EFFECT OF NEEM (*Azadirachta indica*) LEAF EXTRACT IN DRINKING WATER ON THE PERFORMANCE OF COMMERCIAL BROILERS

A THESIS

BY

DR. MD. TAUHIDUR RAHMAN

Registration No. 1605475

Semester: July-December, 2018

MASTER OF SCIENCE (M.S.)

IN

POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR - 5200

NOVEMBER, 2018

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DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR - 5200

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Dedicated to
My
Beloved Parents

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The Author

ABSTRACT

An experiment was carried out with 120 day-old broiler chicks (Lohman Meat) to evaluate the effect of neem (*Azadirachta indica*) leaf extract in drinking water on the performance of commercial broilers. Chicks and feed were procured from Aman Poultry and Hatchery limited. The neem leaves were collected from local area of Dhupchachiya, Bogura.The treatment groups were Group A (5% NLE @ 24 ml/L water), Group B (5% NLE @ 32 ml/L water), Group C (5% NLE @ 40 ml/L water) and Group D (Control). At the end of the trial (35 days) the body weight gain were 1862, 1893.33, 1969.67 and 1800 g in Group A, B, C and D respectively. Group C had significantly higher body weight gain than others. The cumulative feed consumption of Group A, B, C and D were 3172, 3138.33, 3060 and 3177.67g respectively. Group D (control) had higher feed intake than other groups but the differences were non-significant ($P>0.05$). At the end of the experiment FCR values of Group A, B, C and D were found as 1.68, 1.62, 1.52 and 1.73 respectively. Group C (5% NLE @ 40 ml/L water) had significantly better ($P<0.05$) FCR value than other groups. It was clearly found that 5% (w/v) neem leaf extract at 40ml/L drinking water of broiler can significantly improve the live performance.

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LIST OF ABBREVIATION AND SYMBOLS

AM	= Ante meridian
Av.	= Available
HSTU	= Hajee Mohammad Danesh Science and Technology University
Ca	= Calcium
CF	= Crude fiber
Cm	= Centimeter
cm^2	= Square centimeter
Contd.	= Continued
CP	= Crude protein
DM	= Dry matter
Dr.	= Doctor
et al.	= Associates
G	= Gram
i.e.	= That is
kcal	= kilo-calorie
Ltd.	= Limited
Lys.	= Lysine
ME	= Metabolizable energy
Met.	= Methionine
MLM	= Mulberry Leaf Meal
No.	= Number
NLM	= Neem Leaf Meal
°C	= Degree Celsius
P	= Probability
Total P	= Total Phosphorus
PM	= Post Meridian
Pp	= Page
Prof.	= Professor
SEM	= Standard Error of Means
Tk.	= Taka
Try.	= Tryptophan
UFFDA	= Users Friendly Feed Formulation Done Again

USFDA	= United States Food and Drug Administration
WHO	= World Health Organization
%	= Per cent
&	= and
@	= At the rate of
+	= Plus/and
/	= Per/or
>	= Greater than
<	= Less than
±	= Plus-minus
AE	= Aquous Extract
IBD	= Infectious Bursal Disease
ND	= Newcastle Disease
NLE	= Neem Leaf Extract
SD	=Standard Deviation

CHAPTER-I
INTRODUCTION

Poultry industry of Bangladesh has grown tremendously throughout the past two decades. The most produced poultry product is broiler meat that meet the protein demand of the nation to a large extent. The price of broiler meat is low compared to beef and mutton and also other sources of protein such as fish, vegetables etc. This is the cheapest source of protein and large number of people could afford it. Broiler farming needs a lot of research works to overcome the challenges of diseases and growth promoting issues. The main aim of broiler farming is to achieve maximum growth by utilizing the feed nutrients properly and ensuring the maximum livability of the birds by maintaining good health status. A variety of non-nutritive feed additives mostly antibiotics are used in broiler production to improve the overall performance and immune status by eliminating stress. Continuous use of sub therapeutic levels of such feed additives in feed may result in disadvantages like high cost, adverse side effect on health of birds and long residual properties .This is promoting antimicrobial resistance in human health. Antimicrobial resistance has become a burning global issue that is posing a serious threat to the existence of mankind. Such issue has triggered intensive research to find and develop alternative strategies to maintain health and performance in intensive broiler production system.

Researches have identified several beneficial chemical compounds in medicinal plants, which play an important role in improving production of birds and have strong medicinal value and could be effectively utilized as natural growth promoters to replace antibiotics and other synthetic feed additives. Various plant extracts have been used worldwide for a range of medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal and hepatoprotective without adverse effects (Sharma, V., 2018). One of the alternatives could be the leaves extract of *Azadirachtaindica* (Neem) specially in broiler production.

Azadirachtaindica, commonly known as neem, has attracted worldwide attention in recent years, owing to its wide range of medicinal properties. Neem has been extensively used in Ayurveda, Unani and homoeopathic medicine and has become a cynosure of modern medicine (Quraishi *et al*. 2018). In Bangladesh neem is cultivated throughout the country and very much available to the farmers. Weather of Bangladesh is very much favorable for neem tree cultivation. In 1998 an international and unprofitable institute was

formed in our country. This research type institute increases awareness to the people and also gives neem tree to them at free of cost (Karim, 2007).

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. In general, Neem leaves extract may be used therapeutically to control respiratory problems, constipation and also as health promoter (Pandey *et al.* 2018). Aqueous extract of Neem leaves extract has a good therapeutic potential as anti-hyperglycemic agent, antibacterial agent and could be used for controlling airborne bacterial contamination in the residential premise (Mishra *et al*. 2013). The medicinal utilities and wide range of pharmacological activities have been described especially for neem leaf. Neem leaves and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties (Rahmani *et al.* 2018). The leaves extract containnimbin, nimbinene, 6-desacetylnimbiene, nimbandiol, nimbolide and quercetin (Shareef *et al.* 2018). Leaves are carminative and aid in digestion. The tender leaves along with piper nigrum Linn are found to be effective in intestinal helminthiasis. An aqueous extract (10%) of tender leaves is reported to possess anti-viral properties against, fowl pox, IBD and New Castle disease virus (NDV) and significantly enhances the antibodies production against the IBD and NDV (Sadekar *et al.* 1998). The infusion of fresh leaves is stated to be an antiseptic. The hot infusion of leaves is used as anodyne for fomenting swollen glands, bruises and sprains (Sharma, 1997).Since neem leaf increases immunity against some common diseases which assists to reduce mortality , it is likely that the growth pattern of broiler chicks will proceed uninterrupted.

So based on these medicinal properties of neem a study was planned to provide neem leaf extract (NLE) in drinking water to investigate the effect of neem Leaf Extract (NLE) on the performance of broiler chicks.

CHAPTER-II
REVIEW OF LITERATURE

Neem is popularly used in the traditional Unani system of medicine for its various beneficial properties. All parts of neem tree have therapeutic value such as the roots, stem, leaves, fruits, bark and seeds. Among these neem leaves are widely applied in the treatment of a number of human and animal diseases. Neem leaves are also used for manufacturing a number of drugs and medicines because the physio-chemical properties of neem are worthy to maintain overall well being. It is well established that Neem Leaf Extract (NLE) contains valuable chemical components which enhance the immunity in birds as well as in human. A good number of works with neem compounds has been carried out mostly in India and Pakistan and few in Bangladesh, although these studies are very little precisely with poultry. The chemical composition of neem leaves, their medicinal properties and beneficial effect on birds, animal and human are reviewed in this chapter.

