared with un-supplemented ones, also birds fed on 1% and 2% consumed more feed than those fed on 3%. However, Gakuya *et al*. (2014) reported that feed intake of birds fed on 7.5% MOLM was not significantly different from control ones, while increasing level of MOLM to 15% and 30% showed a significant reduction in feed intake. Portugaliza and Fernandez (2012) indicated that *Moringa oleifera* Aqueous Leaf Extract at 30 mL and 60 mL level significantly improved feed intake compared to control diet however, at 90 mL feed intake significantly reduced. Low feed intake in birds supplemented with 8% MOLM compared to other MOLM treated groups could be attributed to presence of some anti- nutritional factors such as mimosine and tannins (Atawodi *et al.*, 2008). However, Makkar and Becker (1997) indicated that, leaves of Moringa are very poor in anti-nutritional factors. Also, low feed consumption may be attributed to high crude fiber content in Moringa which may resulted in decreased palatability (Kakengi *et al*. 2003). According to Swain *et al.* (2017) *Moringa oleifera* leaf meal (MOLM) (0.5kg/100kg) diet can improve significantly ($P<0.05$) the egg production and feed conversion ratio (FCR) of layer chicken. Tesfaye *et al.* (2013) worked on MOLM as an alternative protein feed ingredient in broiler ration and found that there was significantly increase in feed intake with supplemented groups as compared to the control group when they used *Moringa oleifera* leaf meal. However, Divya *et al.* (2014) found that the addition of MOL powder at any level slightly decrease feed intake on 21 and 42 days of age as compared to control, although the decrease was not significant ($p>0.05$).

4.1.3 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) was significantly (P<0.05) lower for birds supplemented with T2 (1.38^{c}±0.01) than T_0 (1.44^{a}±0.01), T_3 and T_4. (Table 5). Onu and Aniebo (2011) who found that FCR was significantly better in birds fed MOLM supplemented diet compared to control birds. Banjo (2012) indicated that, broilers fed 1%, 2% and 3% MOLM had significantly superior FCR in all MOLM supplemented groups compared to control birds. According to Swain *et al.* (2017) *Moringa oleifera* leaf meal (MOLM) (0.5kg/100kg) diet can improve (P<0.05) the egg production and feed conversion ratio (FCR).

4.1.4 Dressing Percentage

The 2% (T_2) MLP (67.31±2.61%) supplemented group had a greater (P > 0.05) dressing percentage compared with the antibiotic group (63.65±0.32%), 2% NLP (T_1), 2% JLP (T_3) and control (T_0) group DP % were 66.26±0.41, 65.34±1.92and 64.24±1.18 respectively (Table 5). However, Ayssiwede *et al.* (2011) and Ochi *et al.* (2015) who studied the effect of *Moringa oleifera* seed powder on broiler chickens did not observe significant differences in the dressing percentage among the treatments. Herb extracts have been reported to significantly improve body weight gain, feed conversion ratio as well as broiler carcass dressing percentages (Omar *et al.*, 2016).

4.1.5 Survivability

The Survivability rate was non- significant. The lowest survivability rate found in T_0 group (99.67±0.00) the highest in T_3 (100.00±00).

4.1.6 Weekly Body Weight Gain

The result revealed that the cumulative weekly body weight gain differed significantly (p<0.05) among various treatment groups. The birds fed 2% M. oleifera leaf powder recorded significantly higher mean weight gain compared to control and other treatment groups, however, slightly reduced mean body weight gain was observed in T_3 group (Figure 1).

