# DIETARY EFFECT OF NEEM LEAF MEAL ON PRODUCTION PERFORMANCE AND BLOOD CHOLESTEROL LEVEL OF BROILER

**A Thesis**
**By**

**SYEDA FAHAMIDA ROKSHANA**

Registration No. 1205102

Session: 2012-2013

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**MASTER OF SCIENCE (M.S.)**

**IN**

**POULTRY SCIENCE**


**DEPARTMENT OF DAIRY AND POULTRY SCIENCE**

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# CHAPTER I

## INTRODUCTION

Poultry is a substantial contributor to food supply of Bangladesh. Many small and medium farmers are rearing poultry (layer and broiler) in Bangladesh. Bangladesh is considered as one of the most appropriate countries in the world for poultry rearing. Development of poultry has generated considerable employment through the production and the marketing of poultry and poultry related products. Broiler meat is superior to other meat available for human consumption for its tenderness, palatability and better digestibility. For the improvement of national health status and the socio-economic condition of the people, broiler production plays an important role in our country. Unemployment and malnutrition are two major problems in our country. Poultry production can plays an important role to solve these problems in the shortest possible time. Poultry production has greatly increased during last one decade in Bangladesh. But, the scarcity and fluctuating quantity of the year-round feed supply and their price is a major constraint to poultry production in developing countries like Bangladesh. By the year, 2020, world population is estimated at 8 billion with most of the population growth coming from the developing countries (Singh *et al*., 2001). Due to rapid growth of the world population and shrinkage of cultivating land, demand for livestock product is increasing day by day. Future hopes of feeding the millions and safe guarding their food security will depend on better utilization of unconventional feed sources that do not compete with food for human beings. In view of this, poultry is aimed at finding alternative to this elusive feed ingredient.

Neem (*Azadirachta indica*) a large evergreen fast growing perennial tree, is native to Bangladesh and inhabitant of South East Asian countries. Neem is moderate size to large, 15-20 meter in height, usually evergreen tree with a fairly dense rounded crown; leaves are glabrous imparipinnate, leaflets, sub-opposite, alternate, exstipalate, and 22.5-37cm long on long slender petiole. Neem leaves have antihelmentics, antiviral, antibacterial properties (Bhowmik *et al*., 1993). The neem leaves have revealed so many different pains, fevers, infections and other complaints that have been called ‘village

pharmacy'. Neem products may also be tested as treatment for intestinal parasite. In general, neem leaf extracts may be used therapeutically to control respiratory problems, constipation and also as health promoter. Each part of the neem tree has some medicinal properties. Among different parts of neem tree, the processed leaves play the most important role in live stock health (Sharma and Reddy, 2002). Biswas *et al.*, (2002) have recently reviewed the biological activities of some neem compounds, pharmacological actions of the neem extracts, clinical study and plausible medicinal applications of neem along with their safety evaluation.

Cholesterol is a soft, waxy substance found among the lipids in the blood stream and in all body cell. It's an important part of a healthy body because it's used to form the cell membranes, some hormones and is needed for the functions. But a high level of cholesterol in the blood, hypercholesterolemia, is a major risk factor for coronary heart disease. People get cholesterol in two ways. The body, mainly the liver, produces varying amounts. Food also can contains cholesterol. Foods from animals contain it. Foods from plants don't contain cholesterol. Cholesterol is in all foods from animal sources, care must be taken to eat no more than six ounces of lean meat, fish and poultry per day and to use fat-free and low-fat dietary products. So, it's through study is essential before commercial use. Therefore, the present study was undertaken to fulfill the following objectives-

1) To investigate the effect of neem leaf meal on the performance of commercial broiler.
2) To determine the effect of neem leaf meal on blood cholesterol of broiler.

# CHAPTER II

# REVIEW OF LITERATURE

Neem (*Azadirachta indica*) is a tree in the mahogany family Meliaceae. It is native to India, Myanmar, Bangladesh, SriLanka, Malaysia and Pakistan. It grows in tropical an d semi-tropical regions. Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), rarely to 35–40 metres (115–131 ft). It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 15–20 metres (49–66 ft) in old, free-standing specimens. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. It is well documented that neem leaf meal (NLM) possesses some chemical compositions which enhance immunity in birds.

## 2.1 The History of neem (*Azadirachta indica)* and its chemical components

Neem leaves have wide spectrum of uses. The development of traditional medical system may be highly beneficial for farmers and also for the overall improvements of poultry industry in Bangladesh because neem leaves may exerts its beneficial effects effectively on the performance of bird at minimum expenses. The commercial use of neem was known from the Vedic period of India over 4000 years B.C. and the domestic uses have been mentioned by *kautilya* in his *Arthashastra* ($4^{th}$ Century B.C.). The varied uses of

Neem as a medicine have been documented in the *Atharva Veda,* the *Grihathyasutras* and also in the *Sutragranthas*. The Puranas cite it as a cure for Kushtaroga (leprosy). In fact, the Sanskrit name, Nimba is a derivative of the term Nimbati savasthyamdadati (to give good health). Its medicinal properties are described in the 'Puranas' and neem is commonly used in 'Ayurvedic' and 'Unani' medicine. The tree has relieved so many different parts, fevers, infections and other complaints that it is often reffered to as village pharmacy.

Agrawal (2002) reported that more than 135 components have been isolated from different parts of neem. The compounds have been divided into two major classes: Isoprenoids (like differpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type compounds and C-secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolic such as flavonoids and their glycosides, dihydrochalcone, coumarins and tannins, aliphatic compounds etc. The neem has been reported to contain several biologically active constituents such as azadirachtin (Naganishi, 1975) meliantriol (Lavie *et al.,* 1975), salanin (Warthen *et al.,* 1978), as well as nimbin and nimbidin (Shin-Foom. 1984). Garg and Bhakuni (1984) reported salanoid (a meliacin) as one of the bitter principles in neem seed oil. Furthermore, Raman and Santhanagopalan (1979) reported tignic acid (5-methyl-2butanoic acid) as part of the seed constituents. This compound is believed to be responsible for the distinctive odour of the neem oil. The limonoids are freely soluble in organic solvents such as hydrocarbons, alcohol and ketones, but are sparingly soluble in water (Aliero, 2003).

Siddiqui *et al.,* (2004) carried out an experiment where two new tetra cyclic triterpenoids, zafaral and meliacinomhydride have been isolated from the methanolic extract of neem leaves along with two known constituents, nimocinol and isomeldemin. Other than these, the major active components are the limonoids azadirachtin, 3-deacetyl-3-cinnamoylazadirachtin, I-tigloyl-3-acetyl-II-methoxyazadirachtin, nimbanal, 3-tigloyaza-dirachtol, 3-acetyl-salano-V, nimbidio-v, margocin, margocinin, margocilin and others. Terpenoids such as isoazadirolide, 6-nimbocinolide, nimbonone, nimbonolone, methylgrevillate, margosinone, margosinolone, nimosone, methyl nimbiol, methyln-imbionone, 14-acetyl-12methoxy-S, 11, 13-podacarpatrienne, sugiol, 12, 13-

dimethoxy-S,11,13-podacarpatriene-3,7-di-ion1 and gedunin have been isolated. The oil contain salannin, 1,3-diacetyl salannin, deacetyl salannin and salannol nimbidin, nimbidinin and nimbinin. Meliacin cinnamates 13 have isolated from root bark of the plant. Isonimolide, isolimbolide and isonimocinolide14 have been reported from twigs.

## 2.2 Biological activity of some neem compounds

Nimbidin, a major crude bitter principle extracted from the oil of seed karnels of A. indica demonstrated several biological activities. From this crude principle some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated. Anti-inflammatory; antiarthritic; antipyretic; hypoglycemic; antigastric ulcer; spermicial; antifungal; antibacterial; diuretic; antimalarial; antitoumor; immunomodulatory activities are also found by the application of neem due to such chemical compounds.

## 2.3 Medicinal properties of neem (*Azadirachta indica*)

### 2.3.1 Immuno-stimulant activity

The aqueous extract of leaf also possesses potent immunostimulant activity as evidenced by both humoral and cell-mediated responses (Sen, 1992). Intraperitoneal administration of neem leaf, bark and seed extracts revealed immune-stimulatory properties of neem which are responsible for their anti-HIV effect (http://www.neemfoundation. org.comp.html, 14/9/2014, 12pm). Patnaik (1993) reported that fresh neem leaves are notorious for their bitterness. But after cooking these leaves are helpful to gain immunity from malaria. The aqueous extract of neem leaves possesses anticomplement and immune-stimulant activity. Leaf extract at 100mg/kg after three weeks of oral administration causes higher IgM and IgG levels along with increases titer of antiovalbumin antibody (*Ray et al.,* 1996).

### 2.3.2 Hypoglycaemic activity

Aqueous extract of neem leaves significantly decrease blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia. Recently, hypoglycaemic effect

was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits.

### 2.3.3 Antiulcer effect

Neem leaf aqueous extracts produced highly potent antiacids scretory antiulcer activity. Chaturvedi (1995) reported that peptic ulcers and duodenal ulcers were treated well with neem leaf extracts. Nimbidin from seed extracts taken orally prevented duodenal lesions and peptic ulcers and provided significant reductions in acid output and gastric fluid activity. He also found that low doses of 20 to 40 mg/kg brought that most relief. Increased dosages reduced the effectiveness of neem's antiulcerative effects. Chattopadhyay *et al.,* (2004) studied the mechanism of the antiulcer effect of neem leaf aqueous extract to stock gastric lesions in rat with emphasis on acid secretion, oxidative damage and apoptosis. The extract dose-dependently inhabits gastric lesions induced by restraint-cold stress, indomethacin and ethanol. In stress ulcer model, it is more effective than ranitidine but less effective than omeprazole. The extract also prevents OH-mediated mucosal DNA damage in vitro by scavenging the of neem leaf extract.

### 2.3.4 Antifertility effect

Intra-vaginal application of neem extract, prior to coitus, can prevent pregnancy. It could be a novel method of contraception. Parshad *et al.,* (1996) studied the antifertility efficacy of both aqueous and steroidal extracts of neem (*Azadirachta indica A. Juss*) leaves in male wistar rats. Intraperitoneal injections of the steroidal extracts at a dose of 100mg/kg body weight, twice a week for 10 weeks resulted in impaired spermiogenesis, increased the number of headless spermatozoa and significantly decreased ($p<0.01$) motility of cauda spermatozoa, leading to a decline in the fertility index. Feeding of a 0.8 (w/v) aqueous neem leaf extracts in drinking water for 7 weeks decreased serum testosterone ($p<0.01$) but no effect was observed in the fertility index. Gowda *et al.*, (1998) reported that the fertility and hatchability were adversely affected by the higher inclusion rates (150 g/kg and 200g/kg) of neem kernel meal (NKM).