2.1 Medicinal properties of neem (*Azadirachtaindica*) leaves in general

The development of traditional medicinal system may be highly beneficial for the farmers and also for overall improvement of the poultry industry in Bangladesh because neem leaves may exert its beneficial effects effectively on performance of birds at a minimum expense. Neem leaves have a wide spectrum of uses. WHO has recognized the necessity of ancient medicinal practice for meeting the primary health needs for the people of the developing countries like Bangladesh. This is specially important for poultry sector where excess use of antibiotics and their residual problem have become a burning question. The most common uses of neem are well recognized in Ayurveda, Unani and Homeopathic systems of medicine (Quraishi *et al*. 2018).

Agrawal (2002) reported that more than 135 components have been isolated from different parts of neem. The components have been divided into two major classes: Isoprenoids (Diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C-secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids which are proteins (amino acids) and carbohydrates (polysaccharides), sulphurous

compounds, polyphenolich such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds etc.

Siddiqui *et al*. (2004) reported that two new tetracyclic triterpenoids, zafaral and meliacinomhydride have been isolated from the methanolic extract of neem leaves along with two known constituents, nimocinol and isomeldemin.

2.1.1 Immuno-stimulant activity

Putri *et al*. (2018) carried out an experiment to evaluate immunostimulant activity of Neem Leaf (A. indica A. Juss) ethanol fraction on Tilapia fish (*Oreochromisniloticus*) and found that the total leukocyte of the fish test increased after treated with fraction 4 of neem leaf (*A. indica* A. Juss) compared with control on day 7 after injection, i.e. 25.29 x 10^4 cells.mL^{-1}.

The aqueous extract of leaf also possess potent immuno-stimulant activity as evidenced by both humoral and cell mediated responses (Sen, 1992 and Ray, 1996)

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). 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 increased titer of antiovalbumin antibody (Ray *et al.* 1996)

2.1.2 Hypoglycaemic activity

Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycemia. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits.

2.1.3 Antiulcer effect

Neem leaf aqueous extracts produced highly potent aniacids secretory and antiulcer activity.

Bhajoni *et al.* (2018) reported that the leaves of *A. indica*possess significant antiulcer activity and act via multiple mechanisms.

Chaturvedi (1995) reported that peptic ulcers and duodenal ulcers were treated well with neem leaf extracts; nimbin 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 the most relief; increased dosages reduced the effectiveness of neem's antiulcerative effects.

2.1.4 Anti cancer activity

Potential of neem (Azadirachtaindica L.) for prevention and treatment of oncologic diseases were reported by Patel *et al.* 2016.

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.

Chemo preventive response was measured by the average number of papillomas per mouse, as well as percentage of tumor-bearing animals. There was a significant inhibition of tumor burden, in both the tumor model system studied (from $P< 0.005$ to $P< 0.001$). Tumor incidence was also reduced by both the doses of *Azadirachtaindica* extract (Dasgupta*et al.* 2004).

Vaccinations of mice with B16MelSAg+NLP more efficiently prevented the growth of B16 melanoma tumor than mice immunized with B16MelSAg or NLP alone. Neem leaf preparation (NLP) might be a potential immune adjuvant for inducing active immunity towards tumor antigens (Baral *et al.* 2005). 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. Unique property of neem may be utilized for the immunotherapy of CEA positive tumors (Sarkar *et al.* 2007).

2.1.5 Effect on nervous system

Xiang *et al*. (2018) reported that *Azadirachtaindica* was demonstrated to exhibit neuroprotective antioxidative and antiapoptotic effects in Parkinson's disease.

According to Ayurveda, neem leaves help in the treatment of vatic disorders (neuro muscular pains) (http://www.neemfoundation.org/comp.html).

Varying degrees of central nervous system (CNS) depressant activity in mice was observed with the neem leaf extract. Fractions of acetone extract of neem leaf showed significant CNS depressant activity. Shafeek *et al.* (2004) studied on the alterations in the activity of the enzyme acetylcholinesterase (AChE) and electrical activity in the nervous system of the cockroach, *Periplanetaamericana* , exposed to azadirachtin. Exposure to azadirachtin produced an excitatory effect on spontaneous electrical activity as well as cercal sensory-mediated giant-fibre responses in the cockroach. Topical exposure to sublethal doses of azadirachtin did not result in any significant alterations in the AChE activity in different regions of the nervous system. They suggest that azadirachtin exerts excitatory action on the electrical activity in the nervous system of cockroach by interfering with the ion channels in the nerve membrane, the probable target of several insecticides.

2.1.6 Antifertility effect

Intra-vaginal application of neem leaf extract, prior to coitus, can prevent pregnancy. It could be a novel method of contraception.

Sadre *et al.* (1983) studied the male antifertility activity of neem leaf in mice, rats, rabbits and guinea pigs by daily oral feeding of a cold-water extract of fresh green neem leaves. They found that in treated male rats there was a 66.7% reduction in fertility after 6 weeks, 80% after 9 weeks, and 100% after 11 weeks. There was a marked decrease in the mortality of spermatozoa and the male antifertility activity was reversible in 4 to 6 weeks.

Parshad *et al.* (1996) studied the antifertility efficacy of both aqueous and steroidal extracts of neem leaves in male wistar rats. Intraperitoneal injections of the steroidal extract 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 extract in drinking water for 7 weeks decreased serum testosterone ($P<0.01$) but no effect was observed in the fertility index.

Gowda (1998) reported that the fertility and hatchability were adversely affected by the higher inclusion rates (150g/kg and 200g/kg) of neem kernel meal (NKM).

2.1.7 Antimalarial activity

Murugan *et al*.(2016) synthesized silver nanoparticles (AgNP) using the *Azadirachtaindica* seed kernel extract as reducing and stabilizing agent and found that the *A. indica*-mediated fabrication of AgNP is of interest for a wide array of purposes, ranging from IPM of mosquito vectors to the development of novel and cheap antimalarial drugs.

Gedunin, contained in whole neem fruit, has been shown to possess antimalarial activity (http://www.neemfoundation.org/comp.html).

Chaturvedi (1995) reported that Irodin A, an active ingredient in neem leaves, was toxic to resistant srains of malaris; 100% of the malaria gamete was died within seventy two hours with a 1 to 20,000 ratio of active ingredients.

Joshi *et al*. (1998) conducted an experiment to investigate the antimalarial activity of neem. From the ethanol extract of fresh neem leaves, four limonoids (meldenin, nimocinol, isomeldenin and nimbandiol) were isolated and these limonoids showed antimalarial activity against chloroquine-resistant *Plasmodium falciparum* strain K1.

Neem leaf extracts are effective against both chloroquine-resistant and sensitive strain of malarial parasites (http://www.neemfoundation.org/comp.html).

Udeinya *et al.* (2004) carried out an experiment where an acetone-water neem leaf extract with anti-malarial activity was evaluated in ten patients with HIV/AIDS at 1000mg 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, and 0 and 2, respectively, with the extract. In the absence and presence of the extract 0% and 75% of lymphocytes were protected respectively.

