DIETARY EFFECT OF NEEM (*Azadirachta indica)* AND MULBERRY LEAF *(Morus alba)* MEAL IN THE PRODUCTION PERFORMANCE AND CHOLESTEROL LEVEL OF LAYING HEN

A THESIS

BY

DR. SWARNO SHEKHOR ROY

SEMESTER: JULY-DECEMBER 2016

REGISTRATION NO.: 1505028

SESSION: 2015-2016

MASTER OF SCIENCE (M.S.)

IN

POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND

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Submitted to the Department of Dairy and Poultry Science
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ABSTRACT

The objective of present study was to determine the effects of various dietary levels of neem (*Azadirachta indica)* and mulberry *(Morus alba)* leaf meal on production performance, egg qualities and egg yolk cholesterol. The study was conducted at the poultry shed on Dairy and Poultry Science Laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. In this study, Sixty (60) weeks old laying hens (Hi-sex brown) were divided into 5 dietary groups each with 3 replications (4 birds/ replication) and offered manually prepared diets supplemented with T_0: (control), T_1: (NLM 1% and MLM 2.5%), T_2 : (NLM 1% and MLM 5%), T_3 : (NLM 1% and MLM 7.5%), T_4 : (NLM 1% and MLM 10%) meal for 8 weeks. Eggs were collected and weighted daily. Laying performance, egg quality and feed conversion ratio were evaluated. Results showed that the feed intake, egg production, egg weight, feed conversion ratio, body weight and egg qualities were insignificant among the treatment groups. However, the egg yolk cholesterol concentration was significantly decreased ($P<0.05$) with higher levels of mulberry leaf supplementary diets. Egg yolk cholesterol was decreased at T_1 (2.27%), T_2 (3.87%), T_3 (7.99%) and T_4 (13.78%) by supplementation respectively. Based on the results, it may be concluded that the supplementation of neem and mulberry leaf meal up to my investigation level (NLM 1% and MLM 10%) has potentiality in reduction of egg yolk cholesterol.

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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

CHAPTER 1

INTRODUCTION

The world populating is growing day by day and the demand of food specially protein is increasing keeping pace with that demand. Poultry industry now playing an outstanding role throughout the world by satisfying the demand of protein for growing population. Poultry sector is supplying both meat and egg, that is consumed by the whole world populations. Poultry egg has been a staple meal and important food ingredient in different parts of the world. There is no doubt that eggs are consumed steadily throughout the world. It can be cooked and used in a various of ways. Eggs can be mixed with other ingredients for meal and ever dessert but they can also be served as a separate and complete meal and can be found in almost every home, cafe diner and restaurant. In 2009 and estimated 62.1 million metric ton egg were produce worldwide from total laying flock of approximately 6.4 billion hens (watt.ag.net word publishing company) nutritional value in per 100 gm chicken egg is energy 647kj

(155kcal), carbohydrate 1.12gm, fat 10.6gm, protein 12.6 gm, vitamins and minerals in significant amount and water 75 gm and cholesterol 373mg. In a research, Mailer and Denton *et al*. (1962) found 15.5 to 17.5 mg cholesterol/gm yolk. Whereas Harris and wilcox *et al.* (1972) repoted value from 22 to 26. Cotterill *et al*. (1977) found 270mg cholesterol per yolk or 14.3 mg/gm yolk. C.K.Han and N. H. Lee *et al*. (1992) reported that birds age 63 weeks and their egg yolk cholesterol mean value was 17.26mg/ gm and the range from 16.40 to 18.18 mg/gm.

The egg white contents little or approximately no cholesterol, but it is the egg yolk that contributes in our dietary cholesterol. Eggs are high in dietary cholesterol. But not as high in saturated fat researches shows that consumption of eggs should have a limit because the more dietary cholesterol we eat, the higher our blood cholesterol rises more artery damaging plaque we accumulate. Excess cholesterol can form plaque between layers of artery walls, making it higher for the hazard to circulate blood. Plaque can open and cause blood clots, if a clots blocks an artery the feeds the brain, it cause a stroke if it block an artery that feeds the heard it causes heard attacks.