### 2.3.5 Antimalarial activity

Joshi *et al.*, (1998) conducted an experiment to investigate the antimalarial activity of neem (*Azadirachta indica).* From the ethanol extract of fresh neem leaves, 4 limonoids (meldenin, isomeldenin, nimocinol and nimbandiol) were isolated and these limonoids showed antimalarial activity against choloroquine-resistant Plasmodium falciparum strain KI. It has also been sated that neem leaf extracts are effective against both chloroquine-resistant and sensitive of malarial parasites. (http://www.neemfoundation.org.html). Udeinya *et al.*, (2004) carried out an experiment where an acetone-water neem leaf extract with anti-malarial activity was evaluated in 10 patients with HIV/AIDS at 1000 mg daily for 30 days. The mean binding of infected erythrocytes and cancer cells per endothelial cell was 15 and 11 respectively, in the absence of the extract, 0 and 2 respectively, with the extract. In the absence and presence of the extract, 0% and 75% respectively, of lymphocytes were protected. They also found that the extract showed antiretroviral activity with a mechanism of action that may involve inhibition of cytoadhesion.

The antimalarial activities of two fractions (IRDN-A and IRDB-B) of an extract from the leaves of neem tree (*Azadirachta indica)* were compared with those of chloroquine, in in-vitro assays against Plasmodium falciparum. The sexual stages of a Chloroquine-sensitive clone (ITG2F6) and a chloroquine-resistant isolate (W2) and the gametocytes of the NF 54 (BD-7) isolate of P. falciparum were used as the drug targets. If they are found safe and effective in vivo, the neem-leaf fractions may from the basis of new antimalarial drugs that not only cure chloroquine-sensitive and chloroquine-resistance malaria but also markedly reduce transmission (Udeinya *et al.*, 2006)

### 2.3.6 Antifungal activity

Neem oil has been reported to be against certain human fungi, which are even difficult to control, by modern synthetic fungicides. These include some Trichophyton, Epidermophyton, Microsporum, Trichosporon, Geotricum and Candida (http://www.neemfoundation.org.comp.html, 12/9/2014, 1pm). Using the groundnut rust disease (causal agent *Puccinia arachidis* Speg.) as the bioassay system, two limonoids from the neem tree (*Azadirachta indica* A.Juss.) which evinced antifungal activity, were isolated through extraction, solvent fractionation and HPLC. A polar extract derived through solvent partitioning reduced the disease intensity considerably. The polar effect and the

impure HPLC fractions were more effective than the pure compounds in reducing the pustule numbers and, consequently, the disease severity (Suresh, 1997). Suresh *et al.*, (2004) conducted an experiment where active extracts of terpenoids (neem compound) were identified by GC-MS, and their interactions in mixtures were studied. They obtain that identification of the mode of action and target sites in fungi, and the ability to induced systemic acquired resistance in host pathogen systems will enable the potential of neem to control plant pathogens to be maximized.

### 2.3.7 Anti-bacterial activity

In toothpaste, it helps to relieve swollen and bleeding gums and kills the bacteria that cause gingivitis. Neem powder can be used in a foot bath to kill fungus and bacteria (http://www.essentialwholesale.com/product/1317/neem-oil-certified-organic, 14/9/2014, 12pm). Neem stick extract can reduce the ability of some streptococci to colonize in tooth surfaces (Wolinsky *et al.*, 1996). Neem oil inhibit the growth of all the three strains of mycobacterium at a concentration of 12.5 mg/ml (http://neemfoundation.org.html, 12/9/2014, 4pm). Among all the bacteria tested, *A. hydrophila*, *P. fluorescent* and *Myxobacteria spp*. exhibit maximum sensitivity to Aquaneem in terms of percentage reduction of bacterial cell population in comparison to E. coli (Das, 1999). Oil from neem leaves found to possess a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *Mycoplasma tuberculosis* and streptomycin resistant strains. Antibacterial effect neem leaf extracts have been demonstrated against *Streptococcus mutants* and *Streptococcus faecalis* (Almas *et al.*, 1999). *In vitro*. It inhibits *Vibrio cholera*, *Klebsiella pneumonia*, *Mycolpasma pyogenes*. The antibacterial effect of Neem mouthwash against the salivary levels of streptococcus mutans and lactobacillus has been tested over a period of two months. Also its effect in reversing incipient carious lesions was assessed. While streptococcus mutans was inhibited by neem mouthwashes, with or without alcohol as well as chlorhexidine, lactobacillus growth was inhibited by chlorhexidine alone (Vanka *et al.*, 2001).

### 2.3.8 Antiviral activity

A 10% aqueous extract of tender leaves of neem has been found to possess anti-viral properties (http://neemfoundation.org./comp.html, 14/9/2014, 12pm). Tanzubil *et al.*, (1987) carried out a number of experiments to evaluate the effectiveness of several

locally available materials for the protection of stored cowpeas (*Vigna unguiculata*) from the bruchid *callosobruchus maculatus*. Experiment with smallpox, chicken pox and fowl pox showed that although neem did not cure these diseases, but it is effective for the purpose of prevention. Crude neem did not extracts absorbed the viruses, effectively preventing them from entering unaffected cells (Patnaik, 1993). Presence of a battery of compounds besides flavonoids, triterpenoids and their glycosides in NCL-11 (methanolic extract fraction of leaves of neem, (*Azadirachta indica A. Juss*) have antiviral for coxsackie B group viruses 'in vitro.' The minimal inhibitory concentrations were non toxic to Vero (African green monkey) cells; subtoxic concentration was 8,000 micrograms/ml and cytotoxic concentration 10,000 micrograms/ml, which was confirmed by trypan blue dye exclusion test. (Badam *et al.*, 1999)

Neem leaf extract reported to possess antiviral properties against fowl pox and Newcastle virus. (http://www.positivehealth.com/article/acne/neem-an-ancient-cure-for-a-modern-world, 14/9/2014, 1pm). Khan *et al.*, (2000) carried out an experiment where crushed neem seeds and leaves (3g/plot) were applied to the top soil layer 7days after germination. After 15 days, they found that viruliferous *Bemisia tabaci* were released on each cotton plant. The in vivo protection studies with neem leaves extracts at its maximum non-toxic concentrations 120-30 mg/ml resulted in hibition of the virus replication as confirmed by the absence of Dangue related clinical symptoms in suckling mice and absence of virus specific 511 hp amplicon in RT-PCR. The pure neem i.e. Azadirachtin did not reveal any inhibition on Dengue virus type-2 replication in both in vitro and in vivo system (Parida *et al.*, 2002).

### 2.3.9 Anti cancer activity

Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7, 12-dimethylbenz [a] anthracene (DMBA), as revealed by reduced incidence of neoplasm. Chemopreventive response was measured by the average number of papillomas per mouse, as well as percentage of tumor-hearing animals. There was a significant inhibition of tumor burden, in the both the tumor model system studied (from $P<0.005$ to $P<0.001$). Tumor incidence was also reduced by both doses of *Azadirachta indica* extract (Dasgupta *et al.*, 2004).

Vaccination of mice with B16MeIsAg+NLP more efficiently prevented the growth of N16 melanoma tumor than mice immunized with B16MeISAg or NLP alone. Neem leaf preparation (NLP) might be potential immune adjuvant for including active immunity towards tumor antigens (Baral *et al.*, 2005). The chemopreventive effects of ethanolic neem extracts in the initiation and post-initiation phases of 7,12-dimethylbenz [a] anthracene (DMBA)- induced hamster buccal pouch (HBP) carcinogenesis was evaluated. The results of the study demonstrate that ethanolic neem leaf extract inhibits the development of DMBA-induced HBP tumors by protecting against oxidative stress (Subapriya *et al.*, 2005). An immune serum generated in Swiss-mice against an aqueous preparation from neem leaf was reactive with carcinoembryonic antigen (CEA) and peptic sequence derived from it. Unique property of neem may be utilized for the immunotherapy of CEA positive tumors (Sarker, 2007)

### 2.3.10 Cardiovascular activity

Administration of the leaf extract of Azadirachta indica was found to reduce a potent and dose-dependent hypotension in rabbits (5-200mg/kg, IP) and guinea pigs (5-40 mg/kg). The extract also exhibited antiarrhythmic activity (40mg/kg IV) against ouabain-induced dysrhythmia in rabbits. The mechanism of action may be due to an increase coronary blood flow in isolated rabbit heart and effect on vascular smooth muscle, giving raise to vesodilation and also effect were not blocked by atropine and mepyramine (Khosla *et al.*, 2002).

### 2.3.11 Effect of nervous system

According to Ayurveda, neem leaves help in the treatment of vatik disorders (neuro muscular pain) (http://www.neemfoundation.org/comp.html, 14/9/2014, 1pm).

## 2.4 Medicinal poperties of neem leaves in human

Neem and its compounds, its extracts and finished product have been known to cure a large number of human aliments. Right from the skin diseases to diabetes, from the cholesterol to hair problem, from the ulcer to dental problems, the way in which it affects our lives cannot be underestimated (http:neem-products.com/neem-benefits.html, 14/9/2014, 1pm). Alam (1989) concluded that neem leaves were effective in treating and

preventing diabetes. Chattopadhay *et al.,* (1992) reported that neem leaf extracts reduced in human blood cholesterol significantly. They also reported that alcoholic extract of neem leaves reduce serum cholesterol by about 30% beginning two hours after administration and keep the level low for an additional four hours until the test ended. Upadhay *et al.,* (1993) reported that the neen leaf extracts and neem bark extracts significantly reduced the P-24 viral proteins and induced in vitro production of IL-I infection. Caldwell (1994) observed that neems have the ability to enhance the cell-mediated immune responses. He also reported that neem could be used as a vaginal lubricant before intercourse to protect the disease due to vaginal concentration. Chattopadhay *et al.,* (1994) reported that the use of small amount of neem leaf extracts might protect the liver from damage when toxic agents were used to induce hepatocellular necrosis. Udeinya (1994) reported that neem leaves might be used to prevent adhesion of concern cell to other cells in the body. Viral hepatitis is deadly disease with effective remedy. Indian tests indicate that as much as 80% of the test cases showed significant improvement when treated with neem leaf (Wagh, 1998). As therapeutic agent, neem is one of the most popular tress in traditional medicinal systems and is increasingly becoming important in herbal alternative therapy. The tree itself considered a "village pharmacy" because of the well established fact that every part of the tree has an application in curing human diseases. The tree has been a constant source of novel and structurally unique phytochemicals that can constitute the basis for the development of novel pharmaco-therapeutic agents against various diseases (Singh, 2002). An ethanolic extract of neem has been shown to cause cell death of prostate cancer cells (PC-3) by including apoptosis as evidenced by a dose-dependent increase in DNA fragmentation and a decrease in cell viability. Western blot studies indicated that treatment with neem extract showed decreased level of Bcl-2, which is anti-apoptotic protein and increased the level of Bax protein. So the neem extract could be potentially effective against prostate cancer treatment (Kumar *et al.,* 2006). Nimbolide, a triterpenoid extracted from the flowers of the neem tree (*Azadirachta indica*), was found to have antiproliferative activity against some cancer cells lines (Roy *et al.,* 2007).