2.1.8 Antifungal activity

Neem oil has been reported to be effective 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. neem foundation.org/comp.html).

Khune *et al.* (1985) had an experiment where Adulsa+Neem+tobacco decoction, neem leaf decoction, calixin (tridemorph) and Aureofungin consistently gave >75% inhibition of fungus (*Capnodiumcitri* and *Chaetothyrium* sp.)

Govindachari *et al.* (1999) conducted an experiment in which the hexane extract of neem leaves and its fractions were studied for antifungal activity against two plant pathogens, *Fusariumoxysporum* and *Colletotrichumlindemuthianum*. Two fractions completely inhibited the growth of the fungi at 400 µg/cm².

Khan *et al.* (2002) showed that the combination of neem leaf extracts and *Verticilliumchlamydosporium* improved plant growth over the control.

Suresh *et al.* (2004) conducted an experiment where active extracts of terpenoids (neem compound) were identified by GC-MS, and their interactions in mixture were studied .They obtained that identification of the mode of action and of target sites in fungi and the ability to induce systemic acquired resistance in host pathogen systems will enable the potential of neem to control plant pathogens to be maximized.

Ilyaset al. (1997) found that the replin (neem product) at a concentration of 0.8 and 1.0% completely inhibited the vegetative growth of the fungus.

2.1.9 Antibacterial activity

Francine *et al.* (2015) examined the *in vitro* effect of extracts of different neem (*Azadirachtaindica*) plant (leaf and bark) on *Staphylococcus aureus* and *Escherichia coli* and found that the effectiveness of the extracts was dependent of the concentration used thus the increase of extract concentration increased the inhibition zone.

Neem oil inhibited the growth of all the three strains of Mycobacterium at a concentration of 12.5 mg/ml (http://www.neemfoundation.org/comp.html). Among all the bacteria tested, *A. hydrophila, P. fluorescens*and *Mycobacteria spp.* exhibited maximum sensitivity to Aquaneem in terms of percentage reduction of bacterial cell population in comparison to *E.coli* (Das *et al.* 1999).

Oil from neem leaves was found to possess a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *Mycoplasma*

tuberculosis and *Streptomycin* resistant strains. Antibacterial effects of neem leaf extracts have been demonstrated against *Streptococcus mutans* and *Streptococcus faecalis* (Almas *et al.* 1999). *In vitro,* it inhibits *Vibrio cholera, Klebsiellapneumoniae, Mycoplasma pyogenes*.

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 both powder to kill fungus and bacteria (http://www.essentialwholesale.com/ingredients/html). Neem stick extract can reduce the ability of some Streptococci to colonize tooth surfaces (Wolinsky *et al.* 1996).

The antibacterial effect of Neem mouthwash against 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).

Prates *et al.* (2003) conducted an experiment where neem leaf aqueous extract against *Spodopterafrugiperda* was evaluated. Bioassays were carried out using artificial feed with various neem extract concentrations and with chlorpyrifos as the control. At 15 days after larvae infestation, extract concentrations from 3.60 to 10.00mg/ml were equally effective against *Spodopterafrugiperda.*

2.1.10 Antiviral activity

Mahmood *et al.* (2017) evaluated the in-vitroand in-ovoantiviral activity of Neem (Azadirachtaindica) bark extract against Newcastle disease virus (NDV) and found that there was no significant difference in the antiviral effects of different concentrations of Neem bark extracts but the exposure time was a significant variable for cytotoxicity.

A 10% aqueous extract of tender leaves of neem has been found to possess anti-viral properties (http://www.neemfoundation.org/comp.html). 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 (*Vignaunguiculata*) from the bruchid *Callosobruchusmaculatus*. Neem fruit dust 2, 5 and 10% neem leaf dust at 5 and 10% neem seed kernel oil at 2 and 5ml/kg of seed protected stored cowpeas for at least 4 months.

Khan *et al.* (2000) carried out an experiment where crushed neem seeds and leaves (4g/plot) were applied to the top soil layer 7 days after seed germination. After 15 days, they found that 10 viruliferous *Bemisiatabaci* were released on each cotton plant.

Epp *et al.* (1993) found that aqueous extracts (AE) and methanolic extracts (ME) of neem seeds were responsible for the inhibition of tobacco mosaic tobamovirus infections of tobacco. ME gave greater inhibition than AE, but results given by ME were in general not much better than those given by methanole alone.

Neem leaf extract reported to possess antiviral properties against fowl pox and Newcastle virus. (http://www.neem.biz/neemcake.html).

Experiments with small pox, chicken pox and fowl pox showed that although neem didn't cure these diseases, but it was effective for the purpose of prevention. Crude neem extracts absorbed the viruses, effectively preventing them from entering unaffected cells (Patnaik, 1993).

2.2 Medicinal properties of neem leaves in human

Neem compounds, its extract and finished products have been used to cure right from skin diseases to diabetes, from cholesterol to hair problems, from ulcers to dental problems. (http://www.neem-products.com/neem-benefits.html). Five parts of neem tree is i.e. bark, root, fruit, flower and leaves together are used in diseases of blood. It is also used in vitiated conditions of excess heat, itching, and wound, burning sensation in body and skin diseases. (http://www.neemfoundation.org/comp.html).

Sing (1979) reported that neem may be used in skin problems to prevent infection. According to their report, neem reduces pain, kills bacteria that can cause infection, stimulates the local immune system and promotes rapid healing with reduced scarring.

Alam (1989) reported that neem leaves were effective in treating and preventing diabetes. Upadhay *et al.* (1993) concluded that neem leaf extracts and neem bark extracts significantly reduced the P-24 viral proteins and induced *in vitro* production 0f IL-1 infection. Chattopadhyay *et al.* (1992) reported that the neem leaf extracts reduced cholesterol levels significantly. They also found that alcoholic extract of neem leaves reduced serum cholesterol by about 30% beginning two hours after administration and kept the levels low for an additional four hours until the test ended.

Wagh (1998) reported that as much as 80% of the test cases of deadly viral hepatitis showed significant improvement when treated with neem leaf.

Udeninya (1994) found in his experiment that the use of neem leaves can prevent the adhesion of concern cell to other cells in the body.

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.

Caldwell (1994) reported that neems have the ability to enhance the cell-mediated immune responses. He also observed that neem could be used as a vaginal lubricant before intercourse to protect the diseases due to vaginal contraction.

An ethanolic extract of nem has been shown to cause cell death of prostate cancer cells (PC-3) by inducing 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 (*Azadirachtaindica*) was found to have antiproliferative activity against some cancer cell lines (Roy *et al.* 2007).

2.3 Medicinal properties of neem leaves in livestock

Pietrosemoli *et al.* (1999) reported that oral administration of neem leaves has an antiparasitic effect on grazing cattle. They found that the addition of neem leaves to the nutritional blocks reduced the number of parasitic egg per gram of faeces of grazing cattle.

Farries (1993-1996) found that the neem oil dilution of 1.0% had the greatest mortality with least egg laying of ticks of cattle. The dilution of 0.1% had least effect on the viability of the eggs laid and had the greatest hatchability, while the dilutions of 0.85 and 1.0% had the greatest effect, with 60-75% of the eggs non-viable.