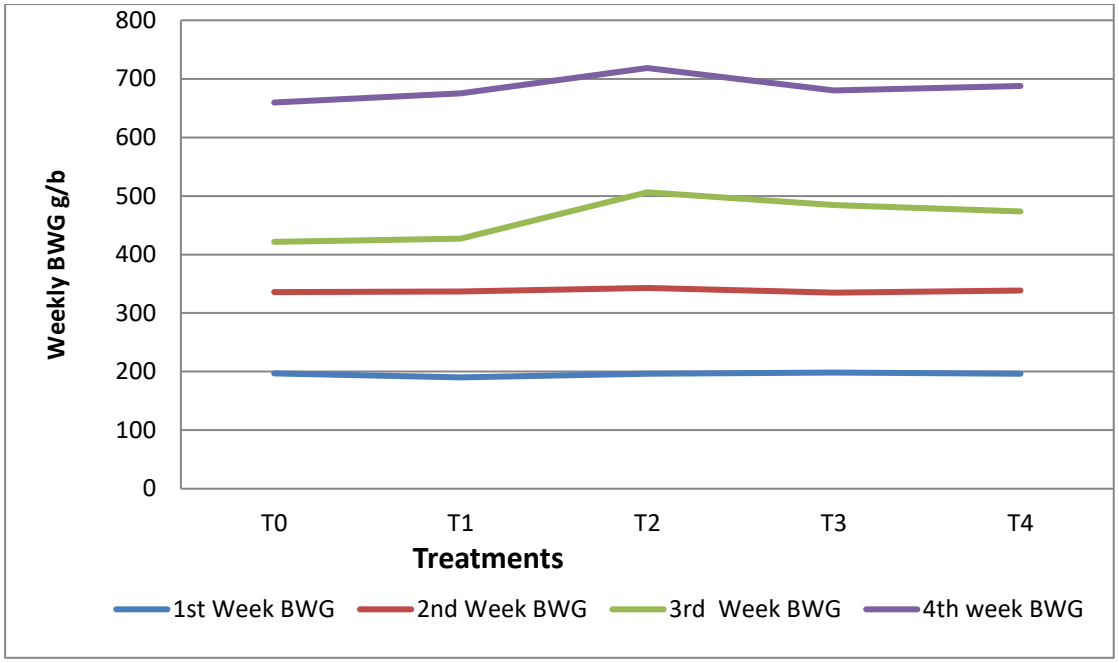

Figure 1. The Effect of supplementation NLP, MLP and JLP to broiler diets on Body Weight Gain (g/bird) of broiler chickens at different week

The mean body weight gains (g) of broiler chicks at the end of 4th week in different groups were $687.75^{ab}\pm8.08$, $675.25^{bc}\pm1.75$, $718.5^{a}\pm2.50$, $680.25^{ab}\pm7.79$, and $659.65^{c}\pm1.14$ respectively. The overall mean body weight gain of different groups showed that there was significant ($P<0.05$) increase in groups T_1 compared to control and antibiotic (Figure 1).

The present findings are in accordance with Okafor *et al.,* (2014) who reported that *M.oleifera* supplemented groups recorded a higher daily weight gain. Banjo (2012); Gadzirayi *et al.,* (2012); Kout *et al*., (2015) showed that birds fed on Moringa leaf powder gained significantly higher body weights than birds fed the control diet. Talha and Mohamed (2012) observed that addition of *M. oleifera* undecorticated seed powder also had significant beneficial effects on weight gain in broilers.The experiment level (2%) of Neem leaf powder found to reduce the weight gain in broiler. Divya *et al*. (2014) reported that addition of Moringa leaves at 0.5%, 1.0%, 1.5% and 2.0% level or antibiotic did not improvement the body weight gain of broiler. Similar reports are also available in the literature of Aderinola *et al*. (2013). Karthivashan *et al*. (2015) found no significant differences in weight gain of broiler at 0.5%, 1.0%, and 1.5% w/v *Moringa oleifera* aqueous leaf extract as a dietary supplement on the growth performance. Zanu *et al.* (2011) and Olugbemi *et al.* (2010)

also observed decline in body weight gain when Moringa was included in maize and cassava based broiler ration. Ochi *et al*. (2015) reported significant reduction in weight gain, feed efficiency and body weight due to addition of 2.0% Moringa oleifera seed powder to broilers' diet during starter period. The reduction in weight gain can be explained by the presence of phytate which acts as an anti-nutritional factor. Results from productive performance in the current study were in close agreement with Onu and Aniebo (2011) who found that birds supplemented with 2.5%, 5% and 7.5% MOLM had significantly higher final BW and BWG at 35 days of age compared to control birds.

