COMBINED EFFECT OF NEEM LEAVES AND APPLE CIDER VINEGAR ON GROWTH AND ANTICOCCIDIAL EFFECT IN BROILER

LIMA KHUNDOKER

DEPARTMENT OF ANIMAL NUTRITION, GENETICS AND BREEDING
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

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BY

LIMA KHUNDOKER

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APPROVED BY:

Prof. Dr. Md. Mufazzal Hossain Supervisor Department of Animal Nutrition, Genetics and Breeding. Sher-e-Bangla Agricultural University, Dhaka-1207	**Associate Prof. Dr. Mofassara Akter** Co-Supervisor Department of Animal Nutrition, Genetics and Breeding. Sher-e-Bangla Agricultural University, Dhaka-1207

Associate Prof. Dr. Mofassara Akter
Chairman
Examination Committee
Department of Animal Nutrition, Genetics and Breeding
Sher-e-Bangla Agricultural University,
Dhaka-1207

Professor Dr. Md. Mufazzal Hossain
Dean (January 2015 to January 2017)
Faculty of Animal Science & Vet. Medicine
Former Chairman
Dept. of Animal Nutrition, Genetics & Breeding
Ex-Proctor
Sher-e-Bangla Agricultural University, Dhaka-1207

অধ্যাপক ড. মোঃ মোফাজ্জল হোসাইন
ডিন (জানুয়ারি ২০১৫-জানুয়ারি ২০১৯)
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এনিম্যাল নিউট্রিশন, জেনেটিক্স এন্ড ব্রিডিং বিভাগ
প্রাক্তন প্রক্টর
শেরেবাংলা কৃষি বিশ্ববিদ্যালয়, ঢাকা-১২০৭

CERTIFICATE

This is to certify that the thesis entitled, "COMBINED EFFECT OF NEEM LEAVES AND APPLE CIDER VINEGAR ON GROWTH AND ANTICOCCIDIAL EFFECT IN BROILER" Submitted to the Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal science and veterinary medicine, Sher-E-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (MS) in Animal Nutrition embodies the result of a piece of bona fide research work carried out by LIMA KHUNDOKER, Registration No. 19-10098 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged by her.

Prof. Dr. Md. Mufazzal Hossain
Supervisor
Department of Animal Nutrition, Genetics & Breeding
Sher-e-Bangla Agricultural University,
Dhaka-1207

Date:
Place: Dhaka, Bangladesh

Mobile: 01912102104, E-mail: mufazzal_hossain@yahoo.com, mhossain.angb@sau.edu.bd, web: www.sau.edu.bd

Dedicated

To

My Beloved Parents

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The Author

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LIST OF ACRONYMS AND ABBREVIATION

ABBREVIATION		FULL MEANING
A.M	=	Anti meridiem
Avg.	=	Average
BCR	=	Benefit cost ratio
BWG	=	Body weight gain
cm^2	=	Square centimeter
CP	=	Crude protein
DOC	=	Day old chick
DP	=	Dressing percentage
e.g.	=	For example
et al.	=	And others/associates
EU	=	European union
FAO	=	Food and agricultural organization
FI	=	Feed intake
FCR	=	Feed conversion ratio
FI	=	Feed intake
g	=	Gram
i.e.	=	That is
IB	=	Infectious bronchitis
IBD	=	Infectious bursal disease
K Cal	=	Kilo calorie
Kg	=	Kilogram
L	=	Litre
M.S.	=	Master of science
ml	=	Mililitre

LIST OF ACRONYMS AND ABBREVIATION (CONT'D)

ABBREVIATION		FULL MEANING
mm	=	Milimetre
LW	=	Live weight
BDT	=	Bangladeshi taka
ACV	=	Apple cider vinegar
DNLP	=	Dry neem leaves powder
No.	=	Number
RH	=	Relative Humidity
ND	=	Newcaste Disease
NS	=	Non-significance
PPB	=	Profit per bird
SAU	=	Sher-e-Bangla agricultural university
SE	=	Statistical Error
SPSS	=	Statistical package for social sciences
Viz.	=	Such as
Vs.	=	Versus
WHO	=	World health organization
Wks.	=	Weeks

LIST OF SYMBOLS

SYMBOLS		FULL MEANING
*	=	5% level of significance
&	=	And
@	=	At the rate of
℃	=	Degree celsius
℉	=	Degree fahrenheit
$	=	Dollar
>	=	Greater than
<	=	Less than
/	=	Per
%	=	Percentage
±	=	Plus-minus
:	=	Ratio

COMBINED EFFECT OF NEEM LEAVES AND APPLE CIDER VINEGAR ON GROWTH AND ANTICOCCIDIAL EFFECT IN BROILER

ABSTRACT

This study was conducted to find out the efficacy of neem leaves and apple cider vinegar on growth and anticoccidial effect in broiler. A total of 120-day old Indian River-Lohmann meat broiler chicks were divided into 4 experimental groups with 3 replicates as 10 chicks in each replication. One of the 4 experimental group fed normal diet without neem leaves and apple cider vinegar (ACV) was as control and the remaining three groups fed diet with neem leaves were T_1 (2g/Kg feed), apple cider vinegar (ACV) T_2 (5ml/L drinking water), combined neem leaves and apple cider vinegar (ACV) T_3 (2g/Kg feed & 5ml/L drinking water). During the experimental periods of 4 weeks, feed intake, body weight gain, feed conversion ratio (FCR), flock uniformity, survivability & coccidial oocyst count were calculated. Growth performance parameters & coccidial oocyst count were significantly ($P<0.05$) affected by experimental diets. Birds fed combined neem leaves and apple cider vinegar (ACV), T_3 gained superior body weights (1734.00±16.37g) compared to control (1581.00±9.54g), T_1 neem (1647.33±10.48g) and other dietary treatments. The mean body weight gains (g) at the 1st, 3rd and 4th week of different treatment groups were significantly higher ($P<0.05$) than control group. The groups fed diets containing combined neem leaves and apple cider vinegar feed (T_3) had better FCR (1.32±0.01) compared to control (1.51±0.00). Highest (100%) survivability was found in T_3 group than control (90%) group. The inclusion of different dietary treatments had significant ($P<0.05$) difference on coccidial oocyst count. However, T_3 group had the better (3.00±1.15) oocyst count compared to control (22.33±1.45). It is concluded that combined neem leaves & apple cider vinegar can be included in broiler diet for better performance with better FCR & lower coccidial oocyst count.

CHAPTER 1

INTRODUCTION

1.1 Background

Poultry industry is one of the important industries in Bangladesh in terms of employment avenue and source of protein supply at a cheaper price for the nation. Commercial poultry production has been growing rapidly in Bangladesh since early 1990 by using improved genetics, manufactured feeds and management. Bangladeshis consume 6.3kg broiler meat per capita per year out of total consumption around 40% share of broiler meat. This profitable business is responsible for meeting up unemployment problem in young generation as we as for growing 198 registered commercial feed mills as per DLS which collectively produces 5.3 – 5.4 million metric ton. According to recent statistics, total poultry population in our country is 3470.35 million of which about 2892.83 million chicken and 577.52 million duck (DLS, 2019).

Poultry meat alone contributes 37% of the total meat production in Bangladesh. Poultry contributes about 22-27% of the total animal protein supply in the country (Prabakaran *et al*., 2003). The progress of the poultry industry in Bangladesh is mainly in the private sector. In the early 90s, a number of private parent stock poultry farms started their operations to produce commercial broiler and layer day-old chicks. During 1970-80, the poultry population growth rate was 0.7% which increased to 4% per year during 1990-2005. Since 1995, a significant annual average growth rate of 15-20% in commercial poultry has been achieved until 2007 and slow down after due to avian influenza outbreak (Da Silva *et al*., 2014).

In Bangladesh, the per capita requirements of meat and eggs are 120 g/day and 104 eggs/ year, respectively however the average per capita availability of meat and eggs are 124.9 g/day and 103.8 eggs/ year (DLS, 2019). The demand of meat consumption per head almost able to fulfill the requirement but egg consumption still lack 0.2%. Poultry can play a pivotal role to retain in meat production level and to achieve the expected egg production.

Chicken is the most common type of poultry in the world. Owing to the relative ease and low cost of raising them in comparison to animals such as cattle or hogs, chickens have become prevalent throughout the cuisine of cultures around the world, and their meat has been variously adapted to regional tastes. Total output from poultry is coming from broiler sector because of its commercialization and also rapid return to the farmers.

1.2 State of the problems

Coccidiosis is a disease of universal importance in poultry production. It has been attributed to the loss of about 1 to 3 US billion dollars annually, on the global record within the poultry industry (Muthamilselvan *et al*., 2016, Cardenus *et al*., 2017). It is caused by gut parasites of the genus Eimeria. The protozoan parasites of the genus Eimeria multiply in the intestinal tract and cause tissue damage with resulting interruption at feeding and digestive processes or nutritional absorption dehydration, blood loss, loss of skin pigmentation and increased susceptibility to other disease agents. Anticoccidial chemicals, coccidiocides, coccidiostats and ionophores have been used as a conventional strategy to control the coccidiosis in modern poultry production (Amare *et al*., 2012, Ritzi *et al*., 2014). Due to the presence of drug resistance and public demands for residue free meat still encourage the development of alternative control strategies (Amare *et al*., 2012). Apple Cider Vinegar and Neem Leaves are widely used as a natural anticoccidial to control and manage the coccidiosis. Apple Cider Vinegar (ACV), which is the most commonly manufactured vinegar globally, contains various organic acid (OA) and phenolic substances. The highest concentration of OA and phenolic agent in ACV are acetic acid (Nater *et al*., 2000) and chlorogenic acid (Budak *et al*., 2011) respectively. Neem has been used since in the folk medicine to treat various parasitic infection of man and animal (Nadkarni *et al*., 1976). Natural products are emerging as an attractive way to control and manage the coccidiosis.