Kukde *et al.* (1999) conducted an experiment involving 12 calves to investigate the effect of neem leaves powder. They found significant differences in animals' growth and an increase in feed intake. Brelin (2002) studied the effect of fresh neem leaves on sheep. He

reported that fresh neem leaves significantly reduced the number of *Haemonchuscontortus* in the abomasum of the treated sheep. Bais*et al.* (2002) found that both sare and neem leaves can be fed to goats as sole roughage, preferably the sares leaves during the lean period. Arunachal *et al.* (2002) carried out an experiment with 75 lambs naturally infected with gastrointestinal helminthes. They reported that antihelminthic efficacy of neem extracts and proziplus were 53, 49 and 87 percent respectively.

Chandrawathani *et al.* (2006) conducted an experiment on the anthelmintic effect of neem on nematodes of sheep and reported that feeding neem had an effect on worm numbers in sheep, but was not reflected in their faecal egg counts.

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. In some cases due to bad husbandry and poor sanitation in stables, subclinical mastitis is prevalent. While conducting AI (artificial insemination), it was noticed that inspite of repeated AI cows or buffaloes sometimes do not conceive due to subclinical metritis that has remained undetected. Regular intra-uterine use of neem oil@30ml for 3 days has proved very much useful on that case. (http://www.neemtree.info/eng/animal_care.html)

Shukla and Deasi (1988) reported that neem seed cake has not proved successful as cattle feed and its use is not recommended until further work on detoxification and processing has not been completed; adverse effects such as weight loss, gingivitis and diarrhea were found.

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 pyrethrum were significantly less effective than cypermethrin (Heath *et al.* 1995).

2.4 Effect of medicinal properties of neem leaves on rabbit

Khosla *et al.* (2000) conducted an experiment on the hypoglycaemic effects of neem lea extract and seed oil in normal rabbit as well as in diabetic rabbit. They reported that *Azadirachtaindica* could be beneficial in diabetes mellitus in controlling blood sugar or might be helpful in preventing or delaying onset of the diseases.

An enzyme-linked immunosorbent assay (ELISA) was developed for azadirachtin (aza), a biopesticide from the neem tree. The immunogen was synthesized by epoxidation using the furan ring in the aza molecule. Rabbits were immunized with either bovine serum albumin (BSA)-azadirachtin or ovalbumin (OA)-azadirachtin conjugate. Evaluation of the antisera by antibody capture assay showed that the antibody titer of antisera raised against OA-aza was 1:30,000. An indirect competitive ELISA was developed with BSA-azadirachtin as coating antigen and aza-specific antibodies raised against OA-azaimmunogen (Hemalatha *et al.* 2001).

2.5 Effect of medicinal properties of neem leaves on rats

Koley *et al.* (1994) tested mature, green leaves of *Azadirachtaindica* (neem) for its anti-inflammatory activity. They found significant and dose dependent anti-inflammatory activities in rats and mice. They also reported that doses which were sufficient to produce an anti-inflammatory, analgesic or antipyretic action, had no ulcerogenic effect on the gastric mucosa of rats.

Chattopadhyay *et al.* (1992) carried out an experiment where rats fed neem leaf extract at 1g/kg, for 5 days prior to the addition of paracetamol to their diet showed much lower serum levels (53.85-88.00% lower than those recorded for rats fed paracetamol alone). The elevated levels of serum enzymes in rats treated with paracetamol alone were due to induced hepatocellular necrosis; the neem leaves extract afforded protection from this paracetamol-induced liver damage.

Mukhopadhyay *et al.* (1998) found that neem leaf extract was a weak clastogen, but it inhibited the clastogenecity of cyclophosphamide (CP; 10mg/kg) and mitomycin C (MMC; 1.5mg/kg). An ANOVA test showed that the extract was much more effective in antagonizing the clastogenic potential of CP. MMC co-administered with the extract showed a trend that was not statistically significant.

Seema *et al.* (2000) conducted an experiment that the neem extract decreased the Red Cell Distribution Width (RDW) by 10.63 and 7.19% in male and female rats, respectively, and decreased Haemoglobin Distribution Width (HDW) by 19.86 and 16.60% respectively. Mean Platelet Volume (MPV) in treated male and female rats increased by 18.46 and 14.81% respectively.

Pari *et al.* (2001) found the antihyperglycemic effect of diameda herbal formulation composed of the aqueous extract of three medicinal plants, *Azadirachtaindica*, *Cassia auriculata and Mondicacharantia* in rats with alloxan induced diadetes. They also found that diamed also prevented a decrease in body weight.

Haptinga and Anunciado (2002) conducted an experiment where organ weight analysis showed that the pancreas, liver and kidneys of neem-treated mice were significantly lighter compared with control mice. Neem has a potent effect in normalizing blood and urinary glucose levels and body weight.

Jayakumar *et al.* (2002) carried out an experiment to determine the possible anti-orexigenic effect of neem leaf extract (NLE) in rats by giving 500mg/kg body weights did not affect the feed intake and live weight of rats.

Baral and Chattopadhyay (2004) found in an experiment that the conditional tumour growth retardation, observed in mice treated with Neem Leaf Preparation (NLP) before tumourinoculation , may be regulated by NLP mediated immune activation, having prominent role in the cellular immune function of the tumour host.

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.

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 colorectal cancer specimens, by anti-CEA monoclonal antibody and NLP sera was documented by immunohistochemistry (Sarkar *et al.*, 2007)

2.6 Medicinal properties of neem leaves and its effects on poultry

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

leaves may be useful for treatment of immune-suppressive diseases, such as infectious bursal Disease (IBD) and Newcastle disease (ND) in broilers.

Nagalakshmi *et al.* (1998) reported 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 diet of broilers didn't depress the feed intake and growth rate of broiler chicks.

Adekanye and sonaiya (1992) conducted a feeding trial to test the responses of layers to three dietary treatments (T_1 -without neem leaves, T_2 -10% 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.

Gowda (1998) conducted an experiment with the incorporation of neem kerenel meal (NKM) into a standard layer diet at 0, 100, 150 and 200 g/kg, replacing part of the soybean meal and de-oiled rice bran. Result indicated significantly lower food intakes ($P<0.01$), rates of egg production and egg weights in birds fed on the diets with NKM at 150 and 200 g/kg. Fertility and hatchability were also adversely affected by the higher inclusion rates of NKM.

Esonu *et al.* (2006) carried out an experiment to evaluate the effect of neem (*Azadirachtaindica*) 0n 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 weights were significantly ($P<0.05$) increased at 5% dietary level of NLM.

Sridhar *et al.* (2003) carried out an experiment in which neem leaf extract (NLE) treated birds showed mild depression and lesser body weight gain after 4 weeks and onwards. Haematological observations revealed lower weeks post treatment. However, there were no significant changes in Total Leukocyte Count (TLC) and Direct Leukocyte Count (DLC) in treated birds.

Chowdhury *et al.* (2004) reported that NLM at a dietary level of 40g/kg with or without supplementation of protexin, a probiotic significantly decreased live weight and FCR didn't differ significantly when NLM were used up to 20g/kg level. But the meat quality

didn't differ significantly and no bitter taste was found in broiler meat when neem leaf meal was used at 20g/kg feed.