## 2.5 Medicinal properties of neem leaves in livestock

It might be increased to reader to know how an outbreak of food and mouth disease is handled in the Indian countryside. First of all, animal is isolated and provide bedding.

Then it is gradually made to walk on hot and sand to help in healing of foot ulcers. Use of coal-tar, kerosene, turmeric and extract of leaves of neem or custard apple is made on the foot ulcer to promote healing and prevent maggot infestation. A special diet of green gram along with chopped onion is fed to the ailing animal. While this does not result in an overnight cure, it helps the healing process and the animal eventually recovers. How much more human and preferable these practices are compared to the killing and destruction of thousands of animals struck by foot and mouth disease in UK and Europe (www.neemhealth@neemtree_info.html, 14/9/2014, 1pm). It is found that neem oil is now being applied with pig lard in cattle sheds as a disinfectant and as an insect repellant and for the treatment of maggot wound. Feeding of ghee with pepper together with betel leaves in fever conditions is found very useful also. These inexpensive remedies for animal health, practiced down the ages in rural India, were recently closely observed by scientists of the Indian Council of Agricultural research. After a careful study it was concluded that by and large most of these practices were effective.

According to neem foundation (http://www.infinityfoundation.com/mandala/t_es/t_es_agraw_neem.htm, 11/9/2014, 4pm), almost every part of the tree is bitter and finds application in indigenous medicine. Records exist that Neem has been used in alarge number of ailments in animal ranging from systemic disorders to infections and injuries. Pet owners, especially dog and cat owners are known to wash their pets in neem leaf extracts mixed in water. This is effective against fleas and other insects that the pets might have. It also heals concealed cuts on the pet's skin. The neem leaf extract has also been used in other pet products (http://arpitaagro.in/neem.html, 14/9/2014, 1pm).

In developing countries, getting clean, toxic free water is problem. This can lead to calf mortality and morbidity in large animals as well as poultry, resulting in huge economic losses. Researchers in India used neem leaf powder in case of cow calves @ 20g/ day for anorexia, fatigue, anaemia and poor weight gain in animal suffering from hepatotxicity. It was observed that feeding neem leaf powder improve appetite, liver function and general health of the calves (http://www.naturecures.co.uk/herbcureslist.htm, 14/9/2014, 1pm).

According to a study in Mumbai, India, neem oil has been used in the Indian countryside to clean the uterine tract in metritis and endometritis for a long time. In some cases due to bad husbandry practices and poor sanitation in stables, sub-clinical metritis is

prevalent. While conducting AI (Artificial insemination), it was noticed that inspite of repeated cows or buffalos sometimes do not conceive due to subclinical metritis that has remain undetected. Regular intra uterine use of neem oil @30ml for 3 days has proved very useful (http://www.equinature.com/Portals/0/Documents/NeemAnimalHlth.pdf, 14/9/2014, 1pm).

The sheep treated with azadirachtin and pyrethrum had significantly fewer lice than either the control or soap treated sheep over the 48 days of the trial. Neither azadirachtin nor pyrethrums were significantly less effective than cypermethrin (health *et al.*, 1995). Neem seed kernel cake (NSKC), a protein rich (34-40% CP) by-product of the oil industry in developing countries; after water washing can be a suitable vegetable protein substitute in diets of goats for profitable chevon production in developing countries, for mitigating chronic shortage of oil cakes for livestock feeding (Verma *et al.*, 1996). Farries (1993) found that neem oil dilatation of 0.1% had the greatest mortality with least eggs laying of cattle ticks. The dilutions of 0.1% had least effect on the viability of the eggs laid and had greatest hatchability, while the dilutions of 0.8% and 0.1% had the greatest effect, with 60-75% of the eggs non-viable.

Gowda *et al.*, (1998) assessed the effect of neem kernel meal (NKM) and mustard meal supplemented diets on carcass traits and meat quality of broiler rabbits. They observed that feeding processed neem kernel meal failed to alter the chemical composition of meat and they also found that sensory evaluation of pressure-cooked meat had scores similar appearance , odor test, texture, tenderness and juiciness. Pietrosemoli (1999) reported that oral administration of neem (*Azadirachta indica*) leaves has an antiparasitic effect on grazing cattle. The authors found that the addition of neem leaves to the nutritional blocks reduced the number of parasites egg per gram of facees of grazing cattle. Kukde *et al.*, (1999) conducted an experiment involving 12 calves to investigate the effects of neem leaves powder. The author found significant differences in animals growth and increase in feed intake. Bais *et al.*, (2002) found that both sares and neem leaves can be fed to goats as sole roughage, preferably the sares leaves during the lean period. Brelin (2002) studied effect of fresh neem leaves on sheep. He reported that fresh neem leaves significantly reduced the number of *Haemonchus contortus* in the abomasum of the treated sheep. Arunachal *et al.*, (2002) conducted an experiment with 72 lambs naturally infected with gastrointestinal helminthes. They observed that antihelmintic efficacy of

neen extracts and proziplus were 53. and 87 percent respectively. The antihelmintic effect of neem (*Azadirachta indica*) on nematode parasites of sheep was showed that feeding Neem had an effect on worm numbers in sheep, but was not reflected in their faecal egg counts (Chandrawathani *et al*., 2006). Fresh neem leaves are regularly fed to camel and goats in India without any untoward effect.

## 2.6 Medicinal properties of neem leaves in poultry

Chicken farmers in Brazil have been known to use 5drops of the neem leaf extract per liter of the chicken's drinking water. Farmers believe that now they don't have to use antibiotics as the neem leaf extract works just as well (http://www.killzrx.com/neem-leaf-extract.pdf, 14/9/2014, 2pm).

In an experiment to evaluate growth and nutrient efficacy of broiler chicks from day 3 to 42, chicks were fed on diet containing alkali treated neem kernel cake as protein supplement, in the place of peanut meal. The trial did not show any qualitative and quantitative in the meat between treated and untreated birds and weight gain was similar both groups. The use of alkali treated neem cake to spare the peanut meal in broiler diets was recommended.

Adekanye and Sonaiya (1992) carried out a feeding trial to test the responses layers to three dietary treatments ($T_1$ –without neem leaves, $T_2$ fresh neem leaves and $T_3$-10% dried neem leaves). They found that layers receiving 10% dried neem leaves had higher feed intake and daily egg production as well as egg weight than those on the other two diets. Injections of NIM-76 resulted in an increase in polymorphonuclear (PMN) leukocytes eith a concomitant decrease in lymphocytes counts. The immunomodulatory activity of NIM-76 was found to be concentration-dependent. At 120 mg/kg body weight, there was an enhanced macrophage activity and lymphocyte proliferation response, while the humoral component of immunity was unaffected. At higher concentrations of NIM-76 (300mg/kg body weight), there was a stimulation of mitogen-induced lymphocyte proliferation, while macrophage activity remained unaffected (SaiRam *et al*., 1997).

Sadeker *et al*., (1998) found that feeding neem leaves to immune-suppressed birds increase their humoral and cell mediated immune responses. They suggested that neem

leaves may be useful for treatment of Immuno-suppressive disease, such as Infectious Bursal Disease (IBD) and Newcastle Disease (ND) in broilers. Nagalakshmi *et al.*, (1998) investigated the effect of alkali treated and urea ammoniated neem seed kernel-cake on the performance of broiler chicks. They found that incorporation of processed neem seed kernel-cake in the diets of broilers did not depress the feed intake and growth rate of broiler chicks.

Gowda *et al.*, (1998) conducted an experiment with the incorporation of neem kernel meal (NKM) into a standard layer diet at 0, 100, 150 and 200 g/kg, replacing part of the soyabean meal and de-oiled rice barn. Result indicated significantly lower food intakes ($P<0.01$), rates of egg production and egg weights in birds fed on the diet with NKM at 150 and 200 g/kg. Fertility and hatchability were also adversely affected by the higher inclusion rates of NKM. Neem fruit 150gm/50kg feed had excellent performance in terms of oocyst count and lower mortality (Murtaza *et al.*, 2002). Sridhar *et al*., (2003) conducted and experiment in which neem leaf extracts (NLE) treated birds showed mild depression and lesser body weight gain after 4 weeks and onwards. Haematological observations revealed lower values of haemogram (hb, PVC and TEC) in NLE treated chicks 4 to 6 weeks post treatment. However, there were no significant changes in Total Leucocytes (TLC) and Direct Leukocyte Count (DLC) in treated birds. Chowdhury (2004) found that in broiler chickens NLM at the highest level (40 g/kg) with or without supplementation of protexin, a probiotic, significantly decreased live weight and increased FCR value. They also reported that live weight and FCR did not differ significantly when NLM were used up to 20g/kg level.

The neem aqueous extracts is well tolerated by the broilers and there is no physiopathological alteration in protein turnover in the liver of the neem extract fed birds (Meenakshi, 2005). Esonu *et al*., (2006) conducted an experiment to evaluate the effect of neem (*Azadirachta indica*) on the productive performance of laying hens. They found that neem leaf meal (NLM) did not show any appreciable difference in weight gain between the birds at 0% and those at 5%, 10% dietary levels. Carcass weight, dressed weight, liver, heart and gizzard weight were significantly ($P<0.05$) increased at 5% dietary level of NLM. Toxicity to cocked chicks of raw full-fat neem seed kernel was tested. The RFK depressed ($P<0.05$) appetite included hemorrhagic anemia ($P<0.01$) and leucocytosis ($P<0.05$) at 150g or more RFK/kg diet. Lesions associated with RFK

included emaciation and paleness of carcasses, kidney congestion and mucous enteritis. Microscopically, there was coagulative necrosis of mucosal cells involving the sub mucosal glands and sloughing of intestinal villi, focal hepatocytic drop-out and hemorrhagic and edematous nephritis. The overall effects was a highly significant ($P<0.01$) mortality of cockerels, which was dose-dependant; and all birds fed on 300 g RFK/kg diet died before day 35 of feeding (Otor John Uko, 2006)

## 2.7 Medicinal properties of neem leaves in rat

Arivazhagan *et al*., (2000) found that the administration of garlic and neem leaf extracts significantly lowered lipid per oxidation and enhanced the hepatic levels of glutathione and glutathione dependent enzymes. Pari *et al.*, (2001) reported the antihyperglycemic effect of a herbal formulation composed of the aqueous extract of the three medicinal plants. *Azadirachta indica, Cassia Auriculata, Mondica charantia* in rats with alloxan induced diabetes. They also reported that diamed also prevented a decrease in body weight. Jayakumar *et al*., (2002) conducted an experiment to determine the possible anti-orexigenic effect of neem leaf extract (NLE) in rats by giving 500mg/kg NLE orally via stomach tube. Result showed that NLE at a dose of 500 mg/kg body weights did not affect the feed intake and live weight of rats. Baral and Chattopadhyay (2004) reported that the conditional tumor growth retardation, observed in mice treated with Neem Leaf Preparation (NLP) before tumour inoculation, may be regulated by NLP mediated immune response activation, having prominent role in the cellular immune function of the tumor host.