Coccidiosis is one of the most expensive and common diseases of poultry production systems in spite of advances in chemotherapy, management, nutrition and genetics (McDougald *et al*., 1987). It remains a big concern for the commercial chicken production because of the high costs to control the disease. Williams (1999) estimated that the total cost of chicken coccidiosis control in UK in 1995 at £38.5 million. McDougald (2003) reported that the current expense for preventive medication exceeds

$90 million in the US and more than $300 million worldwide. Coccidiosis may strike any type of poultry in any type of facility (McDougald, 2003).

Coccidiosis is a major parasitic disease of poultry caused by an Apicomplexan protozoan belonging to the subclass productivity, and high medical cost. Anticoccidial drugs are commonly used to prevent and treat coccidiosis. However, indiscriminate and long-time use of anticoccidial drugs has led to the emergence of drug resistant parasites and presence of residual drugs in chicken products raising concerns about public health and food safety. According to Yang *et al*., (2016) anticoccidial vaccines are an alternative means to prevent coccidiosis. However, efficacy, safety, and cost-effectiveness are still challenging for anticoccidial vaccine use in poultry. Consumers and poultry farmers around the world have voiced concerns about the use of present anticoccidial agents. Therefore, there is an expedient need for an alternative approach to prevent and treat avian coccidiosis necessitating an examination of the potential of natural products from plant extracts.

1.3 Justification of study

The prophylactic use of anticoccidial chemicals in European countries as feed additives has been regulated strictly since 2006. Natural products are emerging as an attractive way to control and manage the coccidiosis. Currently, there are at least four plant products, which are available commercially in the market that can be used as anticoccidial feed additives in chickens and/or other animals (Muthamilselvan *et al.* 2016). The resistance of avian coccidia to drugs has been increasing dramatically. The limitation in the treatment and the rising public concern about drug remains in chicken meat have stimulated the search for new methods to control coccidiosis (Innes and Vermeulen, 2006,-; Tan, 2005,-; Lillehoj *et al.*, 2007). It has been evaluated that the production of flavoured vinegar was initiated 5000 years back. The Babylonians have produced and traded vinegars in the 6th century, along with different flavours, which mainly include honey, malt, and fruit, medical practitioners have mainly indicated that vinegar is helpful for treating various disorders, which include high fever, stomach ache, croup, poison ivy, and oedema (Budak *et al.*, 2014).

Neem (*Azadirachta indica)* is a member of the Meliaceae family and its role as health promoting effect is attributed because it is rich source of antioxidant. It has been widely

used in Chinese, Ayurvedic, and Unani medicines worldwide especially in Indian Subcontinent in the treatment and prevention of various diseases. Earlier finding confirmed that neem and its constituents play role in the scavenging of free radical generation and prevention of disease pathogenesis. The studies based on animal model established that neem and its chief constituents play pivotal role in anticancer management through the modulation of various molecular pathways including p53, pTEN, NF-κB, PI3K/Akt, Bcl-2, and VEGF. It is considered as safe medicinal plants and modulates the numerous biological processes without any adverse effect.

Therefore, combined effect of neem leaves and apple cider vinegar on growth and anticoccidial effect in broiler were measured in this study.

1.4 Objectives

- To determine the growth performance of broiler by using Apple Cider Vinegar and Neem Leaves
- To find out natural alternative to measure anticoccidial effect on broiler production

CHAPTER 2

REVIEW OF LITERATURE

It is essential to review the previous research works which are related to the proposed study before conducting any type of survey or experiment. The literature reviewed here have been limited to those which are considered pertinent and related to the objectives of the present study.

The Babylonians have produced and traded vinegars in the 6th century, along with different flavours, which mainly include honey, malt, and fruit, medical practitioners have mainly indicated that vinegar is helpful for treating various disorders, which include high fever, stomach ache, croup, poison ivy, and oedema (Budak *et al*., 2014). Therefore, the anticoccidial effect of apple cider vinegar, added to broiler water, was measured in this study, along with the variations in the blood antioxidant capacity induced by adding apple cider vinegar to the broiler feed.

2.1 Apple cider vinegar

Vinegar, a kind of acidic condiment, has been used for more than 3000 years (Solieri & Giudici, 2009). Both solid-state and liquid-state fermentation methods are being used in the production of vinegar (Bertelli *et al*., 2015; Huang, *et al*., 2011; Xu, *et al*., 2011). Vinegars are rich in nutrients and bioactive compounds including amino acids, sugars, organic acids, polyphenols, melanoidins, and tetra methylpyrazine (Ho *et al*., 2016; Xia *et al.* 2018). The kinds and concentrations of the substances in vinegars are closely related to the raw materials used and production technology employed, and the chemical reactions, physical changes, and microbial fermentation during the brewing process (Chen, *et al.*, 2016). These functional compounds not only contribute to the flavors of vinegars, but also play important roles in the prevention and treatment of human diseases, through their antibacterial and anti-inflammatory properties (Hindi, 2013).

2.1.1 Composition of apple cider vinegar

The nutritional constituents in vinegars include amino acids, sugars, vitamins, and microelements, (Chen *et al.*, 2015; & Koyama *et al*., 2017), which can maintain acid base balance, regulate cell metabolism, provide energy, and improve the immune system (Budak *et al*., 2014; Shin *et al*., 2016).

Table 1: Composition of apple cider vinegar

Vinegar	Main Material	Fermentation techniques	Country	Detection method	Organic acids	Reference
Apple Vinegar	Apple	Liquid fermentation	China	HPLC	Acetic acid, lactic acid, quinic acid, tartaric acid, malic acid, propanedioic, acid, succinic acid and citric acid	Ren *et al.* (2006)
Apple Vinegar	Apple	Liquid fermentation	Japan	LC-MS	Chlorogenic acid, 4-pcoumaroyl quinic acid, *p*-hydroxybenzoic acid, isomer of chlorogenic acid, protocatechuic acid	Nakamura *et al.* (2010)
Apple Cider Vinegar	Apple	Liquid fermentation	Turkey	HPLC	Gallic acid, catechin acid, chlorogenic acid, caffeic acid, epicatechin and *p*-coumaric acid	Budak *et al.* (2011)

2.1.2 Bioactive components

The bioactive compounds in vinegars consist of organic acids, polyphenols, melanoidins, and tetramethylpyrazine, which exhibit several health benefits, because of their antibacterial and antioxidant activities, aiding in weight loss, blood pressure and glucose control, and expansion of blood vessels (Al-Rousan *et al.*, 2018; Chen *et al.*, 2016; Kondo *et al.*, 2009).

2.1.3 Organic acids

Organic acids in vinegars include volatile organic acids (such as acetic acid, formic acid, propionic acid, butyric acid, and quinic acid) and nonvolatile organic acids (for example lactic acid, malic acid, pyroglutamic acid, citric acid, and succinic acid) (Cocchi *et al*., 2006; Qi *et al*., 2013; and Ren *et al*., 2016)

2.1.4 General uses of apple cider vinegar

- Keeps a body in balance
- Promote water consumption
- Increases egg production
- Increases chicken growth
- Cleaner bumps
- Stress relief for chickens “not feeling on top form”
- Get rid of “microbes” and “nasty toxins”

2.1.5 Medical uses of apple cider vinegar

- Promotes healthy "mucous flow" and therefore "clears the airways" and cures respiratory diseases
- Bad taste seeps through chickens' skin; mites and lice disappear because they hate the taste
- Stops diarrhea
- Prevention of coccidiosis
- Decreases harmful bacteria in the gut
- Increases chicken immune system
- Increases the digestion of nutrients

2.1.6 Nutrient digestion

Because ACV reduces harmful bacteria, it at the same time allows nutrients in the gut to increase. The gut is no longer having to share nutrients with the bacteria. What studies show is that the gut itself then improves. The surface area of the intestine (the "villus") increases, the "intestinal mucosa" which helps preserve the gut's ability to absorb nutrients becomes less inflamed, and the chicken is able to absorb more of her food's nutrients as it passes through the digestive system. This is where the misunderstanding about ACV having benefits for the respiratory system happens. People have seen some of these studies but not understood the terms or the science. Here, the word "mucous" doesn't have anything to do with the chicken's breathing. It's the "mucosa" in the intestine that sees an improvement. So, there is specific, documented evidence that apple cider vinegar with the mother helps chickens digest their food and absorb nutrients more effectively. (F.M.F. Hayajneh *et al*., 2018; and Quiroz-Castaneda *et al*., 2015).