The neem aqueous extract is well tolerated by the broilers and there is no physiological alteration in protein turnover in the liver of the neem extract fed birds (Meenakshi *et al.* 2005).

The effect of neem oil (azadirachtin), originating from the tree *Azadirachtaindica*, was investigated as a potential compound to control the poultry red mite, *Dermanyssusgallinae*. In vitro tests were performed to determine the most appropriate formulation of neem extracts and concentration of the substance to be used. A 92% reduction of *D. gallinae* was recorded (Lundh *et al.* 2005).

2.7 Chemical composition of neem leaves

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

Chemical components (%)	Vietmeyer (1992)	Chaudhary *et al.*(1999)	Sonaiya & Olori (1989)	Bais*et al.* (2002)	Esonu*et al.* (2005)	Laboni (2006)
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	3.81	4.20	2.42	4.13	6.54
Nitrogen free extract	56.40	49.63	-	-	43.91	39.11

2.8 Research gap and the recent study

Considering the valuable pysio-chemical and medicinal properties of Neem Leaf, the use of NLE in the drinking water of commercial broiler may be relevant to gain high performance and provide safe food for human being by discouraging the use of different types of medicines specially antibiotics having residual effects on human. One of the

major constraints to broiler farmers is undoubtedly the existence of various diseases mainly Newcastle Disease (ND) and Infectious Bursal Disease (IBD) or Gumboro. This diseases seriously affect the broiler production and the birds are seldom vaccinated. On the other hand plant products like neem are locally available and free or very cheap to the farmers. This certainly contains immunity enhancing and other beneficial properties. Some research works have been conducted in Bangladesh to observe its effect but certainly further research in this field is required for understanding whether and when traditional practices are effective and should be used for broiler production and when modern veterinary medicine offers a better alternative. Moreover, research works are needed under controlled conditions on the efficacy rates and veterinary properties of such plant products and treatments. Broiler farming is the most dynamic farming which is changing its farm management, disease managements and treatments, quality of chicks and feeds frequently and that is why studies and research data of the most recent broiler farming are very much necessary to cope with the new emerging problems.

So, it seemed worthwhile to investigate the effect of Neem Leaf Extract (NLE) in the drinking water on the performance of modern commercial broilers.

CHAPTER-III
MATERIALS AND METHODS

3.1 Statement of Research Work

The experimental work was conducted at a poultry farm in Dhupchachia, Bogura to investigate the effect of administering Neem Leaf Extract (NLE) in drinking water on the performance of broiler chicks (Lohman meat). The trial was conducted from 1stoctober to 5th November, 2018.

3.2 Collection, processing, preparation and storage of neem leaf extract (NLE)

Fresh green neem leaves were hervested from neem tree grown locally in the experimental area of Dhupchachia, Bogura to produce a 5% (w/v) concentrated neem leaf infusion. Neem leaves were washed, separated and then sundried. Dry leaves were then ground and 50 g of dried ground leaves were taken in a non-metallic jar. One liter of hot boiled distilled water was poured on it and kept at room temperature for 8 hours to prepare an infusion. The mixture was sieved through a Buchner funnel to remove debris and a clear extract was found. For storage NLE was kept in dark bottle maintaining 4°C temperature in refrigerator.

3.3 Preparation of the experimental house and equipment

The experimental house was divided into 12 small pens of equal size (6 ft × 2.5 ft= 15 sq. ft) for 10 birds in every pen. As a result the floor space for each bird was 1.5 sq. ft. In commercial broiler farming in Bangladesh each bird is given 1.1-1.3 sq. ft space according to season. But for small confined pens in this experiment we provided more space for each bird. The experimental house was properly cleaned and washed by forced water using a hose-pipe. After two weeks the room was disinfected with Virocid (6ml/1l water). At the same time all feeders, plastic buckets, waterers and other necessary equipments were also properly cleaned ,washed and disinfected with bleaching powder solution and virocid solution , subsequently dried and left them empty for two weeks before the arrival of chicks.

3.4 Source of feed

The experimental broiler chicks were supplied Broiler starter feed from day 1 to day 15 and broiler grower feed from day 16 to last day of the experiment. Both the starter and grower feed was collected from Aman feed limited. The starter feed was in crumble and grower feed was in pellet form. The nutritional value of the Aman Broiler Feed was collected from the company profile that is presented in Table 3.1

Table 3.1 Nutritional value of Aman Broiler Feed

Nutritional Ingredients	**Starter feed**	**Grower feed**
Moisture % (maximum)	11	11
Crude protein %(minimum)	22.5	21
Metabolic Energy (minimum) Kilocal/kg	3000	3100
Crude Fiber% (maximum)	3	3
Fat% (minimum)	4-5	5-6
Calcium%(minimum)	1	0.96
Phosphorus%(minimum)	0.50	0.48
Methionine%(minimum)	0.50	0.48
Lysine%(minimum)	1.3	1.25
Methionine+Cystine(minimum)	0.89	0.84
Sodium%(minimum)	0.20	0.17
Chloride%(minimum)	0.2	0.2
Vitamins and minerals	Standard level	Standard level

3.5 Collection of experimental birds

One hundred twenty Lohman meat broiler chicks (Day old) were procured from Aman Poultry and Hatchery limited.

Fig.1: Collected fresh neem leaves.

3.6 Layout of the experiment

The chicks were randomly distributed into 4 treatment groups A, B, C and D with three replications in each treatment. The number of birds in each replication was 10. Group A, B and C was given 5% neem infusion @ 24, 32 and 40 ml/liter of drinking water respectively, and group D was kept as control. The layout of experiment is shown in Table 3.2.

Table 3.2 Layout showing the distribution of experimental birds

Treatments	Number of birds in each replication			Total
	R_1	R_2	R_3	
Group A (5% NLE @ 24 ml/L water)	10	10	10	30
Group B (5% NLE @ 32 ml/L water)	10	10	10	30
Group C (5% NLE @ 40 ml/L water)	10	10	10	30
Group D (Control)	10	10	10	30
Total number of birds	40	40	40	120

NLE= Neem Leaf Extract

3.7 Management of experimental birds

The care and management practices were followed according to following description throughout the experimental period. All the management practices were identical to all treatment groups during the experiment.

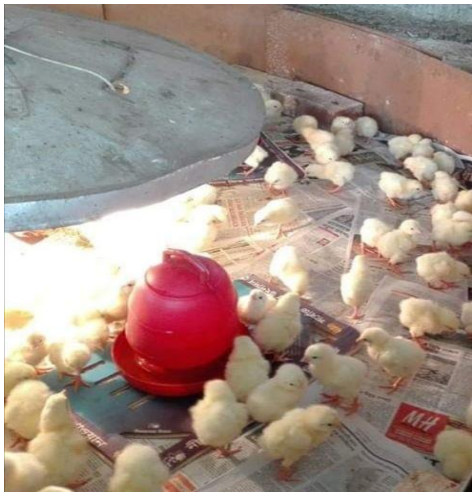


Fig. 2: Brooding of experimental chicks

3.7.1 Feed and water management

The experimental birds were given Aman broiler starter feed from day 1 to day 15 and Amanbroiler grower feed from day 16 to day 35. During the first three days chicks were given feed on newspaper at three hours interval and then on tray feeders up to 9 days old. From day 10, every small pen was provided with a round feeder. Feed was given four times from day 4 to day 14. From day 15 feed was given thrice a day. Water was served *ad libitum*four times a day with a round drinker in each replication. Feeders and waterers were set up in such a way that the birds were able to reach them conveniently. Feeders were cleaned at the end of each week and waterers were cleaned twice daily.