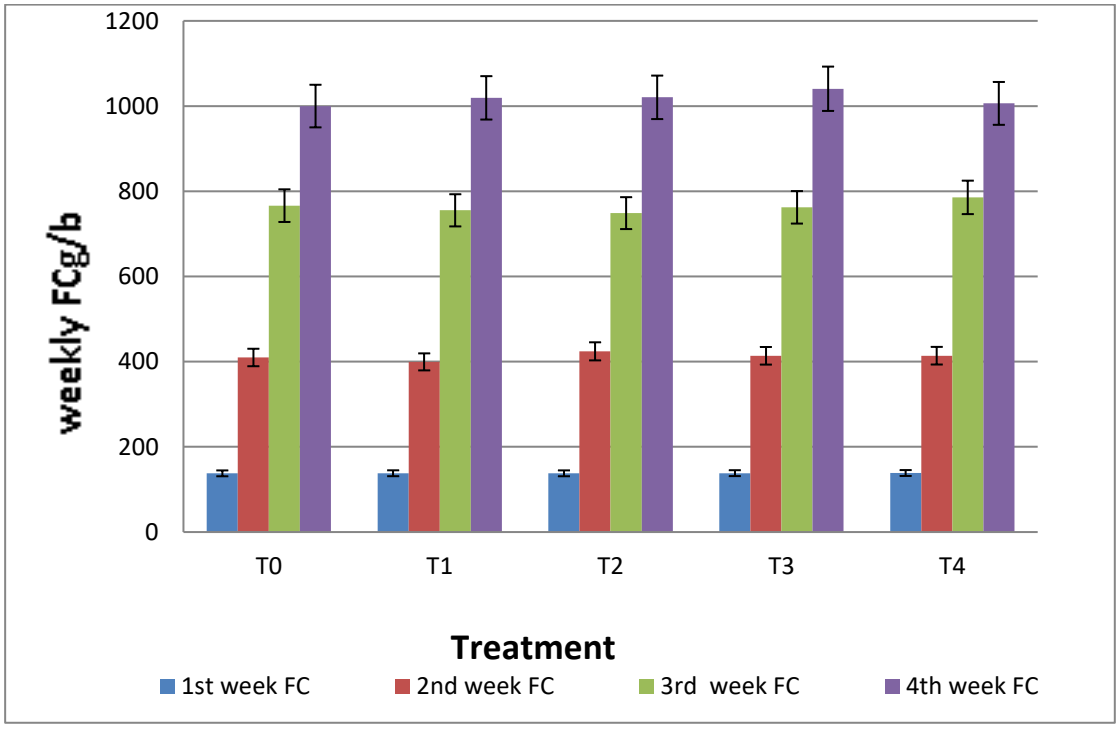

Figure 2. The Effect of supplementation of NLP, MLP and JLP to broiler diets on feed consumption (g/bird) of broiler chickens at different week.

4.1.7 Weekly Feed consumption (FC)

On perusal of the mean weekly feed intake of the present study (Figure 2), it could be seen that during the first week of age the feed intake was lowest in T_0 (137.85±0.65) group and highest in T_4 (138.62±.59) group.

During the second week, feed intake was highest in T_4 group and lowest in T_2 group. Similar trend was seen in third week of age, except that feed intake was lowest in T_2 group.

At the end of the four week of age higher feed intake was found in T_3 group (1040.62±26.54g) and lower in T_0 group (1000.00±0.89g). Wanker *et al.*, (2009) was found that the increased feed intake might be due to its appetite and digestion stimulating, antibacterial and hepatoprotective properties which help to reduce the microbial load of birds and improved the feed consumption. Similar findings with respect to improvement in feed intake were observed by several workers (Onyimonyi *et al*., 2009); Khatun *et al*., (2013); Nodu *et al*., (2016) and Shihab *et al*., (2017). The finding of the present study was contradictory to the findings of Wanker *et al*. (2009). Zanu *et al*., (2011); Adeyemo and Akanmu (2012); Bonsu *et al.* (2012); Nnenna and Okey, (2013) And Ali *et al*., (2015 who reported no significant difference in feed intake between the control and neem leaf fed groups of broiler chicken. The total feed consumption per broiler under different experimental groups was found to be highest in T_3 group (2358.25^{a}±22.14g) followed by T_4 (2337.50^{ab}±2.39g), T_0 (2318.10^{bc}±5.71g) and T_1 (2289.62^{c}±6.71g) group. Shihab *et al*. (2017) reported that supplementation of NLP at the levels of 0.2 and 0.3% increased total feed consumption by 8.05 and 9.63% respectively, as compared to control.Contrary to the present finding. They also found that the highest total feed consumption in 0.2% NLP supplemented group (3281.6 g) and lowest in control group (2592.6 g) during a period of five weeks. However, supplementation of high level of NLP at the dose of 0.5% and above significantly reduced feed intake in broiler chicken (Onyimonyi *et al.*, 2009); (Adeyemo and Akanmu, 2012) and (Bonsu *et al*., 2012).

4.1.8 Weekly Feed Conversion Ratio (FCR)

The mean body FCR of broiler chicks at the end of 4^{th} week in different groups were T_1 (1.40^{bc}±.01), T_2 (1.38^{c}±.01), T_3 (1.44^{a}±.02), T_4 (1.42^{ab}±.01) and T_0 (1.44^{a}±.01) respectively. The overall mean FCR of different groups showed that there was significantly ($P<0.05$) increase in groups T_2 compared to control and antibiotic (Table 6).

Table 6. The Effects of feeding NLP, MLP, JLP and antibiotic on FCR of broiler chickens at different week.