2.1.7 Anticoccidial action of apple cider vinegar

It's been known for some time that the parasite which cause coccidiosis in chickens have become increasingly resistant to drugs. That, together with the public's increasing concern about coccidiosis in meat meant the poultry industry have recently invested in searching for alternatives. They found one in apple cider vinegar (always with the mother). Recent research studies demonstrated that chickens given apple cider vinegar in their drinking water "significantly increased" the percentage of beneficial antioxidants and "significantly decreased" the level of harmful toxic stressors in cells. The result: "In the vinegar group, no clinical signs of coccidiosis were observed. In two control groups, chickens showed clinical signs of coccidiosis and the number of coccidial oocytes in feces increased over time". So, there is specific, researched evidence that ACV does help control coccidiosis. (F.M.F. Hayajneh *et al*., 2018; and Quiroz-Castaneda *et al*., 2015).

2.1.8 Antibacterial action of apple cider vinegar

Other studies, using both adult chickens and day-old chicks followed through to adulthood, have demonstrated the effectiveness of ACV against other bacteria, specifically *Salmonella*, *Campylobacter* and *Escherichia coli* (E-coli). In eliminating those bacteria, it also "enhanced the specific and non-specific immunity in poultry". So,

there is specific, documented evidence that apple cider vinegar with the mother reduces the risk of chickens becoming infected with harmful bacteria and increases their immunity. (F.M.F. Hayajneh *et al*., 2018; and Quiroz-Castaneda *et al*., 2015).

2.1.9 Effect of ACV on chicken growth

Because apple cider vinegar decreases bacteria, which in turn increases the ability to absorb nutrients, there's some evidence that the chicken's general growth over time increases. This is important for the broiler chicken industry where birds are expected to put on growth very quickly. For our backyard chickens, it's clearly a benefit - we want our chickens to reach their full potential - but it's not the most important effect of ACV. The more important benefit is the control of harmful bacteria. (F.M.F. Hayajneh *et al*., 2018; and Quiroz-Castaneda *et al*., 2015).

2.2 Neem

Neem one of the strongest candidates as herbal growth promoter, is known for its antifungal, antifertility, anti-inflammatory, antihypertensive, antioxidant, immune-ostimulant, antigenotoxic, hepatoprotective and antibacterial. Neem leaves and his bark, flowers, seeds and oil has been studied for its medicinal properties. (Mohammad A. Alzohairy , 2016).

Table 2: Taxonomic position of *Azadirachta indica* (neem).

Order	Rutales
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*indica*

2.2.1 Chemical composition of neem leaves:

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

2.2.2 Active compounds of *Azadirachta indica* L. (Neem)

The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7 -benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hy-droxyazadiradione, and nimbiol. Quercetin and ß-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties. (Mohammad A. Alzohairy , 2016).

2.2.3 Pharmacological activities of neem

The main active molecules present in the neem leaves and seeds include the following compounds.

- Nimbin: anti-histamine, anti-fungal, antiinflammatory, antipyretic.
- Nimbidin: antiulcer, analgesic, antibacterial, antiarrhythmic, antifungal
- Ninbidol: antiprotozoan, antitubercular, antipyretic
- Gedunin: antifungal, vasodilator, antimalarial,
- Sodium nimbinate: spermicide, antiarthritic diuretic
- Quercetin: antiprotozoal
- Salannin: insect repellent
- Azadirachtin: antifeedant, antihormonal, insect repellent

2.2.4 Mechanism of action on neem

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

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

Azadirachtin, a complex tetranortriterpenoid limonoid present in seeds, is the key constituent responsible for both antifeedant and toxic effects in insects (Mordue Luntz,

2000). Results suggest that the ethanol extract of neem leaves showed *in vitro* antibacterial activity against both *Staphylococcus aureus* and MRSA with greatest zones of inhibition noted at 100% concentration. (Sarmiento, 2011)

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

2.2.5 Antioxidant properties of neem

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

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

2.2.6 Neem as nutritional and therapeutic supplement in poultry

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

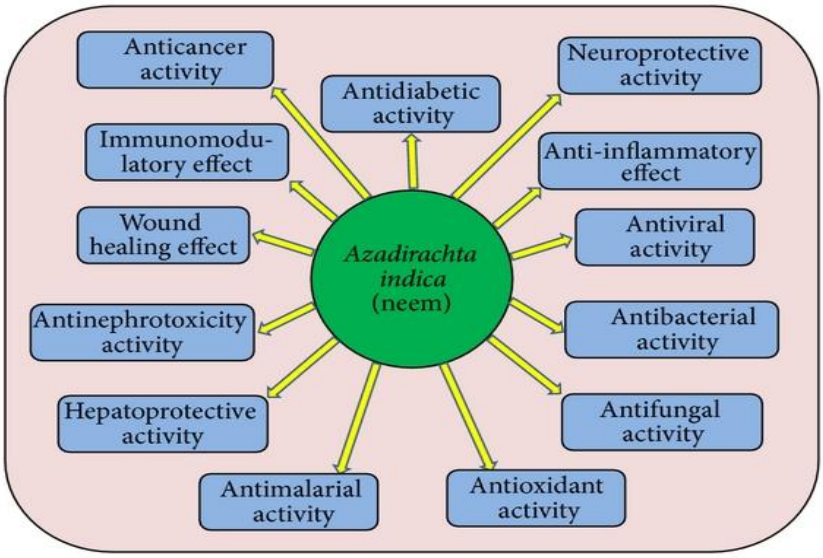

Figure 1. Pharmacological activities of *Azadirachta indica* in diseases management through the modulation of various activities

2.2.7 Effect of neem on internal organs

Medicinal plants and their ingredients play a pivotal role as hepatoprotective without any adverse complications. A study was performed to investigate the hepatoprotective role of azadirachtin-A in carbon tetrachloride (CCl4) induced hepatotoxicity in rats and histology and ultrastructure results confirmed that pretreatment with azadirachtin-A dose-dependently reduced hepatocellular necrosis (Baligar,2014). Furthermore, results of the study show that pretreatment with azadirachtin-A at the higher dose levels moderately restores the rat liver to normal.

Another study was carried out to evaluate the protective effect of active constituent of neem such as nimbolide against carbon tetrachloride (CCl4) induced liver toxicity in rats and results suggest that nimbolide possesses hepatoprotective effect against CCl4 induced liver damage with efficiency similar to that of silymarin standard (Baligar, 2014) and another study finding revealed that leaf extract was found to have protection against paracetamol-induced liver necrosis in rats (Bhanwra, 2000).

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

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

2.2.8 Effect of neem on immune organs

Plants or their isolated derivatives are in the practice to treat/act as anti-inflammatory agents. A study result has confirmed that extract of *A. indica* leaves at a dose of 200 mg/kg, showed significant anti-inflammatory activity in cotton pellet granuloma assay in rats (Chattopadhyay, 1998). Other study results revealed that neem leaf extract showed significant anti-inflammatory effect but it is less efficacious than that of dexamethasone (Mosaddek, 2008) and study results suggest that nimbidin suppresses the functions of macrophages and neutrophils relevant to inflammation (Kaur, 2004). Earlier finding showed immunomodulator and anti-inflammatory effect of bark and leave extracts and antipyretic and anti-inflammatory activities of oil seeds. Experimentation was made to evaluate the analgesic activity of neem seed oil (Arora, 2011; Biswas, 2002) on albino rats and results of the study showed that neem seed oil showed significant analgesic effect in the dose of 1 and 2 mL/kg and oil has dose-dependent analgesic activity (Kumar, 2012).

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

2.2.9 Effect of neem on microbial activity

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

2.2.9.1 Antibacterial activity

In clinical trials neem oil has been shown to inhibit many strains of pathogenic bacteria, such as *Staphylococcus aureus*. Bacteria of some common source of food poisoning and many pus-forming disorders. These bacteria can cause secondary infections in peritonitis, cystitis, and meningitis. Many bacterial strains are now resistant to penicillin

like methicillin resistant *S. aureus* and other antibiotics, one of the major reasons for the widespread transmission of *staphylococcal* infections in hospitals a public health concern for nosocomial infections. *Salmonella typhosa* is a virulent bacteria, common in food and water sources, which is the causal agent of typhoid, food poisoning, and a variety of infections such as blood poisoning and intestinal inflammation. There have been several clinical studies showing that neem has significant effects on several bacterial strains. Among some of the more prominent strains studied are *S. aureus, S. pyogenes, Cornebacterium, E. coli*, and *S. typhimurium*. Oil from the leaves, seed and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, such as *Mycobacterium*. *tuberculosis* and *streptomycin* resistant strains. There has been strong evidence of *in vitro* inhibition of *Vibrio cholerae, Klebsiella pneumoniae, M. tuberculosis* and *M. pyogenes*. Antimicrobial effects of neem extract have been demonstrated against the strains of *Streptococcus mutans* and *S. faecal*is, Most of these bacteria can possibly cause meningitis, cystitis, sore throats, typhoid, blood poisoning, and food poisoning. Neem's biological and pharmacological activities exert significant effects to address the problem of management and control of bacterial pathogens. Antibiotics in the market have low response to address the treatment of this bacterium. The neem however has demonstrated many drawbacks as an antibacterial agent in the sense that in the other preclinical test, neem showed no antibacterial activity against certain strains of the bacteria, and none was effective against *Citrobacter, Escherichia coli, Enterobacter, Klebsiella pneumoniae, Proteus mirabilis, Proteus morgasi, Pseudomonas aeruginosa, Pseudomonas E01,* and *Streptococcus faecalis*.