Fig.3: Feeding and water management

3.7.2 Litter management

Fresh, clean and dried rice husk was used as litter materials at a depth of 2 inch. The litter of each pen was covered with clean newspaper up to day 10. At day 15 the upper part of the litter with droppings were removed and regularly stirred. The litter material was disinfected with Virocid spray (3 ml/L water) in every alternate day. Litter materials, when found wet for any reason, were removed to prevent dampness that could accelerate ammonia and other harmful gases**.**

3.7.3 Brooding management

During brooding period the chicks were provided heat by electric bulbs. A 100 watt bulb was hung in each replication. The temperature was maintained at 34 ^{0}C during the first week and then gradually decreased. The electric bulbs were hanged just above the birds level and were moved up and down to adjust the heat. The room temperature and humidity were measured by an automatic digital thermo-hygrometer. For maintaining room temperature the house was covered by cloth curtain leaving 6 inch to 1 feet gap at the top for ventilation purpose.

Before placing the birds into brooder the temperature was adjusted at optimum level by preheating the brooder about 3 hours earlier of the chicks arrival.

3.7.4 Lighting

The house was provided a light period of 24 hours during first three weeks. After day 21 the light period was 23 hours per day.

3.7.5 Immunization

The experimental birds were vaccinated according to the following schedule of table 3.3

Table 3.3 Vaccination program of the experimental birds.

Age of the bird (Day)	Name of vaccine	For specific disease	Route of administration
3	Cevac BI L	Bronchitis and Newcastle Disease	Eye drop
10	Cevac IBD L	Gumboro	Eye drop
18	Cevac IBD L	Gumboro	Eye drop
21	Cevac NEW L	Newcastle Disease	Eye drop

Vaccines were procured from ACI Animal Health Ltd.

3.7.6 Medication

Besides NLE in drinking water no other antibiotics or growth promoter was used during the experiment. The day old chicks were supplied glucose and vitamin C for 6 hours at a dose of 50 gm per litre drinking water as they were stressed due to journey. The adult birds were supplied saline and vitamin C to reduce stress whenever the mid day temperature was too high.

3.7.7 Sanitation

Adequate hygiene and sanitation were maintained during the experimental period. The entrance point and surroundings of the farm were kept clean and Virocid solution were sprayed on daily basis.

3.7.8 Bio-security

Strict biosecurity measures were taken during the experiment. Equipments were cleaned and disinfected regularly. Entrance of people were restricted except relevant personnel's. Before entrance hands were washed with soap and separate shoes were used. Virocid spray was used for disinfection. Adequate precautions were taken in case of vaccination. Dead birds were buried away from the farm and sick birds were isolated immediately to a separate place from the experimental pens. The farm was kept free of rats, cats, dogs and wild animals.

3.8 Postmortem examination of birds

Incase of dead birds postmortem examination was performed promptly and based on the postmortem lesions necessary measures were taken to remove the problem without applying medications.

3.9 Data collection and record keeping

The following data was recorded throughout the experimental period.

3.9.1 Body weight

The chicks of each replication were weighed at beginning of the experiment. After that birds of all replications were weighed every week in the morning at 7 AM prior to feeding and finally weight was taken at day 35. Replication-wise weekly average body weight was recorded.

3.9.2 Body weight gain

The average body weight gain of birds of each replication was calculated by deducting initial body weight from the final body weight.

3.9.3 Feed consumption

The amount of feed consumed by the birds of different replications were calculated from the amount of supplied feed at each week and the amounts that were retained at the end of the week. Feed intake was adjusted for the birds which died during the experiment by necessary calculation.

3.9.4 Feed Conversion Ratio (FCR)

The feed conversion ratio was calculated by dividing the cumulative feed consumption by average body weight up to certain period of production.

3.9.5 Temperature and relative humidity

The temperature and relative humidity of the experimental house and respective pens were recorded four times a day during the whole experimental period at 6 am, 12 pm, 6pm and 12 am with the help of an automatic digital thermo-hygrometer.

3.10 Statistical analysis

Data were analyzed by Analysis of Variance using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986). The significance differences between the treatment means were calculated by the Duncan's Multiple Ran^{g}e Test (Duncan, 1955). All analysis were performed by SPSS Program.

CHAPTER-IV
RESULTS AND DISCUSSION

4.1 Performance of broiler

The results of productive performance in term of body weight gain, feed consumption, feed conversion ratio, mortality and morbidity of birds supplied 5% Neem Leaf Extract (NLE) in drinking water at different level (24, 32 and 40 ml/L drinking water) are presented and discussed in the following sections.

Table 4.1 Productive performance of broilers receiving Neem Leaf Extract (NLE) in drinking water (0-35 days)

Variables	Group A (5% NLE @ 24 ml/L water)	Group B (5% NLE @ 32 ml/L water)	Group C (5% NLE @ 40 ml/L water)	Group D (Control)	Sig.
Initial body weight (g/bird)	40 ^{a}±1.00	40 ^{a}±1.00	40.33 ^{a}±0.58	40 ^{a}±1.00	NS
Final body weight (g/bird)	1902 ^{a}±33.65	1933.33 $^{a\ b}$±20.82	2010^{b}±65.57	1840 ^{a}±60.83	*
Body weight gain (g/bird)	1862 ^{a}±33.45	1893.33$^{a\ b}$±21.08	1969.67 ^{b}±65.12	1800 ^{a}±61.65	*
Cumulative feed consumption (g/bird)	3172 ^{a}±33.05	3138.33 ^{a}±68.25	3060 ^{a}±60	3177.67 ^{a}±101.1	NS
FCR	1.68 ^{bc}±0.066	1.62 ^{b}±0.025	1.52^{a}±0.023	1.73^{c}±0.101	*

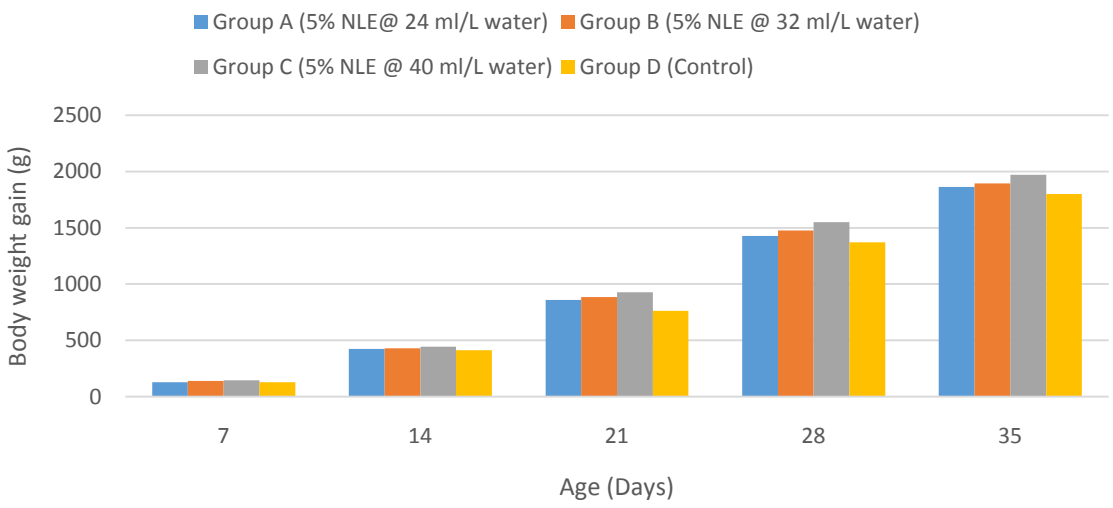
Values of different variables under different programs indicate average ±SD, NS= Non-significant, * = Significant at the 0.05 % level.