*Azadirachta indica A. Juss.* (AI) leaf extracts exerts equipments cardioprotective activity in the experimental model of isoprenalin induced myocardial necrosis in rats as compared to vitamin E, a known cardioprotective antioxidant (Peer *et al.*, 2007). An immune serum generated in Swiss mice against an aqueous preparation from neem leaf was reactive with carcinoembryonic antigen (CEA) and a peptide sequence derived from it. Using ELISA, have demonstrated that CEA reactive antibody titer (chiefly IgG2a) was significantly decreased after absorption of the immune sera with CEA. Neem leaf preparation (NLP) generated immune sera was also reactive with CEA in immunoblotting and CEA reactive component in the NLP sera can be immunoprecipitated. Identical recognition of CEA expressed on human cholesterol

cancer specimens, by anti-CEA monoclonal antibody and NLP sera was documented by immunohistochemistry (Sarker, 2007).

## 2.8 Effect of medicinal properties of neem leaves on rabbit

Hypoglycemic effect was observed with *Azadirachta indica* when given as a leaf extracts and seed oil, in normal as well as diabetic rabbits. The effect, however, was more pronounced in diabetic animals in which administration for 4 weeks after alloxan induced diabetes, significantly reduced blood glucose levels. Data suggest that *A. indica* could be of benefit in diabetes mellitus in controlling the blood sugar or may also be helpful in preventing or delaying the onset of the disease (Khosla *et al*., 2002). They studied the hypoglycemic effects of neem (*Azadirachta indica*) leaf extracts and seed oil in normal rabbits as well as in diabetic rabbit. They suggest that *Azadirachta indica* could be beneficial in diabetes mellitus in controlling blood sugar or might be helpful in preventing or delaying onset of the diseases.

## 2.9 Chemical composition of neem leaves

The comparison of neem leaves as reported by various authors (Table 2.1) indicates that variations in CP, CF, EE and NFE concentration of neem leaves ranges from 16-20,12-26, 2.2-6.5 and 50-56.4 respectively. The greatest variation was for CF which probably resulted from variation in the age of plants.

**Table 2.1 Shows the chemical composition of neem leaves as reported by various workers (DM basis)**

| Chemical components (%) | Vietmeyer (1992) | Chaudhary and singh (1999) | Sonaiya & Olori (1989) | Bais *et al.*, (2002) | Esonu *et al.*, (2006) | Laboni (2007) |
|---|---|---|---|---|---|---|
| Dry matter | 40.60 | 30.10 | – | 45.00 | 92.42 | – |
| Crude protein | 17.48 | 15.96 | 17.50 | 18.67 | 20.68 | 18.21 |
| Crude fiber | 15.27 | 15.35 | 12.30 | 24.57 | 16.60 | 26.31 |
| Ether extract | 2.46 | 3.18 | 4.20 | 2.420 | 4.13 | 6.54 |
| Nitrogen free extract | 56.40 | 49.63 | – | – | 43.91 | 39.11 |

## 2.10 Research gap and present study

Neem leaf extract (NLM) possesses some chemical components which enhance the immune-stimulas in birds as well as in human. So, use of NLM through poultry feed may be relevant to organic farming and provide safe food for human being by discouraging the use of different types of antibiotics and drug. In Bangladesh, a few research works have been carried out on poultry to observe the effect of NLM. Also a good number of experiments on large animal were carried out to test its effect on parasitic infestation. Experiment on the effects of NLM on cholesterol metabolism of laying chickens has also carried out. It may be mentioned here that good number of research works with neem compounds has been conducted in abroad. So, it seemed worthwhile to investigate whether or not neem leaf meal showed better effects on the performance of broiler. This research was aimed to investigate the effect of neem leaf meal on the performance of broiler and cholesterol metabolism in broiler.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 Location of research work

The experiment was conducted to study the effect of neem leaf meal in the diets of broiler chicks and its effects on cholesterol metabolism from 28$^{th}$ August to 5$^{th}$ October 2013, at poultry shed in Hajee Mohammod Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

## 3.2 Collection, processing and storage of neem leaf meal (NLM)

The fresh neem leaves were collected from neem trees which are grown at HSTU campus, Dinajpur. The green leaves were cleaned and sun-dried on a hygienic polyethylene paper. The leaves were ground properly by a grinder machine. The ground neem leaf meal (NLM) packed in polyethylene bags and preserved properly. Proper care was taken to avoid spoilage of NLM.

## 3.3 Preparation of the experimental house and equipment

Open sided houses have been used for rearing the experimental birds. Each experimental room was divided into 12 separate pens of equal size by using wire net and bamboo materials. The experimental room (ceiling, wall, floor and wire net) was probably brushed with the help of a broom and then washed and cleaned by forced water using a hosepipe. Then the rooms were disinfected by bleaching powder solution and the rooms were left vacant for 15 days. The rooms were again disinfected by Fam 30 and kept free to dry up properly. At the same time, all feeders, plastic buckets, waterers and other equipments should be cleaned properly and disinfected with bleaching powder solution, as well as drying and left them empty for one week before the arrival of the chicks. Ceiling, walls and wire nets were also thoroughly disinfected by spraying Virkon-S solution@3m/l water.

## 3.4 Collection of birds

A total 120 experimental day old broiler chicks (Cob 500) were used for this experiment purchased from CP Bangladesh. During purchasing all chicks were examined for any kind of abnormalities and uniform size.

## 3.5 Experimental design

All diets were formulated manually to meet the nutrient requirements of broiler (NRC, 1994). The chicks were fed starter diet from 1 to 17 days, finisher diet from 18 to 35 days. In the experiment, total 120 birds were allocated to four treatment groups with three replicates per treatment groups. There were 30 birds per treatment group and 10 birds per replication. The experimental diets were designed as $T_o$: Control, $T_2$: 4% NLM, $T_1$: 2% NLM and $T_3$: 6% NLM. Layout of the experiment is shown in Table 3.1.

**Table 3.1 Layout of the experiment showing the distribution of DOC to the treatment group and replication.**

| Dietary treatment group | No. of broilers/replications | | Total no. of broilers per treatments |
|---|---|---|---|
| $T_o$ (control) | $R_1$ | 10 | 30 |
| | $R_2$ | 10 | |
| | $R_3$ | 10 | |
| $T_1$ (20gm NLM/kg feed) | $R_1$ | 10 | 30 |
| | $R_2$ | 10 | |
| | $R_3$ | 10 | |
| $T_2$ (40gm NLM/kg feed) | $R_1$ | 10 | 30 |
| | $R_2$ | 10 | |
| | $R_3$ | 10 | |
| $T_3$ (60gm NLM/kg feed) | $R_1$ | 10 | 30 |
| | $R_2$ | 10 | |
| | $R_3$ | 10 | |
| Grand total = | | | 120 |

**Table 3.2 Feed ingredient and chemical composition of the experimental broiler starter diets**

| Item | Dietary level of Neem leaf meal (NLM) | | | |
|---|---|---|---|---|
| Feed ingredient (gm/kg feed) | $T_0$ | $T_1$ (20gm NLM/kg feed) | $T_2$ (40gm NLM/ kg feed) | $T_3$ (60gm NLM/kg feed) |
| Maize | 0.535 | 0.540 | 0.53 | 0.515 |
| Rice polish | 0.10 | 0.085 | 0.08 | 0.08 |
| Soybean meal | 0.23 | 0.21 | 0.20 | 0.19 |
| Jeso-Prot | 0.10 | 0.10 | 0.10 | 0.10 |
| Soybean oil | 0.015 | 0.025 | 0.03 | 0.035 |
| NLM | 0.00 | 0.02 | 0.04 | 0.06 |
| Common salt | 0.005 | 0.005 | 0.005 | 0.005 |
| Oyester shell | 0.0075 | 0.0075 | 0.0075 | 0.0075 |
| DCP | 0.0050 | 0.0050 | 0.0050 | 0.0050 |
| Vitamin mineral premix | 0.0025 | 0.0025 | 0.0025 | 0.0025 |
| Methionine | 0.0025 | 0.0025 | 0.0025 | 0.0025 |
| Lysine | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Chemical composition | | | | |
| ME (%) | 2989.45 | 3004.4 | 2982.88 | 2960.70 |
| CP (%) | 2.2 | 2.1 | 2.1 | 2.1 |
| CF (%) | 0.3695 | 0.378 | 0.396 | 0.40 |
| Ca (%) | 0.11 | 0.18 | 0.12 | 0.13 |
| P (%) | 0.056 | 0.062 | 0.064 | 0.068 |
| Methionine (%) | 0.048 | 0.048 | 0.048 | 0.048 |
| Lysine (%) | 0.118 | 0.118 | 0.118 | 0.118 |

****** Vitamin Mineral Premix provided following per kg diet: Vit. A 5000 IU, $D_3$ 1000 IU, K 1.6 mg, B1 1mg, B2 2mg, B3 16mb, B6 1.6mg, B9 320µg, B12 4.8 µg, H 40mg, Cu 4mg, Mn 40mg, Zn 20mg, Fe 2.4mg, I 160 µg.