2.2.9.2 Antiviral activity

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

2.2.9.3 Antifungal activity

Experiment was made to evaluate the efficacy of various extracts of neem leaf on seed borne fungi *Aspergillus* and *Rhizopus* and results confirmed that growth of both the fungal species was significantly inhibited and controlled with both alcoholic and water extract. Furthermore, alcoholic extract of neem leaf was most effective as compared to aqueous extract for retarding the growth of both fungal species (Mondali, 2009). Another finding showed the antimicrobial role of aqueous extracts of neem cake in the inhibition of spore germination against three sporulating fungi such as *C. lunata, H. pennisetti*, and *C. gloeosporioides* f. sp. *mangiferae* (Anjali, 2013) and results of the study revealed that methanol and ethanol extract of *Azadirachta indica* showed growth inhibition against *Aspergillus flavus*, *Alternaria solani*, and *Cladosporium* (Shrivastava, 2014).

2.2.9.4 Antimalarial activity

Experiment was made to evaluate the antimalarial activity of extracts using Plasmodium berghei infected albino mice and results revealed that neem leaf and stem bark extracts reduced the level of parasitemia in infected mice by about 51–80% and 56–87%, respectively, and other studies showed that azadirachtin and other limonoids available in neem extracts are active on malaria vectors.

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

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

A study result showed that, in the acute toxicity test, the LD50 values of neem oil were found to be 31.95 g/kg (Deng, 2013). Another study was performed to evaluate the toxicity in chicken and finding showed that acute toxicity study of neem leaf aqueous extract revealed an intraperitoneal LD50 of 4800 mg/kg, and clinical signs were dose dependent (Biu, 2011).

A study reported that lethal median doses (LD50) recorded for neem leaf and stem bark extracts were 31.62 and 489.90 mg/kg body weight, respectively (Akin-Osanaiya, 2013). The LD50 of water extract of *A. indica* leaves and seeds were 6.2, 9.4 mL kg−1, respectively (Bakr, 2013). Lethal dose values were calculated with probit analysis and LD50 and LD90 values were found to be 8.4 and 169.8 μg/fly of neem extract, respectively (Khan, 2013). A test for acute oral toxicity in mice revealed that LD50 value of approximately 13 g/kg body weight (Okpanyi, 2011).

2.2.11 Effect of neem on blood parameter

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

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

CHAPTER 3
MATERIALS AND METHODS

3.1 Study area

The experiment was carried out at Sher-e-Bangla Agricultural University (SAU experimental shed during the period of 13th September and 11th October 2020, for a period of 4 weeks using neem leaves and ACV. Neem leaves are not commercially available. The experiment was performed by applying different concentration levels of neem leaves and ACV extract in feed and drinking water respectively.

3.2 Study period

The duration of total research period was about 1 month started from September to October, 2020.

3.3 Collection of experimental broilers

The day-old broiler chicks 120 in number of "Lohman meat (Indian River)" were purchased from Kazi Agro Complex Limited, Savar, Dhaka. All the chicks were examined for abnormalities and uniform size. Average body weight of the chicks was tried to maintain similar (about 40.74g±0.26).

3.4 Experimental materials

The collected chicks were carried to the university poultry farm early in the morning. They were kept in electric brooders equally by maintaining standard brooding protocol. Among 120 DOC, 90 chicks were selected and distributed randomly in three treatments of neem leaves and ACV; remaining 30 chicks were distributed another treatment for control. For proper handling and data collection, the chicks of each treatment group were divided into three replications and in each replication, there were 10 birds. After 28 days of nursing and feeding, data were collected for the following parameters: amount of water intake, feed intake, live weight, body weight gain, feed conversion ratio, flock uniformity, survivability & coccidial oocyst count.

3.5 Experimental treatments

To find out the effect of neem leaves and ACV on broiler production, the experiment was carried out after 3rd days of age. 2g neem leaves were added per kg feed, 5ml ACV were added per liter of drinking water for each time. The experimental treatments were as follows:

T_0: Commercial feed with no apple cider vinegar & neem leaves

T_1: Apple cider vinegar (5ml/L drinking water)

T_2: Commercial feed with neem leaves (2g/Kg feed)

T_3: Commercial feed with apple cider vinegar (5ml/L drinking water) & neem leaves (2g/Kg feed)

Table 3: Lay out of the experiment treatment groups

Treatment groups	No. of Replications			Total
	R_1	R_2	R_3	
T_1	10	10	10	30
T_2	10	10	10	30
T_3	10	10	10	30
T_0	10	10	10	30
Total	40	40	40	120

3.6 Preparation of experimental treatment (ACV & neem leaves)

3.6.1 Collection of ACV

The apple cider vinegar was bought from local market.

3.6.2 Preparation of neem leaves powder

The neem leaves were harvested from middle aged green trees and were sun-dried for three days on hygienic cement floors until they become crispy but still retaining the greenish tint. The turning of leaves was carried out on regular intervals to prevent uneven drying and possible decay of leaves. Then the leaves were hammered and converted into grinded form.

Table 4. Nutrient composition of DNLP

Nutrient component	Amount (%)
Dry matter	90.24
Crude protein	23.40
Ether extract	3.36
Ash	9.90

Nutrient component	Amount (%)
Crude fiber	7.81
Calcium (g)	1.40 g
Phosphorus (g)	0.25 g

Source: Ilango *et al.* (2013)

3.7 Preparation of broiler house

The broiler shed was an open sided natural house. Cross ventilation system was provided by using wire-net. It was a tin shed house with concrete floor. There was 1ft. side wall around the shed with no ceiling. The floor was above 1ft. from the ground and the top of the roof was above 15ft. from the floor. Polythene sheet was hung around the side wall to protect the chicks from cold, storm, dust and heavy rainfall. The house was properly cleaned, rubbed with bleaching powder and washed the floor by using tap water and then disinfected by n-alkyl dimethyl benzyl ammonium chloride (Timsen TM) solution before starting the experiment. After proper drying, the house was divided into pens as per layout of the experiment by polythene sheet so that air cannot pass one pen to another. The height of pens was 5ft. Before placement of chicks the house was fumigated by formalin and potassium permanganate @500 ml formalin and 250g potassium permanganate (i.e. 2:1) for 35 m^3 experimental area.

Starter and grower commercial Kazi broiler feed were purchased from the market. The nutrient content of starter and grower diet are shown in Table 5 & Table 6.

Table 5. Nutrient content of (%) starter diet

Nutrient	Amount (%)
Protein	21.0
Fat	6.0
Fiber	5.0
Ash	8.0
Lysine	1.20
Methionine	0.49
Cysteine	0.40

Nutrient component	Amount (%)
Tryptophan	0.19
Threonine	0.79
Arginine	1.2

Table 6. Nutrient content of (%) grower diet

Nutrient	Amount (%)
Protein	19.0
Fat	6.0
Fiber	5.0
Ash	8.0
Lysine	1.10
Methionine	0.47
Cysteine	0.39
Tryptophan	0.18
Threonine	0.75
Arginine	1.18

Feed was supplied 4 times daily by following "Indian River-Lohmann Meat" Management Manual and *ad libitum* drinking water was supplied 2 times daily.

3.8 Management procedures

Different aspects of the management of chicks, experimental events and management procedures are described in detail below:

3.8.1 Litter management

High absorbing bedding material was used as litter on the floor. Fresh, clean and sundried rice husk was used as shallow litter to absorb moisture from fecal discharge of broiler. The shallow litter was 5 cm (2 inch) in depth. About 250g calcium oxide powder was mixed with rice husk in every pen as disinfectant. At the end of each week the litter was harrowed to prevent accumulation of toxic gases and to reduce moisture

and parasitic infection. At 3rd and 4th week of rearing period, droppings were cleaned from the surface level by removing a thin layer of litter and same amount new litter was placed in each pen.

3.8.2 Care of day-old chicks

Just after arrival of day-old chicks to the poultry house the initial weight of the chicks were recorded by a digital electronic balance, vaccination was done and distributed them under the hover for brooding. The chicks were supplied glucose water with vitamin-C to drink for the first 3 hours to overcome dehydration and transportation stress. Subsequently small feed particles were supplied on the newspapers to start feeding for the first 24 hours.

3.8.3 Brooding of baby chicks

The experiment was conducted during **13th September to 12th October, 2020**. The average temperature was 35°C and the RH was 60% in the poultry house. Common brooding was done for one week. After one week the chicks were distributed in the pen randomly. There were 10 chicks in each pen and the pen space was 1m^2. Due to hot climate brooding temperature was maintained as per requirement. Brooding temperature was adjusted (below 35°C) with house temperature. So, when the environmental temperature was above the recommendation, then no extra heat was provided. At day time only an electric bulb was used to stimulate the chicks to eat and drink. In brooding extra heat was not provided at day time except mid night to morning. Electric fans were used as per necessity to save the birds from the heat stress.