4.1.1 Body weight gain

Table 4.1 shows the differences in final body weight and body weight gain of broilers receiving 5% (w/v) Neem Leaf Extract (NLE) in drinking water (0-35 days) in Group A (5% NLE @ 24 ml/L water), Group B (5% NLE @ 32 ml/L water), Group C (5% NLE @ 40 ml/L water) and Group D (Control). The initial body weight of day old broiler chicks were almost similar (P>0.05). But the final body weight of Group A, B, C and D were

1902±33.65, 1933.33±20.82, 2010±65.57 and 1840±60.83 g respectively (table 4.1). We can see that group C significantly (P<0.05) has higher body weight gain than rest of the three groups. An increasing trend was found in body weight gain with increased levels of neem leaves infusion. The results of present study are in agreement with the study of Pagrut *et al* (2018), who reported that 4% Neem Leaf Infusion at 50ml/L drinking water had higher (P<0.05) body weight gain than control. Similar findings have been reported by Tipu *et al*. (2002), who used salinomycine and neem (*A. indica*) fruit as feed additive and anticoccidial in broilers and reported better results in terms of weight gain. The higher body weight gain in broilers consuming neem leaves infusion could be due to its diversified effect on intestinal micro flora, thereby avoiding stressful conditions. Figure 1 shows the growth pattern of broilers receiving NLE in Drinking water.

Fig.4 Growth pattern of broilers receiving NLE



4.1.2 Feed consumption

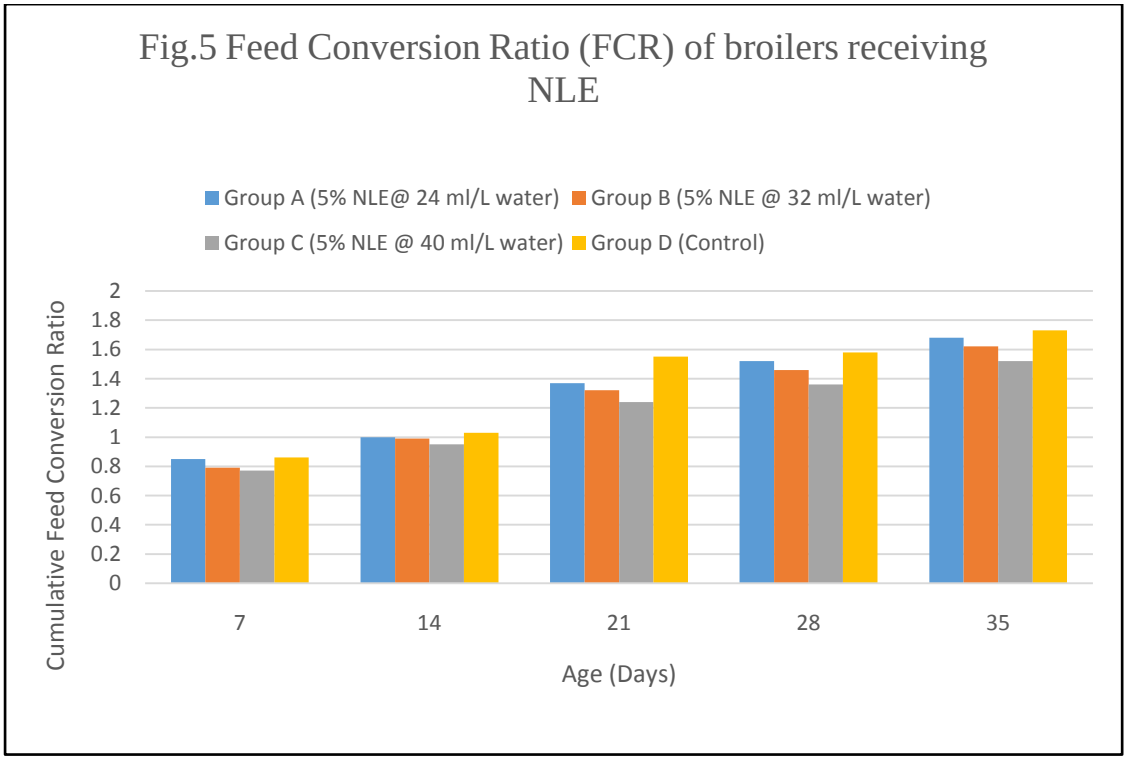
Mean feed intake for group A, B, C and D was 3172, 3138.33, 3060 and 3177.67 g respectively (Table 4.1). Feed intake for group D (Control) was higher than group A, B and C (Table 4.1). Group Chad the lowest feed intake. But these differences of feed consumption of different treatment groups were not significant. No effect of NLE was apparent in the feed consumption of broilers. But it was not in agreement with Pagrut *et*

al. (2018) who reported that mean feed consumption were higher in control and it was significant (P<0.05).

Chowdhury *et al.* (2004) stated that application of NLM at a dietary level of 40g/kg with or without supplementation of protexin, remained the feed consumption unaffected. Nagalakshmi *et al.* (1998) carried out an experiment with neem seed kernel-cake in the diet of broiler and found that it didn't depress the feed intake.

4.1.3 Feed Conversion Ratio

In table 4.1 we see that there is significantly better (P<0.05) feed conversion ratio for group C as compared to the control and other two groups. FCR values at the end of the trial (day 35) of Group A, B, C and D were 1.68±0.066, 1.62±0.025, 1.52±0.023 and 1.73±0.101respectively which suggested economical gains supplemented with neem leaves infusion. This was in agreement with Pagrut *et al*. (2018) who found better (P<0.05) FCR in 4% Neem Leaf Infusion at 50ml/L drinking water of broilers. Chakeravarty and Prasad (1991) also reported that better feed conversion ratio of broilers was found when supplied commercial ration and water containing neem (*A. indica*) infusion than others. In figure 2 the comparative FCR values of different NLE treatments are demonstrated.



4.1.4 Mortality

The mortality in all treatment groups were zero. The livability was 100%. This might be due to proper care and management and the enhanced immunity gained from the experimental treatments of NLE.

CHAPTER-V
SUMMARY AND CONCLUSION

An experiment with 120 day-old broiler chicks (Lohman Meat) was conducted at a poultry farm in Dhupchachia, Bogura to investigate the effect of administering Neem Leaf Extract (NLE) in drinking water on the performance of commercial broiler chicks. The trial was conducted from one-day old to 35 days (1^{st}october to 5^{th} November, 2018). The chicks were randomly distributed to 4 different treatment groups and each group had 3 replications. Each replication had 10 birds in small pen. The groups were named as Group A (5% NLE @ 24 ml/L water), Group B (5% NLE @ 32 ml/L water), Group C (5% NLE @ 40 ml/L water) and Group D (Control). Feed and water were provided *ad libitum* to all birds throughout the experiment. The birds of all treatments were reared under similar care and management.