Treatments	1st week	2nd week	3rd week	4th week
T_0	0.70±.01	1.03±.02	1.38^{a}±.01	1.44^{a}±.01
T_1	0.71±.01	1.01±.03	1.34^{b}±.01	1.40^{bc}±.01
T_2	0.70±.01	1.01±.02	1.34^{b}±.01	1.38^{c}±.01
T_3	0.70±.01	1.04±.02	1.38^{a}±.02	1.44^{a}±.02
T_4	0.71±.01	1.05±.01	1.39^{a}±.00	1.42^{ab}±.01
Mean±SE	0.70^{NS}±.01	1.03^{NS}±.02	1.36*±.01	1.42*±.02

Here, T_0 =(Control), T_1 =(2% NLP), T_2 =(2% MLP), T_3 =(2% JLP) and T_4 =(Antibiotic). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don't differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ * means significant at 5% level of significance (p<0.05)

Aderinola *et al.* (2013) revealed that broiler chicks fed control diet had significantly higher BWG and FCR compared to birds fed 0.5%, 1%, 1.5% and 2% MOLM diets. This assertion is supported by David *et al*. (2012), Safa & El-Tazi (2012) and Ebenebe *et al.*, (2012) who reported better feed conversion ratio for birds on M. Oleifera diets as compared to the control diets.

4.2.1 Glucose

The effects of dietary dried neem, moringa and jute leaf powder supplementation on concentration of glucose of broiler chickens are presented in Table 7. There was no significant (P>0.05) difference among the treatment. Although the highest amount (393.00±27.83) of plasma glucose are found in T_3 (2% JLP) but this was not statistically difference with antibiotic, control and other groups. The results of the present study are compatible with those observed by Velasco *et al.* (2010) and Rezaei *et al*. (2010) who observed reduction in blood glucose level in broilers using 3% MOLM. This may be due to the suppressive effect of herbals plants leaf extracts on glucagon, which otherwise increases blood glucose in chickens, thereby maintaining blood glucose homeostasis. The present results are in line with the findings of Olugbemi *et al*., (2010) who studied the supplementation of herbal plants leaf extracts in broilers and its influence on blood hematology. It was reported that hemoglobin was not affected significantly due to the supplementation of these extracts.

4.2.2 Cholesterol

Total cholesterol concentration (mg/dl) in the serum of different groups ranged from 179.50±15 to 187.50±3.02. Statistical analysis revealed a non significant (P>0.05) deference among the group. However the cholesterol level was lower in T1 fed group(179.50±15.05) followed by T_2 (183.67±8.21), T_3(186.50±11.85), T_0 (186.67±12.31), and T_4 (187.50±3.02) respondingly. While the concentration in T_4(187.50±3.02) was comparable to that of T_1 (179.50±15.05) and T2 (183.67±8.21) (Table 7). Similar results have also been observed by Miao *et al.,* (2008) and Chen *et al.,* (2005) in broilers who observed that addition of different herbal plants leaf extracts as antibiotic replacer significantly decreased the total blood cholesterol level of the experimental birds.Accodding to Ghasi *et al.,* (2000) who was found that the leaf extract was found to regulate cholesterol level in rats.

Table 7. The Effect of supplementation NLP, MLP and JLP to broiler diets on blood parameters.

Parameters	T_0	T_1	T_2	T_3	T_4	Mean±SE
Glucose (mg/dL)	307.00±17.61	352.17±24.64	379.50±37.67	393.00±27.83	369.17±30.87	360.17^{NS}±13.06
Cholesterol (mg/dL)	186.67±12.31	179.50±15.05	183.67±8.21	186.50±11.85	187.50±3.02	184.77^{NS}±4.56
Hemoglobin (g/dL)	12.68±.61	12.81±.26	12.567±.49	12.18±.58	12.65±.16	12.58^{NS}±0.19

Here, T_0 =Control, T_1 = 2% NLP, T_2 =(2% MLP, T_3 =2% JLP and T_4 =Antibiotic. Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don't differ (P>0.05) significantly
- ✓ SE= Standard Error

- ✓ * means significant at 5% level of significance (p<0. 0)

4.2.3 Hemoglobin

The effects of dietary NLP, MLP and JLP supplementation on concentration of Hemoglobin of broiler chickens are presented in Table 11 and Figure 4. Feeding dietary NLP, MLP and JLP had no significant (P>0.05) difference among the treatment. Although the highest amount (12.81±.26) of Hemoglobin are found in T_1 (2% NLP) than antibiotic, control and other groups.

Neem preparations fed to laying hens have been reported by Sadre *et al.,* (1984) and Gowda *et al.,* (1998) to significantly reduce the content of hemoglobin, erythrocyte count and packed cell volume. Observation of Alam *et al.,* (2015) was found that the hematological parameter (RBC, Hb, PCV, ESR) on 21st day and 42 day did not show any significant difference (P<0.05) between the control and treated groups.