3.8.4 Feeding and drinking

Crumble feed was used as starter (0-2 wks.) and pellet feed for grower (3-4 wks.) ration. *Ad libitum* feeding was allowed for rapid growth of broiler chicks up to the end of the four weeks. Fresh clean drinking water was also supplied Ad libitum. Feeds were supplied 3 times: morning, noon and night; water 2 times: morning and evening daily. Left over feeds and water were recorded to calculate actual intake. Digital electronic balance and measuring plastic cylinder was used to take record of feed and water. Daily water consumption (ml) and weekly feed consumption (g)/bird were calculated to find out weekly and total consumption of feed and water. All feeders and drinkers were washed and sundried before starting the trial. One plastic made round feeder and one drinker were kept in the experimental pen. Feeder and drinker size were changed

according to the age of the birds. Feeders were washed at the end of the week and drinkers once daily.

3.8.5 Lighting

At night there was provision of light in the broiler house to stimulate feed intake and rapid body growth. In summer and rainy season at night 4 energy lights were provided to ensure 24 hours light for the first 2 weeks. Thereafter 23 hours light and one-hour dark were scheduled up to marketable age. At night one-hour dark was provided in two times by half an hour.

3.8.6 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation it was easy to remove polluted gases from the farm. Besides, on the basis of necessity ventilation was regulated by folding polythene screen.

3.8.7 Biosecurity measures

Biosecurity is a set of management practices that reduce the potential for introduction and spread of diseases causing organisms. To keep disease away from the broiler, farm the following vaccination, medication and sanitation program was undertaken. All groups of broiler chicks were supplied Vitamin B-Complex, Vitamin-ADEK, Vitamin-C, Ca and Vitamin-D enriched medicine and electrolytes.

3.8.8 Vaccination

The vaccines collected from medicine shop (Ceva Company) and applied to the experimental birds according to the vaccination schedule. One ampoule vaccine was diluted with distilled water according to the recommendation of the manufacturer. The cool chain of vaccine was maintained strictly up to vaccination. The vaccination schedule of broiler is shown in Table 7.

Table 7. Vaccination schedule

Age day	Name of disease	Name of vaccine	Routine vaccine
0	Infectious Bronchitis+ Newcaste disease (IB+ND)	CEVAC BIL	One drop in eye

Age day	Name of disease	Name of vaccine	Routine vaccine
09	Gumboro (IBD)	CEVAC IBDL	Drinking water
17	Gumboro (IBD)	CEVAC IBDL	Drinking water

3.8.9 Medication

The broiler chicks were fed antibiotic drug against bacterial diseases. Ampicillin and oxytetracycline antibiotics were used only for antibiotic groups of birds. Besides vitamin B complex, vitamin-A, D3, E and sinacal-D were used against deficiency diseases. Electromin and vitamin-C also used to save the birds from heat stress. The medication program is presented in the table below:

Table 8. Medication program

Medication	Purpose	Dose	Period
Electromin powder	Electrolytes	1g/2L water	Only in hot climate (all groups)
Calplex	Ca, P and Vit-D	10 ml/100 bird	3-5 days (all groups)

3.8.10 Sanitation

Proper hygienic measures were maintained throughout the experimental period. Cleaning and washing of broiler shed and its premises were under a routine sanitation work. Flies and insects were controlled by spraying Phenol and Lysol to the surroundings of the broiler shed. The attendants used farm dress and shoe. There was a provision of Foot Bath at the entry gate of the broiler shed to prevent any probable contamination of diseases. Strict sanitary measures were followed during the experimental period.

3.9 Recorded parameters

Weekly live weight, weekly feed consumption and death of chicks to calculate mortality percent were taken during the study. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter carcass weight and

gizzard, liver, spleen and heart were measured from each broiler. Dressing yield was calculated for each replication to find out dressing percentage.

3.10 Data collection

3.10.1 Consumption of DW

During the study, the data of consumption of treated and non-treated DW from broiler litter was collected daily at morning from each replication of all treatment groups and untreated also. The average of the daily recorded water consumption was calculated.

3.10.2 Live weight

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

3.10.3 Dressing yield

Dressing yield of bird was obtained from live weight subtracting blood, feathers, head, shank and inedible viscera.

3.10.4 Feed intake

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

3.10.5 Survivability of chicks

Daily death record for each replication was counted up to 28 days of age to calculate mortality if occurred that indicated the survivability of the bird.

3.10.6 Dressing procedures of broiler

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

removed from the liver. Cutting it loose in front of the proventriculus and then cutting with both incoming and outgoing tracts removed the gizzard. Dressing yield was found by subtracting blood, feathers, head, shank and digestive system from live weight.

3.10.7 Oocyst output

The fecal samples were collected and stored at 4°C to determine the oocyst per gram (OPG) count and perform fecal oocyst reduction test (FORT). This method is carried on through the McMaster counting chamber technique that uses saturated NaCl as the flotation medium. The infectious dose of coccidian oocytes/chicken was $1x10^5$ of *Eimeria tenella* given via oral administration at the age of 22th day (Arabkhazaeli *et al*., 2011).

3.11 Calculations

Each data was collected by the following formula:

3.11.1 Live weight gain

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

$$Body\ weight\ gain = Final\ Weight - Initial\ Weight$$

3.11.2 Feed intake

Feed intake was calculated as the total feed intake in a replication divided by number of birds in each replication.

$$\text{Feed intake (g/bird)} = \frac{Feed\ intake\ in\ a\ replication}{No.of\ birds\ in\ a\ replication}$$

3.11.3 Feed conversion ratio

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

$$FCR = \frac{Feed\ Intake\ (Kg)}{Weight\ Gain\ (Kg)}$$

3.11.4 Dressing percentage

Dressing yield was found by subtracting blood, feathers, head, shank and digestive system from live weight. Liver, heart, gizzard and neck were considered as giblet. Dressing percentage of bird was calculated by the following formula-

$$DP = \frac{Dressing\ Yield\ (gm)}{Live\ Weight\ (gm)} \times 100$$

Dressing yield= breast, thigh, drumstick, back, wing, giblet, abdominal fat weight (g)

3.11.5 Flock uniformity

Flock uniformity of bird was calculated by the following formula-

$$Flock\ uniformity = \frac{No.of\ birds\ in\ weight\ range\ in\ a\ replication}{Total\ no.of\ birds\ in\ a\ replication} \times 100$$

3.12 Statistical analysis

Total data were compiled, tabulated and analyzed in accordance with the objectives of the study. Excel Program was practiced for preliminary data calculation. The collected data was subjected to statistical analysis by applying one-way ANOVA using Statistical Package for Social Sciences (SPSS version 26.0). Differences between means were tested using Duncan's multiple comparison test and significance was set at $P<0.05$.

Some photograph of chick management and experimental procedure is represented in plate 1-11 below:

Plate 1. Washing of floor by water & disinfectant

Plate 2. Preparation of chick brooder guard **Plate 3. Arrival of day-old chick**

Plate 4. Giving feed of day-old chick

Plate 5. Measuring temp. & humidity

Plate 6. Distribution of chick

Plate 7. Vaccination of chick

Plate 8. Monitoring of research activities by the supervisor

Plate 9: Neem leaves & apple cider vinegar

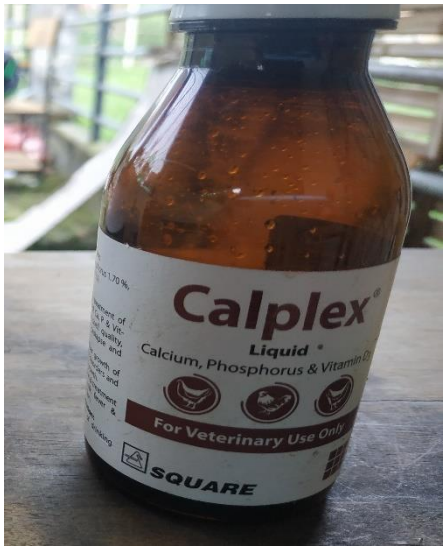

Plate 10. Electromin powder and calplex

Plate 11. IB and IBD vaccination vial

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production performances of broiler chicken

Supplementation of neem leaves and ACV to broiler diets improve growth performance in terms of feed consumption, body weight gain and feed conversion ratio (FCR). The chicks were randomly divided into four experimental treatment groups. The four groups were T_1 (neem leaves, 2gm/kg feed), T_2 (ACV, 5ml/L), T_3 (neem leaves, 2gm/kg feed & ACV, 5ml/L) and T_0 (control). The performance traits *viz.* final live weight, body weight gain, feed consumption, FCR, survivability, flock uniformity, were discussed in this chapter.

4.1.1 Final live weight

The relative final live weight (g) of broiler chickens in the different groups T_0, T_1, T_2, and T_3 presented in Table 9 & Figure 2, which were $1581.00^{c}\pm9.54$, $1647.33^{b}\pm10.48$, $1660.67^{b}\pm29.41$ and $1734.00^{a}\pm16.37$ respectively. The highest result was found in T_3 group ($1734.00^{a}\pm16.37$g) and lowest result was in T_0 ($1581.00^{c}\pm9.54$g) group and that was significantly ($P<0.05$) difference. It might be due to the combined effect of neem leaves and ACV.