**Table 3.3 Feed ingredient and chemical composition of the experimental broiler finisher diets**

| Item | Dietary level of Neem leaf meal (NLM) | | | |
|---|---|---|---|---|
| Feed ingredient (gm/kg feed) | $T_0$ | $T_1$ (20gm NLM/kg feed) | $T_2$ (40gm NLM/kg feed) | $T_3$ (60gm NLM/kg feed) |
| Maize | 0.57 | 0.58 | 0.575 | 0.572 |
| Rice polish | 0.10 | 0.10 | 0.10 | 0.085 |
| Soybean meal | 0.18 | 0.15 | 0.11 | 0.105 |
| Jeso-prot | 0.10 | 0.10 | 0.12 | 0.12 |
| Soybean oil | 0.03 | 0.03 | 0.035 | 0.038 |
| NLM | 0.00 | 0.02 | 0.04 | 0.06 |
| Common salt | 0.005 | 0.005 | 0.005 | 0.005 |
| Oyster shell | 0.0075 | 0.0075 | 0.0075 | 0.0075 |
| DCP | 0.005 | 0.005 | 0.005 | 0.005 |
| Vitamin mineral premix | 0.0025 | 0.0025 | 0.0025 | 0.0025 |
| Methionine | 0.0025 | 0.0025 | 0.0025 | 0.0025 |
| Lysine | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Chemical composition | | | | |
| ME (%) | 3094 | 3090.3 | 3095.4 | 3058.5 |
| CP (%) | 1.90 | 1.93 | 1.90 | 1.90 |
| CF (%) | 0.33 | 0.318 | 0.30 | 0.29 |
| Ca (%) | 0.11 | 0.18 | 0.12 | 0.13 |
| P (%) | 0.056 | 0.062 | 0.064 | 0.068 |
| Methionine (%) | 0.048 | 0.048 | 0.048 | 0.048 |
| Lysine (%) | 0.118 | 0.118 | 0.118 | 0.118 |

****** Vitamin Mineral Premix provided following per kg diet: Vit. A 5000 IU, $D_3$ 1000 IU, K 1.6 mg, B1 1mg, B2 2mg, B3 16mb, B6 1.6mg, B9 320µg, B12 4.8 µg, H 40mg, Cu 4mg, Mn 40mg, Zn 20mg, Fe 2.4mg, I 160 µg.

Photo 3.1 Brooding condition of chick

Photo 3.2 Housing condition of chick

## 3.6 Floor Space

Out of 14 small pens from two rooms 12 small pens were randomly considered for this trial. Each pen was 10 sq ft and 10 sq ft allocated for 10 birds. Therefore each bird was provided 1sq ft floor space.

## 3.7 Brooding

The experiment was conducted during 28$^{th}$ August to 5$^{th}$ October. The environment was lower than the brooding temperature during the experimental period. Additional heat was provided to chicks. Chicks were brooded in respective pens using two 100 watt electric bulbs. The chicks were provided with a temperature of 35 degree Celsius at first week of stage, decreasing gradually at the rate of 3 degree Celsius per week up to four weeks of age. The room temperature was measured by an automatic digital thermometer.

## 3.8 Feeding and watering

The chicks were fed starter diets from day old to 17 days and finisher diets from 18-35 days. All mash feed were supplied ad lithium to birds of all dietary treatments. Feed should be supplied four times daily, once in the morning, noon, afternoon and again at night in such a way that feeder was not kept empty. Clean and fresh water should be supplied two times in a day.

## 3.9 Lightings

The birds were exposed to a continuous lighting period of 23 hours and 30 minutes and a dark period of 30 minutes in each 24 hours. The dark period provision was done to make broilers familiar with possible darkness.

## 3.10 Routine Management

The experiment birds were exposed to similar care and management in all treatment groups throughout the experimental periods. The following management practices were followed during the entire period and those practices were identical for all dietary groups.

## 3.11 Medication

During the time of experimental period multi vitamins, saline, electropak, vitamin-c, Rena-C and vinegar were added to drinking water to reduce stress because of high environmental temperature.

## 3.12 Vaccination

All birds were vaccinated properly against Newcastle disease on the $10^{th}$ days and against Infectious Bursal Disease on $14^{th}$ days. (Table 3.4)

## Table 3.4 Schedule of vaccination

| Age of birds | Name of diseases | Name of vaccines | Route of administration |
|---|---|---|---|
| $10^{th}$ days | New castle Disease | BCRDV (Live) | One drop in one eye |
| $14^{th}$ days | Infectious bursal disease (Gumboro) | IBD (Live) | In drinking water |

## 3.13 Sanitation

Adequate hygiene and sanitation were maintained during the experimental period. The entrance and the veranda were kept clean and solution of bleaching powder and potassium permanganate (KMnO4) was sprayed alternatively. The outside wall of the experimental house and the feed storage of room have to be kept clean.

## 3.14 Bio security

Proper bio security measures were taken during the experimental period. Equipments kept clean and disinfected. Entrance of personnel was restricted except workers, researchers, supervisors and co supervisor who had to follow special care at the time of visiting farm:

- Hands should be washed with liquid soap and shoes were changed.
- Feet were dipped in a footbath containing disinfectant solution and it should be in the entrance point.

- Special dress should be used inside of the house when they will work with the birds.
- Adequate precautions were taken in case of vaccination.

## 3.15 Temperature and relative humidity

During experimental period, the temperatures and the relative humidity was recorded four times a day at the same time by using automatic thermometer and hygrometer respectively.

## 3.16 Record keeping

### 3.16.1 Body weight

The broiler chicks were weighted as a group at the beginning of the experiment and then after at 7 days intervals until the termination of the experiment at 35 days of age. The birds were weighted prior to afternoon feeding. The weekly average live weight recorded and that of live weight gain of broiler chicks on different dietary treatments were calculated.

### 3.16.2 Feed intake

The amount of feed consumed by the experimental birds of different groups was recorded on weekly basis.

### 3.16.3 Feed conversation ratio

Feed conversation ratio was calculated by using the following formula

$$\text{Feed conversati on ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Live weight gain (g)}}$$

### 3.16.4 Mortality

It was calculated on the basis of total number of birds housed and number of birds died during the experimental period.

## 3.17 Collection of blood serum

Sample blood was collected at age 28 day and 35 day with a syringe and needle directly through wing vein puncture without using any anticoagulant. By the end of each treatment, all the birds were sacrificed.

## 3.18 Separation and storage of blood serum

Each of the syringes with blood sample was kept at normal temperature in an inclined position. After 20 minutes the serum was collected and centrifuged for 15 minutes at 2500 RPM. After centrifugation, the supernatant was carefully collected by a micropipette and preserved in eppendrof vial. The collected serum was stored -15°C. These serum samples were used to determine total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides.

## 3.19. Assay estimation methods of various lipid parameters of blood samples

### 3.19.1 Estimation of total cholesterol (Tch) from blood plasma using kit method

**Principle**

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolizes the esters and $H_2O$ is formed in the subsequent enzymatic oxidation of cholesterol- oxidase according to the following reaction:

$$1.\ \text{Cholesterol ester} + H20 \xrightarrow{\text{CHE}} \text{cholesterol} + \text{fatty acid}$$

$$2.\ \text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{cholesterol-3-one} + H_2O_2$$

$$3.\ 2H2O2 + \text{Phenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{POD}} \text{quinonemine dye} + 4H_2O$$

**Reagent concentration**

| | |
|---|---|
| Pipes buffer, pH6.9 | 90mmol/l |
| Phenol | 26mmol/l |
| Cholesterol oxidase | 200U/I |
| Cholesterol esterase | 300U/I |
| Perqxidase | 1250U/I |
| 4-Aminoantipyrine | 0.2mmol/l |

**Procedure**

**Assay:**

Wavelength: Hg 546mm (500-550)nm

Temperature: +25/ + 30 / +37°C

Cuvette: 1cm light path

Zero adjustment: Reagent blank, one reagent blank per series only

| | Blank | Standard | Sample |
|---|---|---|---|
| Standard | _ | 10µl | _ |
| Sample | _ | _ | 10µl |
| Working reagent | 1000µl | 1000µl | 1000µl |

Mixed and measured after incubating at + 37°C for 5 minutes or 10 minutes at +20 to +25°C.

Within 60 minutes read absorbance of sample against reagent blank.

**Calculation:**

$$\text{Cholesterol concentration} = \frac{\Delta \text{A sample}}{\Delta \text{A standard}} \times \text{standard conc}$$

### 3.19.2 Estimation of HDL cholesterol from blood plasma using kit method

**Principle:**

The chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated by addition phosphotungstic acid and magnesium chloride. After centrifugation the supernatant fluids contains the HDL (high density lipoproteins)-fraction, their cholesterol content is determine enzymatically.

**Reagent composition:**

Phosphotungstic acid 0.55mmol/l

Magnesium chloride 25mmol/l

**Procedure:** Precipitation

| Pipette into centrifuge tube | Semi macro |
|---|---|
| Sample/standard | 200µl |
| HDL reagent undiluted | 500µl |

It was mixed well, allowed to stand for 10 minutes at +15°C to 25°C and centrifuged for 2 minutes at 10000g. The cholesterol content of HDL supernatant was determined.

**Assay:**

Wavelength: Hg 546mm (500-550) nm

Temperature: +25/ + 30 / +37°C

Cuvette: 1cm light path

Zero adjustment: Reagent blank

| Pipette into cuvettes | Reagent blank | Sample |
|---|---|---|
| Distilled water | 100µl | _ |
| HDL supernatant | _ | 100µl |
| Cholesterol reagent | 1000µl | 1000µl |

**Calculation:**

HDL cholesterol concentration = ΔA

Factor: Semi-macro: 327 mg/dl

## 3.19.3 Estimation of LDL from blood plasma using kit method

**Principle:**

The low density lipoproteins (LDL) are precipitated by heparin at their isoelectric point. After centrifugation the high density lipoproteins (HDL) and the very low density lipoproteins (VLDL) remains in the supernatant and can then are determined enzymatically. The LDL-cholesterol can then be calculated as the difference between supernatant cholesterol and total serum.

**Reagent concentration**

| | |
|---|---|
| Heparin | 0.68g/l |
| Sodium citrate | 0.064 mol/l |
| Stabilizer | 50 mg/dl (1,29 mmol/l) |

**Procedure:**

| Pipette into centrifuge tube | Semi-macro |
|---|---|
| Standard/sample | 100µl |
| LDL reagent | 1000µl |

It was mixed well, allowed to stand for 10 minutes at +15°C to +25°C and centrifuged for 15minutes at 4000 rpm. The cholesterol content of LDL supernatant was determined.

| | |
|---|---|
| Wavelength: | Hg 546mm (500-550) nm |
| Temperature: | +25/ + 30 / +37°C |
| Cuvette: | 1cm light path |
| Zero adjustment: | Reagent blank |

| Pipette into cuvette | Reagent blank | Sample |
|---|---|---|
| Distilled water | 100µl | _ |
| Supernatant | _ | 100µl |
| Cholesterol reagent | 1000µl | 1000µl |

**Calculation:**

LDL cholesterol concentration: ΔA factor

Factor: Semi-macro =1028 mf/dl

## 3.19.4 Estimation of triglycerides from blood plasma using kit method

The triglycerides are enzymetically hydrolyzed to glycerol according to the following reactions

Triglycerides ⟶ LPL⟶ glycerol + free fatty acid

Glycerol + ATP ⟶GK⟶ G-3-P + ADP

E-3-P + $O_2$ ⟶GPO⟶DAP + $H_2O_2$

$H_2O_2$ +4-aminoantipyrine + p-chlorophenol ⟶POD⟶ Quinoneimine dye

**Reagent**

| | |
|---|---|
| P-chlorophenol | 2mmol/l |
| Lipoprotein lipase | 150000U/I |
| Glycerolkinase | 800U/I |
| Glycerol-3-P-oxidase | 4000U/I |
| Peroxidase | 440U/I |
| 4-aminoantipyrine | 0.7 mmol/l |
| ATP | 0.3 mmol/l |
| Pipes buffer pH 7.2 | 50mmol/l |

**Procedure**

Assay:

Wavelength: Hg 546mm (500-550) nm

Temperature: +25/ + 30 / +37°C

Cuvette: 1cm light path

Zero adjustment: Reagent blank, one reagent blank per series only

| | Blank | Standard | Sample |
|---|---|---|---|
| Standard | _ | 10µl | _ |
| Sample | _ | _ | 10µl |
| Working reagent | 1000µl | 1000µl | 1000µl |

It was mixed and incubated for 5 minutes at 37°C. The absorbance of the sample (As) and the standard (Asd) was measured against the reagent blank within 60 minutes.