The body weight gain of different groups at day-35 were 1862, 1893.33, 1969.67 and 1800 g in Group A, B, C and D respectively. Group C (5% NLE @ 40 ml/L water) had significantly ($P<0.05$) higher body weight gain than control. The cumulative feed consumption of Group A, B, C and D were 3172, 3138.33, 3060 and 3177.67g respectively. Group D (control) had higher feed intake than other groups but the differences were non-significant ($P>0.05$). At the end of the experiment FCR values of Group A, B, C and D were found as 1.68, 1.62, 1.52 and 1.73 respectively. Group C (5% NLE @ 40 ml/L water) had significantly better ($P<0.05$) FCR value than control (Group D) and other groups. The mortality in all treatment groups were zero and livability was 100%.

From the results of the present research study it can be concluded that5% Neem Leaf Extract @ 40ml/L of fresh drinking water could be effectively used as a potential natural growth promoter contributing to better body weight gain and FCR.

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APPENDICES

Appendix1: Body weight (g/bird) of broilers at different treatments

Treatments	Replication	Age(days)					
		D-1	**D-7**	**D-14**	**D-21**	**D-28**	**D-35**
Group A (5% NLE @ 24 ml/L water)	**R_1**	41	170	465	912	1477	1890
	R_2	39	165	470	900	1450	1876
	R_3	40	168	460	885	1480	1940
Mean		**40**	**167.67**	**465**	**899**	**1469**	**1902**
Group B (5% NLE @ 32 ml/L water)	**R_1**	41	180	470	920	1500	1940
	R_2	39	175	472	905	1550	1950
	R_3	40	181	465	950	1500	1910
Mean		**40**	**178.67**	**469**	**925**	**1516.67**	**1933.33**
Group C (5% NLE @ 40 ml/L water)	**R_1**	41	188	485	950	1600	2070
	R_2	40	184	480	980	1590	2020
	R_3	40	180	490	970	1580	1940
Mean		**40.33**	**184**	**485**	**966.67**	**1590**	**2010**
Group D (Control)	**R_1**	40	170	460	800	1400	1800
	R_2	41	167	445	820	1405	1810
	R_3	39	165	450	790	1425	1910
Mean		**40**	**167.33**	**451.67**	**803.33**	**1410**	**1840**

Appendix 2: Body weight gain (g/bird) of broilers of different treatments

Treatments	Replication	Age(days)				
		0-7	**0-14**	**0-21**	**0-28**	**0-35**
Group A (5% NLE @ 24 ml/L water)	$\mathbf{R_1}$	129	424	871	1436	1849
	$\mathbf{R_2}$	126	431	861	1411	1837
	$\mathbf{R_3}$	128	420	845	1440	1900
Mean		**127.67**	**425**	**859**	**1429**	**1862**
Group B (5% NLE @ 32 ml/L water)	$\mathbf{R_1}$	139	429	879	1459	1899
	$\mathbf{R_2}$	136	433	866	1511	1911
	$\mathbf{R_3}$	141	425	910	1460	1870
Mean		**138.67**	**429**	**885**	**1476.67**	**1893.33**
Group C (5% NLE @ 40 ml/L water)	$\mathbf{R_1}$	147	444	909	1559	2029
	$\mathbf{R_2}$	144	440	940	1550	1980
	$\mathbf{R_3}$	140	450	930	1540	1900
Mean		**143.67**	**444.67**	**926.33**	**1549.67**	**1969.67**
Group D (Control)	$\mathbf{R_1}$	130	420	760	1360	1760
	$\mathbf{R_2}$	126	404	779	1364	1769
	$\mathbf{R_3}$	126	411	751	1386	1871
Mean		**127.33**	**411.67**	**763.33**	**1370**	**1800**

Appendix 3: Cumulative feed consumption (g/bird) of broilers of different treatments

Treatments	Replication	Age(days)				
		0-7	**0-14**	**0-21**	**0-28**	**0-35**
Group A (5% NLE @ 24 ml/L water)	$\mathbf{R_1}$	140	460	1200	2245	3156
	$\mathbf{R_2}$	144	469	1230	2255	3210
	$\mathbf{R_3}$	142	465	1250	2190	3150
Mean		**142**	**464.67**	**1226.67**	**2230**	**3172**
Group B (5% NLE @ 32 ml/L water)	$\mathbf{R_1}$	145	460	1250	2240	3200
	$\mathbf{R_2}$	140	465	1210	2210	3150
	$\mathbf{R_3}$	140	470	1205	2200	3065
Mean		**141.67**	**465**	**1221.67**	**2216.67**	**3138.33**
Group C (5% NLE @ 40 ml/L water)	$\mathbf{R_1}$	143	455	1207	2116	3120
	$\mathbf{R_2}$	140	460	1200	2227	3060
	$\mathbf{R_3}$	141	465	1190	2150	3000
Mean		**141.33**	**460**	**1199**	**2164.33**	**3060**
Group D (Control)	$\mathbf{R_1}$	140	470	1250	2250	3149
	$\mathbf{R_2}$	145	465	1260	2260	3290
	$\mathbf{R_3}$	148	460	1230	2185	3094
Mean		**144.33**	**465**	**1246.67**	**2231.67**	**3177.67**

Appendix 4: Cumulative feed conversion ratio of broilers at different treatments

Treatments	Replication	Age(days)				
		0-7	**0-14**	**0-21**	**0-28**	**0-35**
Group A (5% NLE @ 24 ml/L water)	$\mathbf{R_1}$	0.82	0.99	1.32	1.52	1.67
	$\mathbf{R_2}$	0.87	1.00	1.37	1.56	1.75
	$\mathbf{R_3}$	0.85	1.01	1.41	1.48	1.62
Mean		**0.85**	**1.00**	**1.37**	**1.52**	**1.68**
Group B (5% NLE @ 32 ml/L water)	$\mathbf{R_1}$	0.81	0.98	1.36	1.49	1.65
	$\mathbf{R_2}$	0.80	0.99	1.34	1.43	1.62
	$\mathbf{R_3}$	0.77	1.01	1.27	1.47	1.60
Mean		**0.79**	**0.99**	**1.32**	**1.46**	**1.62**
Group C (5% NLE @ 40 ml/L water)	$\mathbf{R_1}$	0.76	0.94	1.27	1.32	1.51
	$\mathbf{R_2}$	0.76	0.96	1.22	1.4	1.51
	$\mathbf{R_3}$	0.78	0.95	1.23	1.36	1.55
Mean		**0.77**	**0.95**	**1.24**	**1.36**	**1.52**
Group D (Control)	$\mathbf{R_1}$	0.82	1.02	1.56	1.61	1.75
	$\mathbf{R_2}$	0.87	1.04	1.54	1.61	1.82
	$\mathbf{R_3}$	0.90	1.02	1.56	1.53	1.62
Mean		**0.86**	**1.03**	**1.55**	**1.58**	**1.73**