Alam *et al.*, (2015) and Ansari (2012) found significantly higher live weight in neem leaf treated groups compared to control group. However, birds supplemented with neem leaf powder had higher body weight and feed efficiency. These results may be due to antimicrobial and antiprotozoal properties of neem leaves, which help to reduce the microbial load of birds and improved the feed consumption and feed efficiency of the birds (Kale *et al.*, 2003). Similar observation was found in the study of Parviz *et al.*, (2018) who supplemented ACV in drinking water and reported significant increase body weight of broilers acv fed groups when compared with probiotic group.

4.1.2 Weekly body weight gains (WBWG)

In every week the BWG was significantly affected by the neem leaves & ACV. The mean body weight gains (g) at the 1st week of different treatment groups were significantly higher ($P<0.05$) than control. The mean body weight gains (g) of broiler chicks at 4th week in different groups were T_0 ($531.00^{b}\pm6.56$), T_1 ($562.67^{b}\pm9.53$), T_2 ($572.33^{b}\pm27.47$), and T_3 ($629.00^{a}\pm6.56$) respectively. At the 4th week the highest result

was found in T_3 (629.00^{a}±6.56g) group and lowest result was found in T_0 (531.00^{b}±6.56g) group and that was significantly (P<0.05) difference. The data of weekly body weight gains of broiler chicks presented in Table 10 & Figure 3.

These results are in agreement with those of previous researchers Deka, *et al.*, (2019), showed that broilers feeding on diet neem leaves powder had a body weight gain significantly higher than the control (without neem leaves).

4.1.3 Total feed intake (FI)

Result in total feed intake demonstrated that treatment groups showed significantly (P<0.05) difference. Total feed intake of different treated groups and control group have been presented in Table 9. Feed intake of T_0 (2317.07^{a}±21.00g) group was significantly (P<0.05) higher than T_2 (2219.60^{b}±10.66g) group, whereas feed intake of T_1 & T_3 were 2259.17^{b}±7.45g & 2232.43^{b}±12.58g respectively.

Bawa *et al.*, (2007) who fed broiler with DNLP and reported reduced feed consumption among the broiler on the test diets. Edens *et al*., (2000) has also established the presence of bitter triterpenoids in the neem leaf.

4.1.4 Feed conversion ratio (FCR)

The result of FCR of broilers under different treatment groups have been shown in Table 9. The lowest (best) FCR was (P<0.05) found in T_3 (1.32^{c}±0.01) group (2g neem leaves & 5ml ACV) than T_0 (1.51_{a}±0.00) group. However, FCR was significantly (P<0.05) higher in T_0 group and lower in T_3 group. Lower FCR indicates better feed efficiency. It might be due to the combined effect of neem leaves and ACV.

Ansari *et al.*, (2012) reported that at 28 days birds fed diets supplemented with 2.5 g/kg of leaf meal had significantly better FCR than those fed diets with 1.25, 5.0 g/kg of *Neem* leaf meal and controls. But Alam *et al.*, (2015) & Zanu *et al.*, (2011) found contrary result and reported that no significant effect of neem leaves on FCR. However, Parviz *et al.*, (2018) reported significant effect of ACV on FCR.

4.1.5 Flock uniformity

Flock Uniformity of different treatment groups were not affected significantly (P>0.05) by neem leaves & ACV. Flock uniformity of broiler chicken were presented in Table 12. The higher flock uniformity was found in T_3 (76.67±3.33%) group. The lower flock

uniformity was found in T_1 (60.00±5.77%) group and T_0 & T_2 were (66.00±6.35%) & (70.00±5.77%). We know 80% uniformity indicates uniform flock. The present finding is 76.67% which is above average and almost uniform flock.

4.1.6 Dressing percentage

Dressing percentage of different treatment groups were insignificantly (P>0.05) difference. Dressing percentage of broiler chicken was presented in table 11. The highest dressing percentage was (P>0.05) found in T_3 (63.25±1.17%) group & lowest dressing percentage was found in T_0 (62.47±0.33%) group. The dressing percentage of other treated groups was 63.17±0.74% (T_1 group) and 62.65±0.70% (T_2 group).

4.1.7 Survivability

Survivability of broiler chicken was presented in table 9. No significant difference was found in survivability of broiler chicken treated with neem leaves & ACV. But relatively highest (100%) survivability was found in T_3 group than control (90%) group.

4.2 Effects of Neem leaves and ACV on coccidial oocyst count in broiler with *Eimeria tenella* (x1000).

Total viable count of oocyst from caecal faeces of broiler chicken treated with neem and ACV presented in Table 13. Different treatment groups showed significantly (P<0.05) difference among treatments. The highest (P<0.05) viable oocyst was found in T_0 group (22.33^{a}±1.45) and lowest (P<0.05) viable oocyst found in T_3 group (3.00^{c}±1.15) than T_1 (7.67^{b}±0.88) & T_2 (6.00^{bc}±0.58) groups. However, T_3 indicate the lower oocyst, it might be due to the combined effect of neem leaves and ACV. This finding confirmed by Rao (2010).

Table 9: Effects of neem leaves and ACV on production performances of broiler

Treatments	Final live weight (g/bird)	Total FI (g/bird)	Average BWG (g/bird)	Final FCR	Survivability
T_0	1581.00^{c}±9.54	2317.07^{a}±21.00	1535.00^{c}±9.54	1.51^{a}±0.00	90±0.00
T_1	1647.33^{b}±10.48	2259.17^{b}±7.45	1601.33^{b}±10.48	1.41^{b}±0.01	100±0.00
T_2	1660.67^{b}±29.41	2219.60^{b}±10.66	1614.67^{b}±29.41	1.37^{bc}±0.03	100±0.00
T_3	1734.00^{a}±16.37	2232.43^{b}±12.58	1688.00^{a}±16.37	1.32^{c}±0.01	100±0.00
Mean±SE	1655.75±18.14	2257.07±12.75	1609.75±18.14	1.27±0.02	97.5±1.30

Here, T_0 = Control group, T_1= Neem leaves powder (2gm/kg feed) T_2= Apple cider vinegar (5ml/L), T_3 = (Neem leaves powder 2gm/kg feed & 5ml/L ACV). Values are Mean ± SE (n=12), one-way ANOVA (SPSS, Duncan Method)

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

Table 10: Effects of Neem leaves and ACV on body weight gain (BWG) (g/bird) of broiler at different weeks

Treatments	1st week BWG	2nd week BWG	3rd week BWG	4th week BWG
T_0	204.33^{b}±4.05	300.67^{bc}±1.33	499.00^{b}±4.51	531.00^{b}±6.56
T_1	215.33^{ab} ±3.76	292.00^{c}±4.04	531.33^{a}±8.21	562.67^{b}±9.53
T_2	220.33^{a}±2.91	303.33^{ab}±2.60	518.67^{ab}±9.87	572.33^{b}±27.47
T_3	229.33^{a} ±5.61	312.00^{a}±4.36	517.67^{ab}±7.84	629.00^{a}±6.56
Mean±SE	217.33±3.26	302.00±2.57	516.67±4.83	573.75±12.49

Here, T_0 = Control group, T_1= Neem leaves powder (2gm/kg feed) T_2= Apple cider vinegar (5ml/L), T_3 = (Neem leaves powder 2gm/kg feed & 5ml/L ACV). Values are Mean ± SE (n=12), one-way ANOVA (SPSS, Duncan Method)

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

Table 11: Average live weight, eviscerated weight and dressing percentage of broiler chicken under different treatments

Treatments	*Average Live weight%	*Eviscerated weight %	Dressing percentage%
T_0	1581.00^{c}±9.54	997.67^{b}±7.22	62.47±0.33
T_1	1647.33^{b}±10.48	1040.67^{ab}±10.48	63.17±0.74
T_2	1660.67^{b}±29.41	1040.67^{ab}±24.73	62.65±0.70
T_3	1734.00^{a}±16.37	1097.33^{a}±30.75	63.25±1.17
Mean±SE	1655.75±18.14	1044.08±13.86	62.89±0.35

Here, T_0 = Control group, T_1= Neem leaves powder (2gm/kg feed) T_2= Apple cider vinegar (5ml/L), T_3 = (Neem leaves powder 2gm/kg feed & 5ml/L ACV). Values are Mean ± SE (n=12), one-way ANOVA (SPSS, Duncan Method)

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

Table 12: Flock uniformity of broiler chicken under different treatment

Treatments	Uniformity
T_0	66.00±6.35
T_1	60.00±5.77
T_2	70.00±5.77
T_3	76.67±3.33
Mean±SE	68.17±2.95

Here, T_0 = Control group, T_1= Neem leaves powder (2gm/kg feed) T_2= Apple cider vinegar (5ml/L), T_3 = (Neem leaves powder 2gm/kg feed & 5ml/L ACV). Values are Mean ± SE (n=12), one-way ANOVA (SPSS, Duncan Method)

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

Table 13: Effects of neem leaves and ACV on coccidial oocyst count in broiler with *Eimeria tenella* (x1000).