4.3.1 Relative giblet weight (liver, heart and gizzard)

The relative weight of liver (g) of broiler chicks in the dietary group T_0, T_1, T_2, T_3 and T_4 were 37.33±.60, 39.20±.89, 40.47±.58, 38.33±1.02 and 38.33±.44 respectively. The highest results were obtained in T_2 and lowest was in T_0 group. However, there was no significant (P>0.05) difference in the relative weight of liver between the groups (Table 8).

4.3.2 Weight of intestine

The results of different groups showed that there was no significant (P>0.05) difference between the groups and the values were ranged from 83.77±3.79 to 102.13±3.28 (Table 8). The present results are akin to that of Hernandez *et al.* (2004), who observed no difference in the mean weight of proventriculus, gizzard, intestine, liver and pancreas in broilers fed on two herbal extracts. The results of Ayssiwede *et al.* (2011) was found that there were significant differences (p<0.05) in the weight of the large intestine and lungs.

Table 8. The Effect of supplementation NLP, MLP and JLP to broiler diets on on Liver, Gizzard, Intestine and heart weight of different Treatment.

Parameters	T_0	T_1	T_2	T_3	T_4	Mean±SE
Liver weight(g)	37.33±.601	39.20±.89	40.47±.58	38.33±1.02	38.33±.44	38.73^{NS}±.395
Gizzard Weight (g)	37.50±.44	38.90±.67	38.33±.73	37.67±.44	37.50±.76	37.85^{NS}±.31
Intestine weight (g)	90.10^{b}±1.85	86.53^{c}±2.65	83.77^{c}±3.79	102.13^{a}±3.28	85.57^{c}±1.26	89.62*±1.15
Heart(g)	9.17±.44	9.83±.16	10.17±.44	9.67±.67	9.00±.86	9.57^{NS}±.24

Here, T_0 =(Control), T_1 =(2% NLP), T_2 =(2% MLP), T_3 =(2% JLP) and T_4 =(Antibiotic). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don't differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ * means significant at 5% level of significance (p<0.05)

4.4 Immune organs

The effect of NLP, MLP and JLP supplementation on immune organs of Cobb 500 strain broiler chicks during the period from 0 to 28 days of age are summarized in Figure 3.

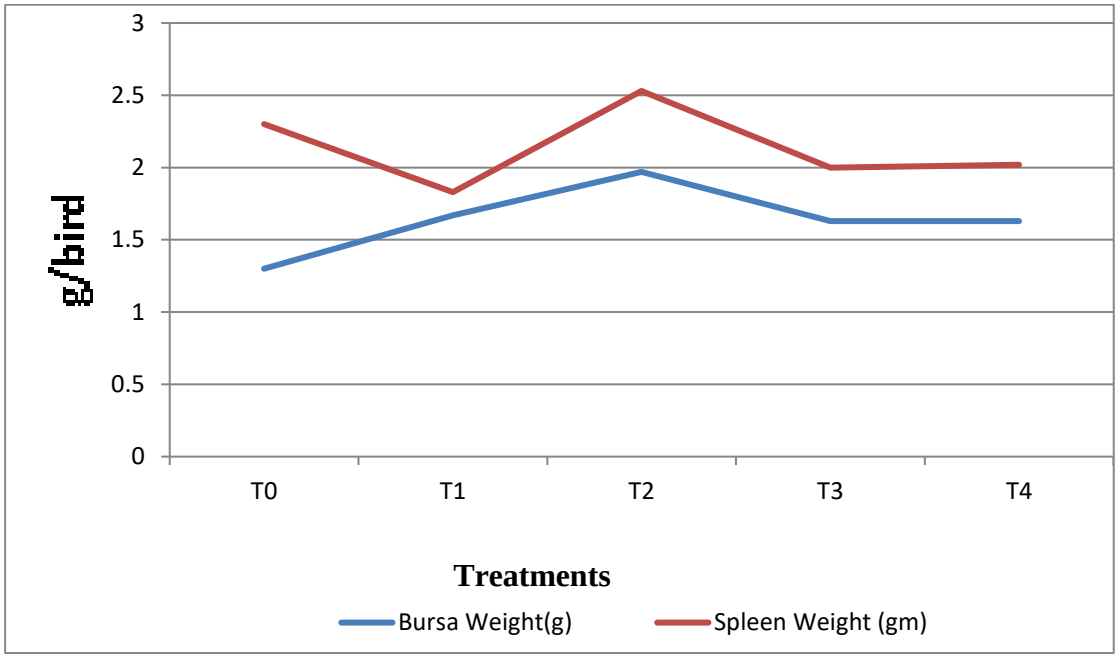

Figure 3:The Effect of supplementation NLP, MLP and JLP to broiler diets on some immune organs.