**Calculation:**

$$\text{Triglyceride concentration (mg/dl)} = \frac{\Delta \text{ A sample}}{\Delta \text{ A standard}} \times \Delta\text{A standard concentration}$$

## 3.20 Statistical analysis

Statistical analyses were conducted with the Statistical Package for Social Science (SPSS for Windows Version 20) to determine if variables differed between treatment groups. Results are expressed as mean ± SEM. The body weight, feed intake and feed conversation were compared among the groups by 1-way ANOVA and subsequent Duncan's Multiple Range Tests (DMRT).

# CHAPTER IV

# RESULTS AND DISCUSSION

The study was conducted to evaluate the effects of varying doses of neem leaf meal and their extract supplemented diets on growth rate, changes in body weight, feed intake, mortality and serum lipid profile in broiler. The formulated diets were supplemented with 0%, 2%, 4%, 6% NLM. The bird fed diets for a period of 5 weeks. Physical parameters were recorded weekly and the chemical parameters were measured also weekly at the end of the feeding trial. All results are expressed as mean ± standard errors means.

## 4.1 Effect of Neem leaf meal (NLM) on body weight of broilers

The body weights of broilers on neem leaf meal containing diet at different ages are presented in table 4.1. There were significant ($P<0.05$) difference in body weight gain of broilers among the experimental groups at age $2^{nd}$ weeks. However significant differences ($P<0.01$) were evident up to the $5^{th}$ weeks of age. The highest body weight body weight was observed on the control group and the lowest body weight was observed on the groups fed 60gm NLM/kg. Body weight gain were significantly reduced ($P<0.01$) in birds fed 40g NLM/kg and 60g NLM/kg. The non significant effect of NLM on body weight gain was not agreed with the findings of Nagalakshmi *et al.*, (1998). But agreed with Sridhar *et al.*, (2003) who reported that neem leaf extract treated birds showed mild depression and lesser body weight gain 4 weeks and onwards. Chowdhury (2004) reported that NLM at 40g/kg might depress growth rate of broilers but NLM might be safely used up to 20g/kg dietary level to get most of the effects similar to control. In our experiment we found, body weight gain was significantly reduced ($P<0.01$) in birds NLM at 20g/kg.

**Table 4.1 Weekly body weight (gm/broiler) of broiler on different treatment groups (Basal diet-control, Neem leaf meal)**

| Age | $T_0$ (control) Mean±SEM | $T_1$ (20gmNLM/kg) Mean±SEM | $T_2$ (40gm NLM/kg) Mean±SEM | $T_3$ (60gm NLM/kg) Mean±SEM | Level of significance |
|---|---|---|---|---|---|
| 1st week | 178.5±9.43 | 180.13±5.22 | 173.73±6.20 | 171.73±12.14 | NS |
| 2nd week | $463.87^{a}$±11.55 | $415.30^{ab}$±15.15 | $398.60^{b}$±14.16 | $400.13^{b}$±10.00 | * |
| 3rd week | $814.0^{a}$±5.34 | $810.37^{a}$ ±20.25 | $767.33^{b}$±10.36 | $728.07^{b}$±36.38 | ** |
| 4th week | $1317.67^{b}$±102.40 | $1263.00^{ab}$±63.38 | $1147.00^{bc}$±50.86 | $1061.33^{c}$±36.90 | ** |
| 5th week | $1851.67^{a}$±53.93 | $1726.67^{b}$±52.65 | $1662.33^{bc}$±49.66 | $1571.00^{c}$±58.89 | ** |

Mean values having uncommon superscripts differ significantly. SEM=Standard error of mean, * = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS = Not significant.

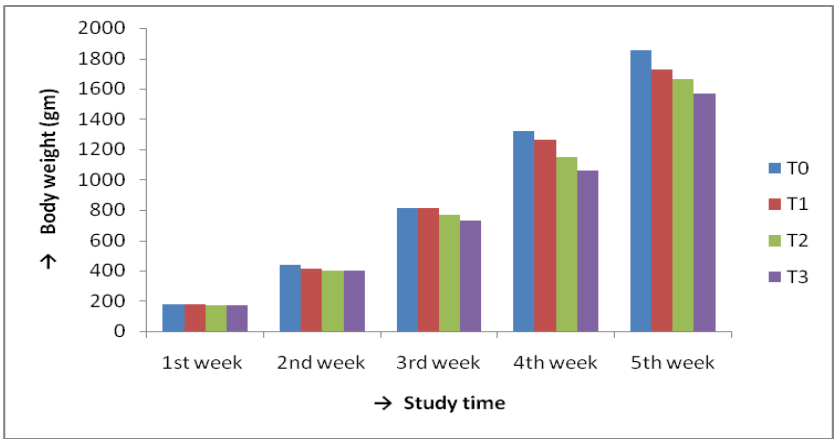


**Fig. 1: Bar graph showing weekly body weight (gm/broiler)**

## 4.2 Effect of neem leaf meal (NLM) on feed intake of broilers

Supplementation of Neem leaf meal (NLM) diet on weekly feed intake of broilers is presented in table 4.2. Tabular results showed that 2nd and 3rd weeks feed intake of broilers did not differ significantly ($P>0.05$) among the experimental groups. At the age of 4th weeks significant differences ($P<0.05$) were observed decrease feed intake in Neem leaf meal groups comparing to the control group and the age of 5th weeks significant differences ($P<0.01$) were observed decreased feed intake in Neem leaf meal groups comparing to the control group. The lowest feed intake was observed on Neem leaf meal diet and highest feed intake was observed on the control group. Significant

effect of NLM (P<0.01) on feed consumption was not in agreement with the findings of Baidy *et al.*, (1993). Nagalakshmi *et al.*, (1998) reported that processed neem seed kernel-cake did not reduce feed intake of broiler chicks. But in this experiment found dissimilar feed consumption in all diet groups.

**Table 4.2 Weekly feed intake (gm/broiler) of broiler on different treatment groups (Basal diet-control, Neem leaf meal)**

| Age (weeks) | $T_0$ (Control) Mean±SEM | $T_1$ (20gm NLM/kg) Mean±SEM | $T_2$ (40gm NLM/kg) Mean±SEM | $T_3$ (60gm NLM/kg) Mean±SEM | Level of significance |
|---|---|---|---|---|---|
| 2nd week | 543.33±51.32 | 523.33±46.19 | 466.67±28.87 | 496.77±50.19 | NS |
| 3rd week | 1133.33±57.74 | 1100.00±50.00 | 1023.33±25.17 | 1043.33±51.32 | NS |
| 4th week | $2050.00^{a}$±50.00 | $2016.67^{a}$±57.74 | $1916.67^{b}$±28.87 | $1933.33^{b}$±15.28 | * |
| 5th week | $3243.33^{b}$±58.59 | $3150.00^{ab}$±50.00 | $3050.00^{bc}$±50.00 | $2973.33^{c}$±107.86 | ** |

Mean values having uncommon superscripts differ significantly. SEM=Standard error of mean,* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS = Not significant.

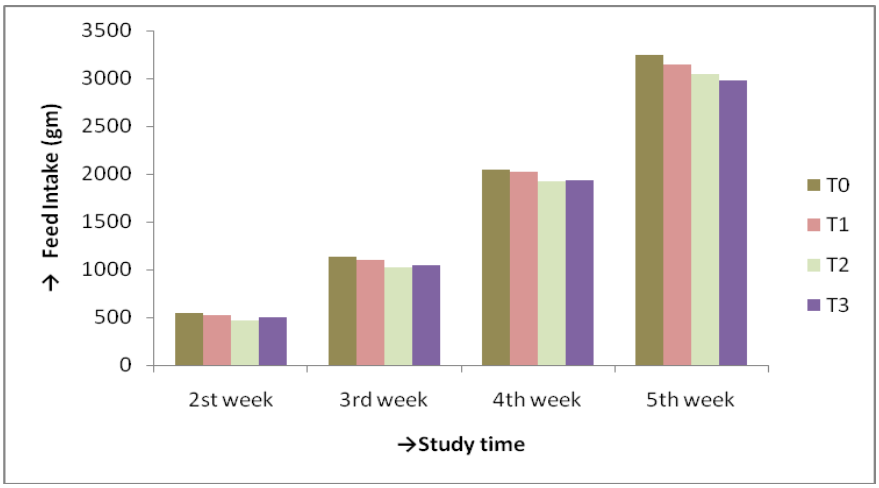


**Fig. 2 Bar graph showing weekly feed intake (gm/broiler)**

## 4.3 Effect of neem leaf meal on feed conversion (FCR) of broiler

The feed conversation (FC) of broiler during different stages of growth under different dietary groups on Neem leaf meal diet is given in table 4.3. There were no significant (P>0.05) difference among the groups on 1st weeks of age. However, at the end of 2nd weeks and 3rd weeks there were no significant (P>0.05) difference on FC among the treatment groups. Again, after the end of 4th and 5th weeks, there were significant (P<0.01) differenc on FC among the groups. There was significant difference on feed conversation among different dietary groups (P<0.01), which was not in agreement with the findings of Baidy *et al.*, (1993). Hamid *et al.*, (1994) found significant improvement in feed conversation of broiler.

**Table 4.3 Weekly feed conversation (FCR) of broiler on different treatment groups (Basal diet-control, Neem leaf meal)**

| Age (weeks) | $T_o$ (control) Mean±SEM | $T_1$ (20gm NLM/kg) Mean±SEM | $T_2$ (40gm NLM/Kg) Mean±SEM | $T_3$ (60gm NLM/kg) Mean±SEM | Level of significance |
|---|---|---|---|---|---|
| 2nd week | 1.25±0.05 | 1.26±0.01 | 1.17±0.04 | 1.25±0.14 | NS |
| 3rd week | 1.39±0.02 | 1.38±0.07 | 1.34±0.04 | 1.44±0.03 | NS |
| 4th week | $1.58^{b}$±0.11 | $1.60^{b}$±0.11 | $1.69^{ab}$±0.08 | $1.82^{a}$±0.07 | ** |
| 5th week | $1.75^{b}$±0.04 | $1.83^{ab}$±0.06 | $1.82^{ab}$±0.05 | $1.89^{a}$±0.01 | ** |

Mean values having uncommon superscripts differ significantly. SEM=Standard error of mean,* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS = Not significant.