Treatments	Oocyst count per gram of feces
T_0	22.33^{a}±1.45
T_1	7.67^{b}±0.88
T_2	6.00^{bc}±0.58
T_3	3.00^{c}±1.15
Mean±SE	9.75±2.29

Here, T_0 = Control group, T_1= Neem leaves powder (2gm/kg feed) T_2= Apple cider vinegar (5ml/L), T_3 = (Neem leaves powder 2gm/kg feed & 5ml/L ACV). Values are Mean ± SE (n=12), one-way ANOVA (SPSS, Duncan Method)

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

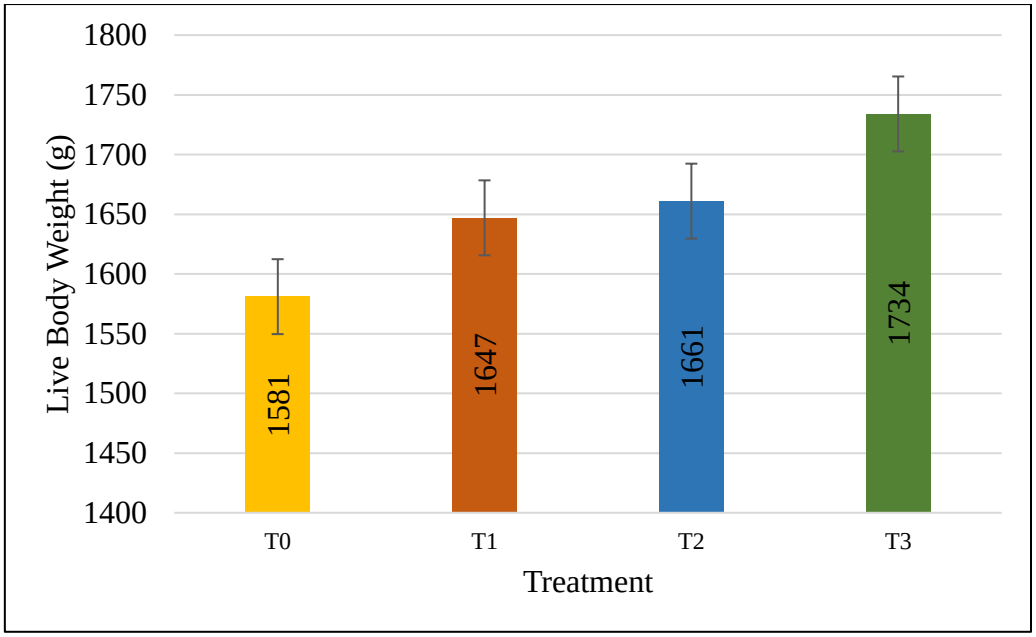

Figure 2. Effects of neem and ACV on average LBW on broiler under different treatments

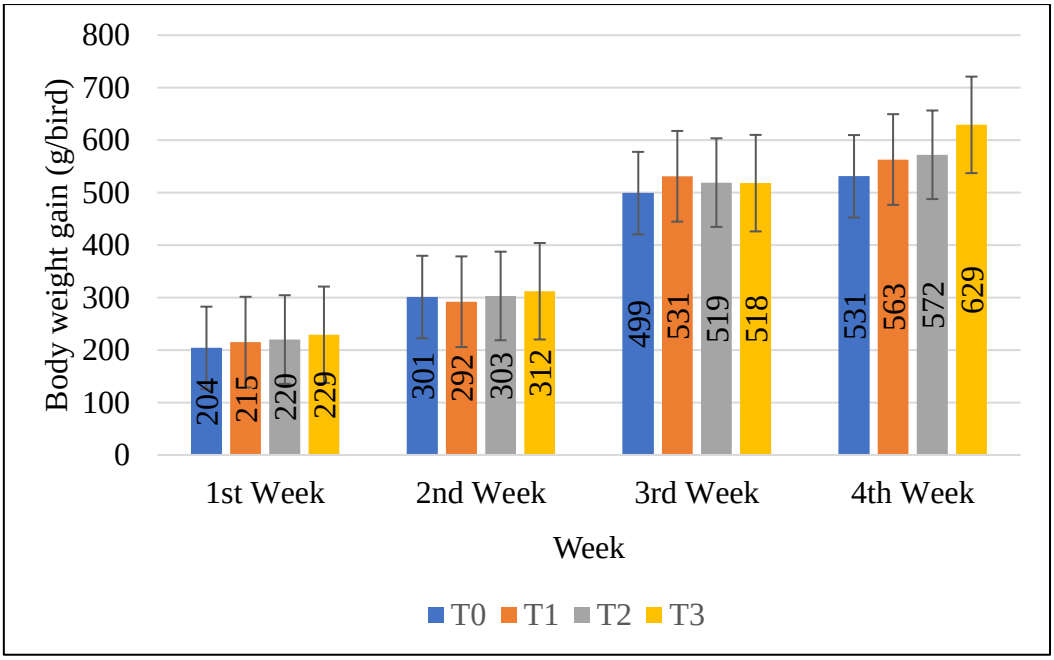

Figure 3. Effects of neem and ACV on weekly average body weight gain under different treatments

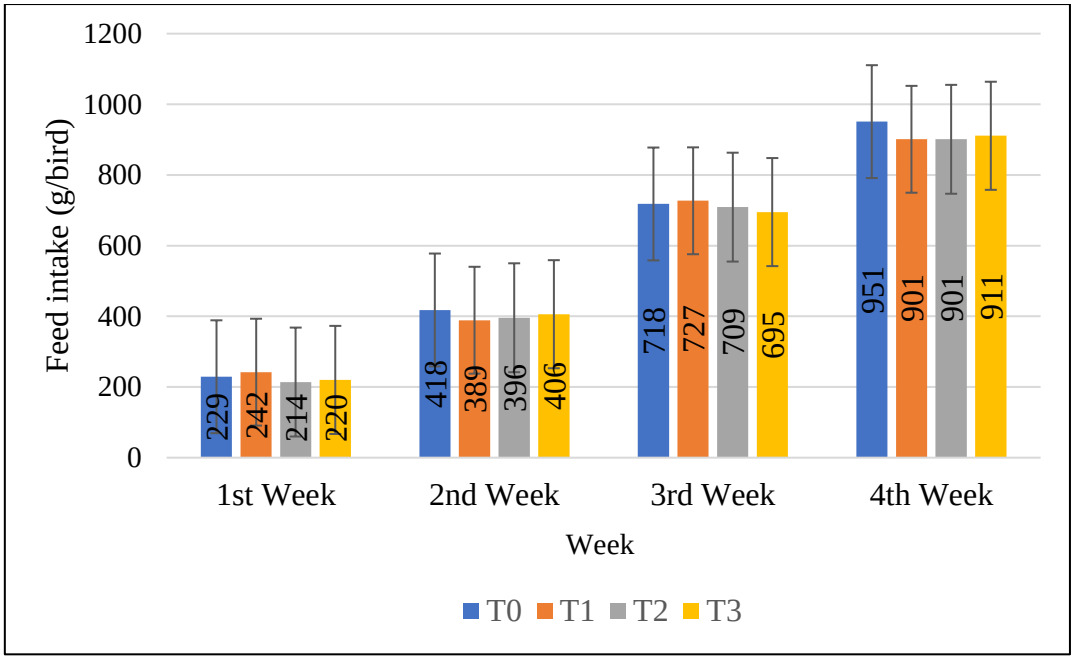

Figure 4. Effects of neem and ACV on weekly average feed intake on broiler

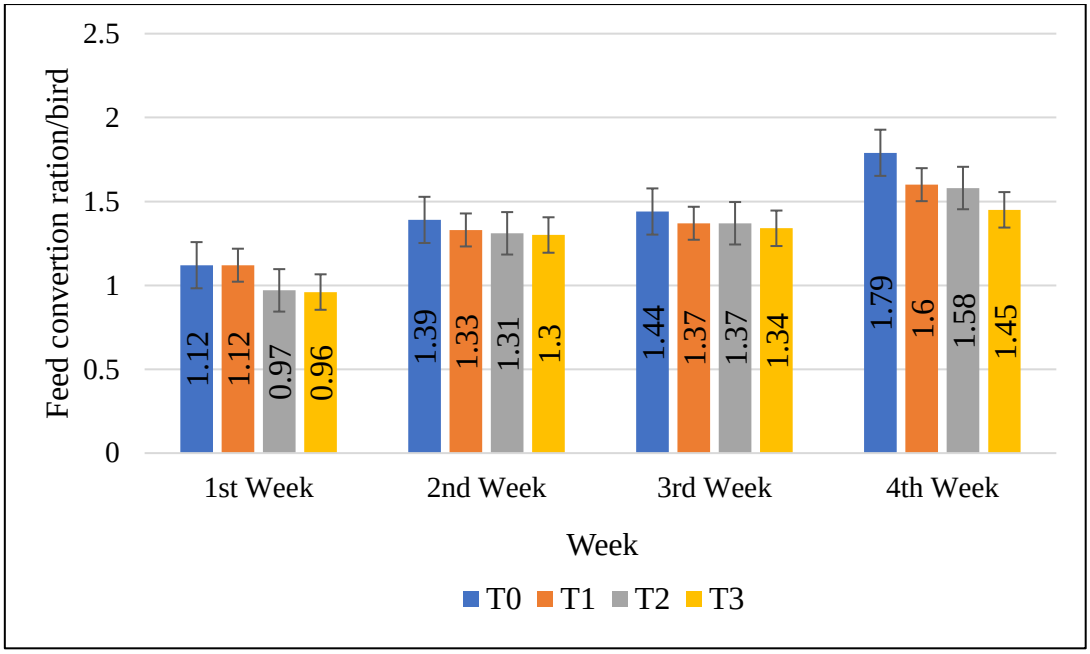

Figure 5. Effects of neem and ACV on weekly average feed conversion ratio on broiler