The comparative weight of spleen (g) of broiler chicks in the dietary group T_0, T_1, T_2, T_3 and T_4 were 2.30±.17, 1.83±.16, 2.53±.14, 2.00±.29 and 2.02±.28 respectively. The highest value was T_2 (2.53±.145) and lowest value was T_3 (2.00±.29). On the other hand, the relative weight of spleen of different groups showed that there were no significant (P>0.05) difference and the values were ranged from 1.83±0.17 to 2.53±.145. The weight of bursa was higher in T_2 group (1.97±.24) compared to the other group which values were T_0 (1.30±.17), T_1 (1.67±.16), T_3 (2.00±.29), and T_4 (2.02±.29) correspondingly. But these values are not significantly differing among the treatments (Figure-3). Ghazalah and Ali (2008) was showed that *Moringa oleifera* has Immune-stimulant activity.

Recent biological trials of certain herbal formulations in India as growth promoter have shown encouraging results and some of the reports have demonstrated

improvement with respect to weight gain, feed efficiency, lowered mortality, increased immunity and increased livability in poultry birds (Kumar, 1991). Muhammd *et al.* (2016) observed that Moringa oleifera leaf meal may replace dietary soya beans meal up to 15%, with optimum level of 5% in growing Japanese quails, its effect on growth performance, immune function, and ileum microflora in broilers was studied by Yang *et al.* (2007) and they found a significant enhancement of duodenum traits, and enhancements of the immune system in broilers were observed. In addition of Low dose of neem leaves powder have an inhibitory action on wide spectrum of microorganisms (Talwar *et al.*, 1997) and immuomodulator actions that induce cellular immune reaction (Devakumar and Sukett, 1993).

Summary and Conclusion

Summary

A study was conducted with broilers to investigate the effects of three herbal natural feed additives as alternative to an antibiotic growth promoter. The study was planned to determine the comparative efficacy of leaf powder of Neem (*Azadirachta indica*), Moringa (*Moringa oleifera*) and Jute (*Corchorus olitorious*), on the productive performance, haematology and health status of commercial broilers. A total of 200 day-old Cobb 500 broiler chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. Chicks were divided randomly into 5 experimental groups of 5 replications and each replication contains 10 chicks. These groups were allotted to five treatment designated as T_0, T_1, T_2, T_3 and T_4 Group. T_0 was offered basal feed without any supplementation and served as a control. Whereas, group T_1, T_2, T_3 and T_4 were offered basal feed supplemented with Neem leaf powder (NLP) 2%, Moringa leaf powder (MLP) 2%, Jute leaf powder (JLP) 2% and antibiotics respectively. The results showed that the weekly body weight in 4th week was significantly higher in 2% MLP treated group ($T_{2)}$ than control group ($T_{0)}$. Final live weight was significantly higher in 2% MLP (1664.30±6.29g) than control group. Weekly feed consumption (FC) was insignificant in different group but total FC significantly lower in 2% MLP than Antibiotic treated group. Weekly FCR was significantly lower in T_2 group than T_3, T_4, and T_0 group in 4th week. In case of final FCR significantly lower in T_2 than control, T_3 & T_4 group. Dressing percentage and survivability were non-significant ($P>0.05$) by the dietary inclusion of NLP, MLP and JLP as compared to control fed broilers. However, a linear increase in DP had found with the T_2 group. Survivability rate was lower in Control group than others. The relative weight of spleen and bursa of different groups showed that there was no significant ($P>0.05$) difference between the groups. In addition, the present study showed that feeding dietary NLP, MLP, JLP and antibiotics had no significant ($P>0.05$) effects on liver, gizzard and heart weight among the treatments except intestines which were significantly higher ($p<0.05$) in T_3 group compared with control and antibiotic. The results of glucose, Cholesterol and hemoglobin studies showed no significant ($P>0.05$) differences due to supplementation of dried NLP, MLP, JLP and antibiotics. But comparatively lowest cholesterol found in 2% NLP than control and antibiotic. Therefore, it could be concluded that though the NLP and JLP both have the positive feedback but 2% MLP

can significantly affect the productive performance and health status of broiler chicken.