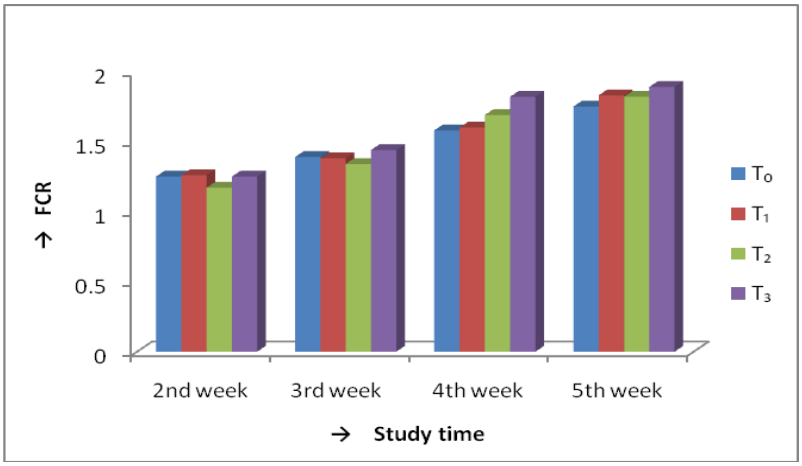


**Fig. 3 Bar graph showing feed conversation in different treatment groups**

## 4.4 Effect of neem leaf meal on mortality rate

There was no significant effect observed in mortality rate with the supplementation of neem leaf meal compared to control treatment. One bird died at 17 days of the experimental period from control feed group due to heat stress. It was noted that at that time the temperature of the experimental broiler shed was 36°C.

## 4.5 Effect of neem leaf meal on serum lipid profile of broiler

### 4.5.1. Total cholesterol

Effects of Neem leaf meal on total cholesterol were shown in table 4.4. It showed a significant ($P<0.01$) decrease in the level of total cholesterol in all levels of NLM at 4$^{th}$ week $T_1$ (137 mg/dL), $T_2$ (125 mg/dL) and $T_3$ (122.00 mg/dL) in comparison to the control $T_0$ (146.33 mg/dl) at fourth week post treatment. At fifth week post treatment there was significant ($P<0.01$) decrease in total cholesterol concentration in NML treatments $T_1$ (137.33 mg/dl), $T_2$ (130.00 mg/dl) and $T_3$ (124.67 mg/dl) as compared to control $T_0$ (154.0 mg/dl). NLM supplementation inhibited the increase in cholesterol level with advancement of age. Bapon (2007) reported that 16 to 18% reduction in egg yolk cholesterol could be made possible by feeding NLM at 10 gm/kg diet in layer hen. He also reported that, supplementation of NLM in the diet had depleting effects on serum cholesterol but a negative quadratic effect ($P<0.05$) was only noted 38 weeks of age.

## Table 4.4 Effect of neem leaf meal on total cholesterol of broiler

| Age (days) | $T_0$ (control) Mean±SE | $T_1$ (20gm NLM/kg) Mean±SEM | $T_2$ (40gm NLM/kg) Mean±SEM | $T_3$ (60gm NLM/kg) Mean±SEM | Level of significance |
|---|---|---|---|---|---|
| 28 day | $146.33^{a}$±5.13 | $137.67^{a}$±2.52 | $125.67^{b}$±6.03 | $122.00^{b}$±5.29 | ** |
| 35 day | $154.67^{a}$±3.51 | $137.33^{b}$±4.04 | $130.00^{c}$±1.00 | $124.00^{c}$±3.51 | ** |

Mean values having uncommon superscripts differ significantly. SEM=Standard error of mean, * = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS = Not significant.

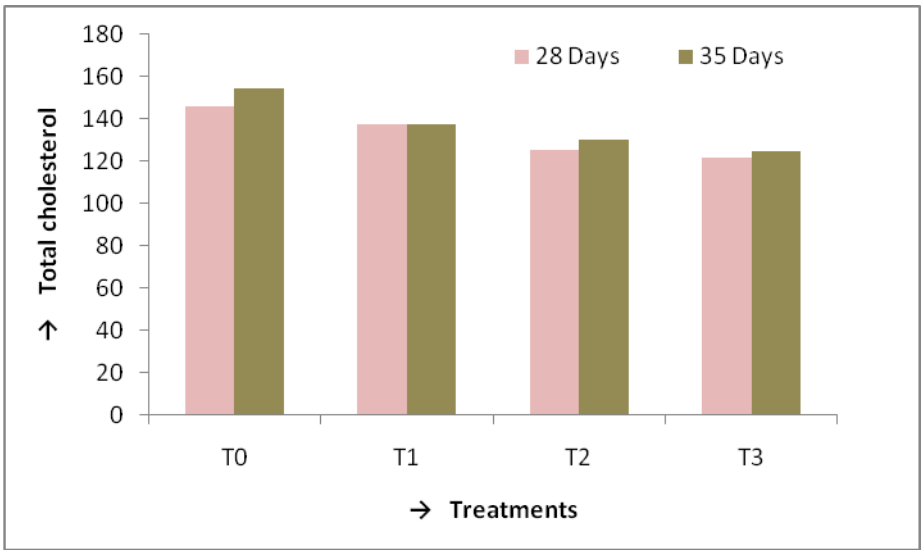


**Fig. 4 Bar graph showing total cholesterol in different treatment groups**

### 4.5.2 HDL Cholesterol

Effects of Neem leaf meal on HDL cholesterol were shown in table 4.5. Results of this research showed a significant (P<0.01) decrease in the level of HDL in birds fed the three levels of NLM treatments $T_1$ (85.33 mg/dl), $T_2$ (75.00 mg/dl), $T_3$ (66.00mg/dl) in comparison to the control birds $T_0$ (98.33 mg/dl) at 4th weeks post treatment. On 5th week of post treatment there was a significant (P<0.01) decrease in HDL concentration in NLM treatments groups $T_1$ (73.67 mg/dl), $T_2$ (65.00mg/dl) and $T_3$ (57.67mg/dl) as compared to control $T_0$ (78.67 mg/dl).

## Table. 4.5 Effects of neem leaf meal on HDL cholesterol

| Age (days) | $T_0$ (control) Mean±SEM | $T_1$ (20gm NLM/kg) Mean±SEM | $T_2$ (40gm NLM/kg) Mean±SEM | T3 (60gm NLM/kg) Mean±SEM | Level of significance |
|---|---|---|---|---|---|
| 28day | $98.33^{a}$±3.51 | $85.33^{b}$±3.31 | $75.00^{c}$±3.00 | $66.00^{d}$±5.00 | ** |
| 35 day | $78.67^{a}$±3.51 | $73.67^{a}$±3.06 | $65.00^{b}$±3.00 | $57.67^{c}$±2.52 | ** |

Mean values having uncommon superscripts differ significantly. SEM=Standard error of mean, * = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS = Not significant.

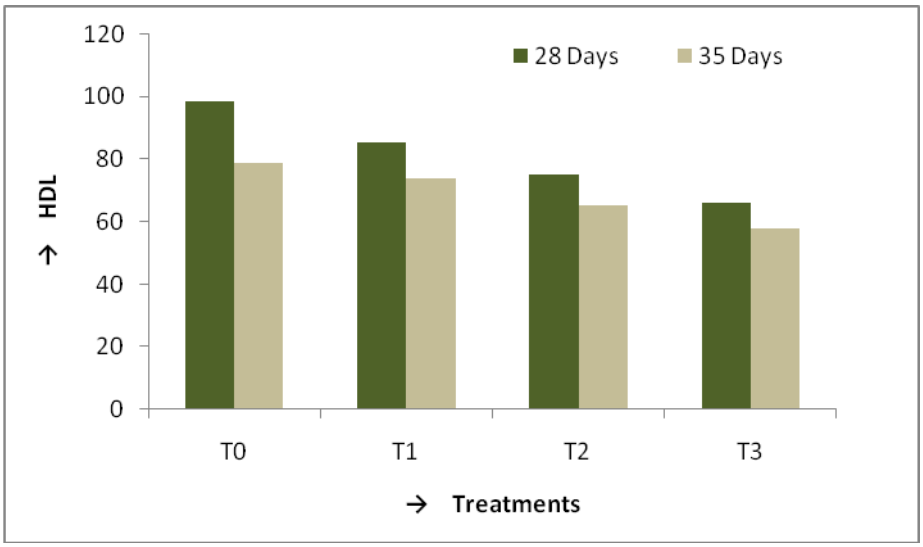


**Fig 5. Bar graph showing HDL cholesterol in different treatment groups**

### 4.5.3 LDL cholesterol

Effects of Neem leaf meal on LDL cholesterol were shown in table 4.6. It showed a significant (P<0.01) decrease in the level of LDL in birds fed the three levels of NLM $T_1$ (139.67 mg/dl), $T_2$ (132.33mg/dl), $T_3$ (121.00mg/dl) in comparison to the control birds $T_0$ (146.67 mg/dl) at four weeks post treatment. On 5th week of post treatment there was a significant (P<0.01) decrease in LDL concentration in NLM treatment groups as $T_1$ (134.33mg/dl), $T_2$ (125.33mg/dl), $T_3$ (122.00mg/dl) compared to control $T_0$ (139.67 mg/d).

## Table 4.6 Effects of neem leaf meal on LDL cholesterol

| Age (days) | $T_0$ (control) Mean±SEM | $T_1$ (20gm NLM/kg) Mean±SEM | $T_2$ (40gm NLM/kg) Mean±SEM | $T_3$ (60gm NLM/kg) Mean±SEM | Level of significance |
|---|---|---|---|---|---|
| 28 day | $146.67^{a}$±10.26 | $139.67^{ab}$±2.52 | $132.33^{b}$±2.52 | $121.00^{c}$±1.00 | ** |
| 35 day | $139.67^{a}$±1.53 | $134.33^{a}$±2.08 | $125.33^{b}$±3.51 | $122.00^{b}$±4.00 | ** |

Mean values having uncommon superscripts differ significantly. SEM=Standard error of mean,* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS = Not significant.