CHAPTER 5

SUMMARY AND CONCLUSION

A total of 120-day old chicks of "Indian River-Lohmann Meat" were reared in Sher-e-Bangla Agricultural University, Dhaka Poultry Farm for a period of four weeks using neem leaves and ACV. Chicks were divided randomly into 4 experimental groups of 3 replicates (10 chicks were allocated in each treatment group). One of the 4 experimental group was fed diet without neem leaves and ACV were considered as control while, the remaining three groups were fed diet with 2g/Kg neem leaves of feed, 5ml/L ACV in water, 2g/Kg neem leaves of feed and 5ml/L ACV in water. The specific objectives of this experiment were, i) To determine the growth performance of broiler by using Apple Cider Vinegar and Neem Leaves. ii) To find natural alternative to measure anticoccidial effect on broiler production. The performance traits *viz.* body weight, weight gain, feed consumption, FCR, survivability, flock uniformity and coccidial oocyst count of broiler on different replications of the treatments were recorded and compared in each group.

A statistically significant difference ($P<0.05$) was noted on final live weight, feed intake, BWG, FCR value of the birds treated with different dietary treatment. Birds fed combined neem leaves and apple cider vinegar (ACV), T_3 gained superior body weights ($1734.00^{a}\pm16.37$g) compared to control ($1581.00^{c}\pm9.54$g), T_1 neem ($1647.33^{b}\pm10.48$g) and other dietary treatments. The mean body weight gains (g) at the 1st, 3rd and 4th week of different treatment groups were significantly higher ($P<0.05$) than the control group. The groups fed diets containing combined neem leaves and apple cider vinegar feed (T_3) had better FCR ($1.32^{c}\pm0.01$) compared to control ($1.51^{a}\pm0.00$). The inclusion of different dietary treatments had significant ($P<0.05$) difference on coccidial oocyst count. Highest (100%) survivability was found in T_3 group than control (90%) group. However, T_3 group had better ($3.00^{c}\pm1.15$) oocyst count compared to control ($22.33^{a}\pm1.45$). It is concluded that combined neem leaves & apple cider vinegar can be included in broiler diet for better performance with better FCR & lower coccidial oocyst count.

Analyzing the above research findings, combined apple cider vinegar & neem leaves were used in T_3 groups showed better results than control and other treatment groups in terms of improved growth performance with better FCR & lower coccidial oocyst count.

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APPENDICES

Appendix 1: Recommended level of nutrients for broiler

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

Source: Broiler Management Guide

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

Ingredients	DM	ME (K.Cal/kg)	CP %	CF %	P %	Lys %	Meth %	Tryp %
Soybean meal	90	2710	44.5	7.5	0.23	2.57	0.76	0.57
Maize	89.5	3309	9.2	2.4	0.40	0.18	0.15	0.09
Soybean oil	100	8800						
Protein concentrate (jeso-prot)	91.64	2860	63.3	8.1	3.24	3.87	1.78	0.53
Meat and bone meal	95.5	1044	14.6	2.5	12.11	0.66	0.24	0.12

Source: Broiler Management Guide

Appendix 3: Recorded temperature during experiment

Age in weeks	**Temperature (^{0}C)**	
	High	**Low**
1st	37.64	29.64
2nd	33.57	28.20
3rd	35.34	27.63
4th	36.14	27.93

Appendix 4: Weekly feed intake (g/bird) by birds

Treatments	Replications	1st week	2nd week	3rd week	4th week	Total
	R_1	236.0	430.0	729.0	962.0	2357.0
T_0	R_2	222.0	406.0	717.8	940.0	2285.8
	R_3	230.0	418.0	708.1	952.3	2308.4
	R_1	241.0	387.0	732.5	890.0	2250.5
T_1	R_2	238.0	389.0	728.0	898.0	2253.0
	R_3	246.0	392.0	721.0	915.0	2274.0
	R_1	214.0	395.7	707.1	898.0	2214.8
T_2	R_2	205.0	387.0	711.0	901.0	2204.0
	R_3	222.0	405.0	708.0	905.0	2240.0
	R_1	209.0	397.0	692.0	913.5	2211.5
T_3	R_2	213.0	403.8	698.0	916.0	2230.8
	R_3	239.0	418.0	695.0	903.0	2255.0

Appendix 5: Weekly body weight gain (BWG) (g/bird) of birds under different treatments

Treatments	Replications	1st week	2nd week	3rd week	4th week	Final
	R_1	211	302	495	544	1552
T_0	R_2	197	302	494	526	1519
	R_3	205	298	508	523	1534
	R_1	209	285	515	579	1588
T_1	R_2	222	299	538	563	1622
	R_3	215	292	541	546	1594
	R_1	215	303	530	520	1568
T_2	R_2	221	308	527	613	1669
	R_3	225	299	499	584	1607
	R_1	227	304	526	621	1678
T_3	R_2	221	319	502	624	1666
	R_3	240	313	525	642	1720

Appendix 6: Weekly feed conversion ratio (FCR) of birds under different treatments

Treatments	Replications	1st week	2nd week	3rd week	4th week	Final
	R_1	1.12	1.42	1.47	1.77	1.52
T_0	R_2	1.13	1.34	1.45	1.79	1.50
	R_3	1.12	1.4	1.39	1.82	1.50
	R_1	1.15	1.36	1.42	1.54	1.42
T_1	R_2	1.07	1.3	1.35	1.6	1.39
	R_3	1.14	1.34	1.33	1.68	1.43
	R_1	1.00	1.31	1.33	1.73	1.41
T_2	R_2	0.93	1.26	1.35	1.47	1.32
	R_3	0.99	1.35	1.42	1.55	1.39
	R_1	0.92	1.31	1.32	1.47	1.32
T_3	R_2	0.96	1.27	1.39	1.47	1.34
	R_3	1.00	1.34	1.32	1.41	1.31

Appendix 7: Production performances of broiler under different treatment

Treatments	Replications	Final live weight (g/bird)	Total FC (g/bird)	Average BWG (g/bird)	Final FCR	Survivability (%)
	R_1	1598.0	2357.0	1552.0	1.52	90.0
T_0	R_2	1565.0	2285.8	1519.0	1.50	90.0
	R_3	1580.0	2308.4	1534.0	1.50	90.0
	R_1	1634.0	2250.5	1588.0	1.42	100.0
T_1	R_2	1668.0	2253.0	1622.0	1.39	100.0
	R_3	1640.0	2274.0	1594.0	1.43	100.0
	R_1	1614.0	2214.8	1568.0	1.41	100.0
T_2	R_2	1715.0	2204.0	1669.0	1.32	100.0
	R_3	1653.0	2240.0	1607.0	1.39	100.0
	R_1	1724.0	2211.5	1678.0	1.32	100.0
T_3	R_2	1712.0	2230.8	1666.0	1.34	100.0
	R_3	1766.0	2255.0	1720.0	1.31	100.0

Appendix 8: Flock uniformity of broiler chickens under different treatments

Treatments	Replications	Uniformity%	AVG Uniformity%
	R_1	77	
T_0	R_2	55	66
	R_3	66	
	R_1	60	
T_1	R_2	50	60
	R_3	70	
	R_1	60	
T_2	R_2	80	70
	R_3	70	
	R_1	80	
T_3	R_2	80	77
	R_3	70	

Appendix 9: Average live weight, eviscerated weight and dressing percentage of broiler chicken under different treatments

Treatments	Replications	Average live weight	Eviscerated weight	Dressing percentage %
	R_1	1598	998	61.82
T_0	R_2	1565	985	62.93
	R_3	1580	1010	62.65
	R_1	1634	1024	62.66
T_1	R_2	1668	1038	62.23
	R_3	1640	1060	64.63
	R_1	1614	1019	63.13
T_2	R_2	1715	1090	63.55
	R_3	1653	1013	61.28
	R_1	1724	1084	62.87
T_3	R_2	1712	1052	61.44
	R_3	1766	1156	65.45

Appendix 10: Production cost of the birds under different treatments

Parameter	Amount (BDT)
Day-old chick cost (120 no.)	3000
Feed cost (around 6 bags)	13200
Medicine & vaccine cost	1000
litter cost	900
ACV cost	750
Neem leaves cost	200
Others	1500
Total	20550

Appendix 11: Coccidial oocyst count in broiler with *Eimeria tenella* (x1000).

Treatment	Replication	Oocyst count per gram of feces
	R_1	25
T_0	R_2	20
	R_3	22
	R_1	8
T_1	R_2	9
	R_3	6
	R_1	6
T_2	R_2	5
	R_3	7
	R_1	5
T_3	R_2	3
	R_3	1