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Appendix 1. Relative humidity (%) during experiment in September-October, 2018

Age in weeks		Relative humidity (%)				
	Period (day)	8 A.M	12A.M	4 P.M.	8 P.M.	Average
1st	19.09.18-25.09.18	85	82	73	74	78.67
2nd	26.09.18-02.10.18	85	83	71	72	77.83
3rd	03.10.18-09.10.18	86	85	74	75	80.67
4th	10.10.18-16.10.18	87	86	83	77	83.83

Appendix2. Recorded temperature (^{0}C) during experiment

Age in weeks	Room temperature (^{0}C)					
	Period	8 A.M	12A.M	4 P.M.	8 P.M.	Average
1st	19.09.18-25.09.18	28.9	29.5	31.6	31.5	30.08
2nd	26.09.18-02.10.18	28.3	28.5	32.1	31.6	29.87
3rd	03.10.18-09.10.18	27.0	27.2	28.8	27.2	27.00
4th	10.10.18-16.10.18	26.8	27.0	28.6	28.5	27.58

Appendix 3. Average Live weight, Eviscerated Weight and Dressing Percentage of different replication of broiler chicken under different treatment

Treatment	Replication	Live weight (g)	Eviscerated Weight(g)	Dressing Percentage (%)
T_0	R1	1641.8	1181.5	71.9637
	R2	1621.8	1148.6	70.82254
	R3	1631.8	1150.34	70.49516
	R4	1638.8	1138.5	69.47156
T_1	R1	1661.8	1203.4	72.41545
	R2	1681.8	1197.4	71.19753
	R3	1651.8	1165.5	70.55939
	R4	1661.8	1199.7	72.1928
T_2	R1	1621.8	1090.67	67.25059
	R2	1638.8	1114.56	68.01074
	R3	1621.8	1165	71.83376
		1651.8	1155.65	69.96307
T_3	R1	1631.8	1105	67.71663
	R2	1671.8	1200	71.77892
	R3	1655.8	1109.5	67.00688
	R4	1634.8	1143.6	69.95351
T_4	R1	1601.8	1080.7	67.46785
	R2	1611.8	1080.5	67.03685
	R3	1617.8	1100.5	68.02448
	T4	1611.8	1091.7	67.73173

Appendix 4. Weight of internal organs of broiler chicken under different treatment groups (g/bird).

Treatment	Replication	Liver weight	Splen weight	Heart weight	Intestine Weight	Gizzard Weight	Bursa Weight
T_0	R1	36.5	1.5	10	90	37.5	1
	R2	37	2	8.5	85	36	1.6
	R3	38.5	1.5	9	84	37	1.3
T_1	R1	37.5	1.5	9.5	95	38	2
	R2	40.5	2	10	89	38.5	1.5
	R3	39.6	2	10	86	40.2	1.5
T_2	R1	39.5	2.5	10	85	37	2.3
	R2	40.4	2.3	11	93	39.5	2.1
	R3	41.5	2.8	9.5	98	38.5	1.5
T_3	R1	36.5	1.5	9	96	37.5	1.7
	R2	38.5	2.5	9	85	38.5	2
	R3	40	2	11	93	37	1.2
T_4	R1	38.5	1.5	10.5	90.5	36.5	1.5
	R2	37.5	2	7.5	87	39	2
	R3	39	2.5	9	91	37	1.4

Appendix 5. Biochemical data in different treatment groups

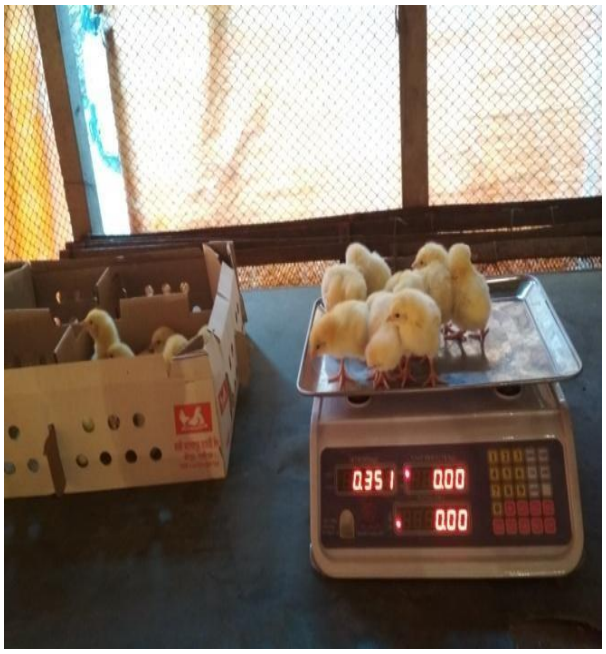
Treatment	Replication	Glucose	Cholesterol	Hemoglubin
T_0	R1(1)	347	137	13
	R1(2)	310	194	12.7
	R1(3)	301	167	13.4
	R3(1)	355	222	12.7
	R3(2)	235	204	13.4
	R3(3)	294	196	11.7
T_1	R1(1)	276	181	12.7
	R1(2)	296	185	15.6
	R1(3)	348	188	11.7
	R3(1)	369	174	12.1
	R3(2)	382	232	12.5
	R3(3)	442	117	11.5
T_2	R1(1)	500	196	12.3
	R1(2)	343	208	10.4
	R1(3)	244	198	13.7
	R3(1)	351	168	12.4
	R3(2)	373	156	13.2
	R3(3)	466	176	13.4
T_3	R1(1)	502	178	11
	R1(2)	361	225	12.1
	R1(3)	451	214	11.1
	R3(1)	364	187	11.7
	R3(2)	324	147	12.3
	R3(3)	356	168	14.9
T_4	R1(1)	335	184	12.6
	R1(2)	361	195	12.5
	R1(3)	515	187	12.9
	R3(1)	351	196	12.4
	R3(2)	359	187	12.2
	R3(3)	294	176	13.3

Appendix 6. Feed consumption (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments.