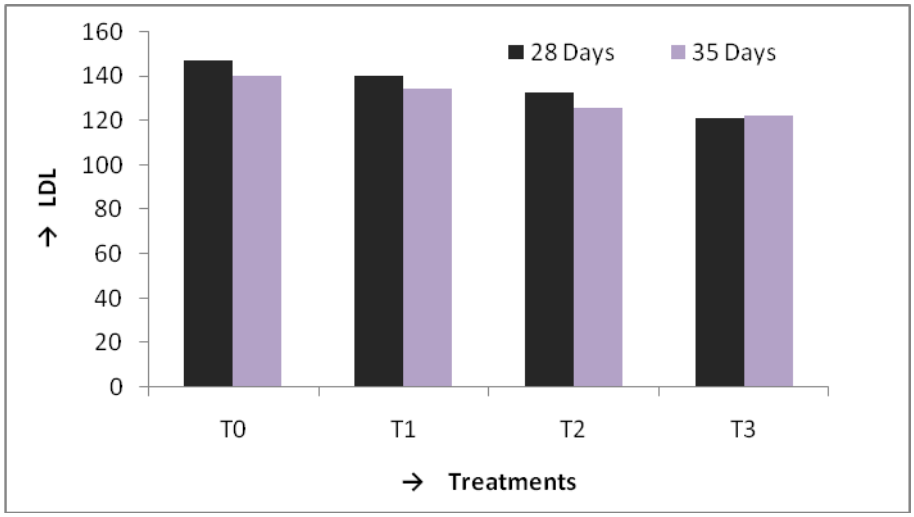


**Fig. 6 Bar graph showing LDL cholesterol in different treatment groups**

### 4.5.4 Triglycerides

Effects of Neem leaf meal on LDL triglycerides were shown in table 4.7. There was a significant (P<0.01) decrease in the level of TG in birds of NLM group treatments $T_1$ (168.00 mg/dl), $T_2$ (154.00 mg/dl), $T_3$ (142.33 mg/dl) in comparison to control birds $T_0$ (179.33 mg/dl) at four weeks of treatment. On 5$^{th}$ week of post treatment there was a significant (P<0.01) decrease in TG concentration in NLM birds $T_1$ (159.00 mg/dl), $T_2$ (147.33 mg/dl), $T_3$ (141.33mg/dl) as compared to control birds $T_0$ (165.67 mg/d).

## Table 4.7 Effects of neem leaf meal on triglycerides

| Age (days) | $T_0$ (control) Mean±SEM | $T_1$ (20gm NLM/kg) Mean±SEM | $T_2$ (40gm NLM/kg) Mean±SEM | $T_3$ (60gm NLM/kg) Mean±SEM | Level of significance |
|---|---|---|---|---|---|
| 28 days | $179.33^{a}$±3.79 | $168.00^{b}$±4.58 | $154.00^{c}$±3.61 | $142.33^{d}$±2.52 | ** |
| 35 days | $165.67^{a}$±4.04 | $159.00^{a}$±9.00 | $147.33^{b}$±2.08 | $141.33^{b}$±3.21 | ** |

Mean values having uncommon superscripts differ significantly. SEM=Standard error of mean, *= Significant at 5% level of probability, ** = Significant at 1% level of probability, NS = Not significant

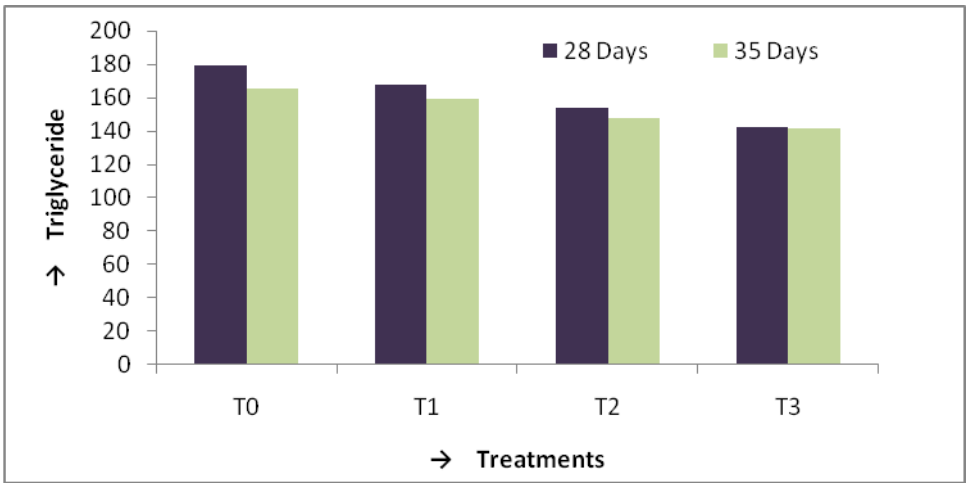


**Fig 7. Bar graph showing triglycerides in different treatment groups**

## 4.6 Effect of neem leaf meal on different parts weight (gm) of broiler

Effect of Neem leaf meal on different parts weights were shown in table 4.8. At the end of the experiment thigh, drumstick and live weight were differ significantly ($P<0.01$) from different experimental groups. Shank and carcass weight were differ significantly ($P<0.05$) among the experimental groups. Head, neck, skin, wing, breast, thigh length, gizzard, liver, heart, fat and dressed weight were not differ significantly ($P<0.05$) among all treatments. Among the different groups, control group show better result than others groups. Birds fed 60gm NLM/kg show poor result from all the treatments.

**Table 4.8 Different body parts weight of different treatment groups (Basal diet-control, Neem leaf meal)**

| Body parts | Treatments | | | | |
|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | Level of sig. |
| Live weight | $1533.33^{a}$±57.74 | $1518.00^{a}$±66.84 | $1416.00^{b}$±13.53 | $1356.60^{b}$±2.52 | ** |
| Head | 41.00±1.50 | 41.00±1.00 | 42.67±1.08 | 40.67±1.53 | NS |
| Neck | 42.67±2.08 | 41.33±2.31 | 42.33±1.79 | 39.00±2.29 | NS |
| Skin | 70.67±1.06 | 75.33±2.21 | 73.67±2.04 | 72.67±2.08 | NS |
| Wing | 50.33±1.53 | 48.67±2.08 | 49.00±1.00 | 49.67±2.52 | NS |
| Breast | 260.00±11.53 | 265.33±2.31 | 257.33±31.79 | 261.00±40.63 | NS |
| Shank | 54.33±7.57 | 57.33±3.51 | 53.67±2.52 | 53.00±3.00 | NS |
| Thigh length(Inch) | 5.33±0.29 | 5.33±0.29 | 5.33±0.29 | 5.33±0.29 | NS |
| Thigh | 123.33±1.53 | 119.33±7.57 | 122.3±2.89 | 117.24±6.24 | NS |
| Drumstick | 128.33±8.50 | 127.00±4.36 | 132.00±2.00 | 130.00±2.00 | NS |
| Gizzard | 55.33±3.06 | 53.33±1.53 | 52.00±10.82 | 51.33±1.15 | NS |
| Liver | 39.67±0.58 | 40.33±0.58 | 46.67±7.64 | 38.00±2.65 | NS |
| Heart | 7.00±1.00 | 7.33±0.58 | 7.33±1.53 | 6.33±0.58 | NS |
| Fat | 36.33±0.58 | 37.33±1.53 | 36.67±2.51 | 37.33±2.02 | NS |
| Dressed weight | $1373.33^{a}$±25.17 | $1377.00^{a}$±117.89 | $1278.67^{ab}$±10.02 | $1203.00^{b}$±30.55 | * |
| Carcass weight | 1067.00±24.02 | 1098.00±46.23 | 1004±16.44 | 986.67±84.54 | NS |
| Dressing % | 69.60% | 72.33% | 70.90% | 72.71% | NS |

Mean values having uncommon superscripts differ significantly. SEM=Standard error of mean, *= Significant at 5% level of probability, ** = Significant at 1% level of probability, NS = Not significant.

# CHAPTER V

## SUMMARY AND CONCLUSION

A study was conducted from 28th August to 5th October 2013, at the poultry shed and Dairy and Poultry Science Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The objectives of this experiment were to evaluate the varying doses of NLM supplemented diets on broiler chicks. In feeding trial, four (4) diets were prepared including NLM at levels of 0, 20, 40 and 60 gm/kg. Body weight gain and feed consumption were recorded weekly basis and serum lipid was measured at day 28 and day 35. Mortality rate was also recorded throughout the study.

In experimental diets, the growth performances of birds were not improved. The experimental birds exhibited weight gain during feeding trail but the rates of gain were different than that of control group. Average body weight gain was found to be highest in treatment $T_0$ (1851.67 gm) and $T_1$ (1726.67 gm) compared with $T_2$ (1662.33 gm) and $T_3$ (1571.00 gm). Feed intake trend from first day to last day of the experimental period in different treatment groups was recorded and expressed as gm/day. Although the rate of feed intake varied day to day but the feed intake (gm/day) was lowest in $T_3$ compared to other treatments. Highest feed intake found in control group $T_0$ (3243.33 gm) compared to other treatment groups $T_1$ (3150.00 gm), $T_2$ (3050.00 gm) and $T_3$ (2973.33 gm). Feed intake decrease significantly ($P<0.05$) after 3rd weeks. This variability in feed intake might be due to different palatability of feeds. Feed conversion ratio (FCR) was lowest in $T_0$ (1.75) and $T_2$ (1.82) treatments compared to $T_1$ (1.83) and $T_3$ (1.89). Mortality rate were same in control and NLM supplemented treatments.

Total cholesterol, HDL cholesterol and triglycerides were statically significant ($P>0.01$) at day 28 to day 35. Total cholesterol was increased gradually from fourth week to fifth week but decreased in NLM supplemented diets compared to control treatments. HDL cholesterol and triglycerides were decreased at fourth and fifth week in NLM supplemented treatments compared to control treatments. At the end of the experimental period, total cholesterol were higher in control treatment $T_0$ (154.67 mg/dl) compared to NLM supplemented diets $T_1$ (137.33 mg/dl), $T_2$ (130.00 mg/dl) and $T_3$ (124.67 mg/dl). HDL cholesterol were highest in $T_0$ (78.67 mg/dl) and $T_1$ (73.67 mg/dl) compared to $T_2$

(65.00 mg/dl) and $T_3$ (57.67 mg/dl). The triglycerides were decreased at final period by NLM supplemented diets compared to $T_0$ (165.67 mg/dl) and there was significant differences ($P<0.01$) between control and NLM supplemented diet.

It may be concluded that the NLM supplemented diets had positive effect on mortality rate of different treatment groups of broiler. The performances of broiler i.e. final body weight, feed intake and feed conversation ratio decreased by feeding NLM supplemented diets at different levels. Among the NLM supplemented diets, our finding suggest that supplementation of 2% NLM powder have high potential as commercial applications for production of low-cholesterol and healthy broilers. It is recommended that the use of NLM as feed supplements to broilers in their starter and finisher diets supplementation with the levels (2% NLM powder) used in this experiment. Broiler feed contains 2% NLM had better result than other treatments contained 4% and 6% NLM.

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