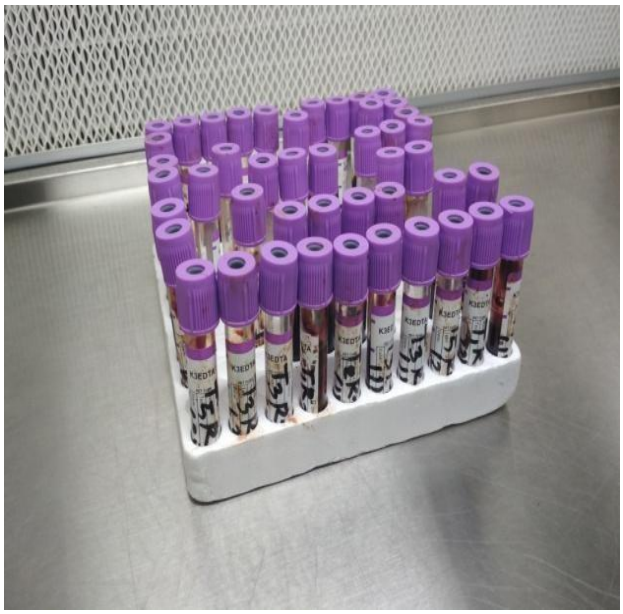
Treatment	Replication	1st week Feed Consumption/ Bird(g)	2nd week Feed Consumption /Bird(g)	3rd week Feed Consumption/ Bird(g)	4th week Feed Consumption/Bird (g)
T_0	R1	136	375.9	783.1	1015.9
	R2	139	415.3	758.7	998.5
	R3	138	415.4	761.6	1000
	R4	138.4	432.6	761.5	1002.5
T_1	R1	137.2	362	777	1028.8
	R2	138	399.7	742.3	996.9
	R3	138.5	418.1	746.4	977
	R4	138.5	418.1	735.5	1004.5
T_2	R1	134.5	392.1	752.4	982
	R2	139.5	400.7	751.5	999.7
	R3	138.5	418.5	742	992
	R4	139	421.7	749	1000.3
T_3	R1	139.3	430.2	745.5	1085
	R2	137	410	768	998.5
	R3	138.8	408.6	757	1088
	R4	138	406.3	768.7	1014.1
T_4	R1	137	413.5	769.5	1014
	R2	138.5	426.7	768.8	1010.5
	R3	139.5	432.2	765.3	999.5
	R4	139.5	423.5	771	1001

Appendix 7. Body weight (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments.

Treatment	Replication	1st week body weight/Bird(g)	2nd week body weight/Bird(g)	3rd week body weight/Bird(g)	4th week Body weight/Bird(g)
T_0	R1	187.9	521.8	940.7	1601.8
	R2	199.9	536.8	955.3	1611.8
	R3	199.9	535.8	958.3	1617.8
	R4	195.9	532.8	959.3	1611.8
T_1	R1	194.9	531.8	968.3	1641.8
	R2	193.9	526.8	948.3	1621.8
	R3	197.9	533.8	958.3	1631.8
	R4	190.9	532.8	958.3	1638.8
T_2	R1	197.9	537.8	1038.3	1761.8
	R2	195.9	541.8	1038.3	1761.8
	R3	200.9	541.8	1038.3	1771.8
	R4	191.9	536.8	1068.3	1761.8
T_3	R1	197.9	526.8	983.3	1621.8
	R2	199.9	535.8	1018.3	1638.8
	R3	198.9	536.8	1037.3	1621.8
	R4	196.9	533.8	1033.3	1651.8
T_4	R1	192.9	530.8	985.3	1631.8
	R2	199.9	537.8	993.3	1671.8
	R3	195.9	532.8	1023.3	1655.8
	R4	196.9	537.8	1031.3	1634.8



Appendix 8. Some photograph of NLP, MLP and JLP experiment conducted at SAU poultry farm



Appendix 8: Collection of blood at the age of 28 days of old and rapid kit test

Appendix 8. Medicine used during the experiment period