The list of possible feed alternatives includes tree fodder mulberry leaves (*Alorits alba*) as a source of dietary protein for commercial livestock and poultry operations. If is reported to have excellent nutritional value as forage. It is grown extensively for its leaves which are used for raising silkworms in the sericulture industry. Mulberry leaves are rich in protein (15-35%), minerals (Ca 2.42-4.71%, P 0.23-0.97%) and metabolizable energy (1130-2240 kcal/kg) with absence of or negligible anti-nutritional factors (Sarita *et al*., 2006). Mulberry leaves contain carotene which can be converted with varying efficiency by animals to vitamin A and the

xanthophylls which may have potentiality in pigmentation of egg yolk (Sarita *et al*., 2006).

On the other hand various part of neem tree have been reported to contain chemical like azadiracin, nimbin, nimbidin, quercein among other (Makeri *et al*., 2007; Gandhi *et al*., 1998; Blaney *et al*., 1990) which have antimicrobial, anthelminth, antioxidant, antifungal, insecticide, antiprotozal and spermicidal activities (Elangovan *et al*., 2000) porpertis (Bonsu *et al*., 2012). Neem (*Azadirachta indica)* is a first growing evergreen tree which has a potential to provide medicial and nutritive value to broilers (Schmutterer, 1990). Broiler given neem leaf extract in water showed improved nutrient conversation efficiency and weight gain (Chakaravarty and Prasad, 1991). Neem also plays an important role in strengthening the immune system of the body. Increase in antibodies against new castle and infectious bursal disease viruses have been observed when neem is incorporated in poultry feeds (Durrani *et al*., 2008). Water based extract (10%) of neem leaves is reported to have anti-viral propertis against fowl pox, infectious bursal disease 9 IBD) and Newcastle disease virus (NDV) and it significantly enhances the antibodies production against the IBD and NDV (Sadekar *et al*., 1998).

Now days, many people of the world are suffering from various heart diseases. There is a high relationship between cholesterol and atherosclerosis. Plasma total cholesterol and low-density lipoprotein (LDL) are closely related to atherosclerosis and excessive concentration of these two materials may lead to coronary artery disease or death. Ordinary chicken eggs provide protein, vitamins, and lipids that contain high levels of cholesterol. Thus, eggs are considered to be a high-cholesterol food. The American Heart Association recommended that cholesterol consumption for each person should be limited up to 300 mg per day and the whole egg yolk

consumption should be limited to three to four per week. In recent days, consumers pay more attention to health and are thus lowering their consumption of high-cholesterol food. But, the consumers have to intake eggs at regular interval which contain cholesterol that risk for health. Therefore, low-cholesterol eggs would not only be beneficial to public's health but also bear business advantage. Egg cholesterol is first biosynthesized in the liver of laying hens and secreted into the plasma in the form of very low-density lipoproteins (VLDL) which transfer to the ovary. Egg cholesterol has been shown to vary with species of bird, breed or strain as well as age of fowl. Egg cholesterol contents can be altered by (i) genetic selection such as upward direction method or selection of hens that produce low cholesterol eggs and (ii) diet alteration. Mulberry leaf also contains phytosterols (plant sterols) which are structurally similar to cholesterol that act in the intestine to lower cholesterol absorption and helps in reduction of cholesterol in the blood vessels (Ray Sahelian, M.D. 2003). So, mulberry leaf diets may inhibit the synthesis of cholesterol and fatty acids in the liver. Thus, the mulberry leaf could be supplemented in laying hen diet at different levels to investigate the efficiency of this unconventional feed ingredient for the reduction of egg yolk cholesterol. Here we combined Mulberry leaf with neem leaf in different diets and fed to the laying hen.

Therefore, present piece of research work was undertaken with the following objectives:

1) to observe whether the combined of NLM and MLM has potentiality in reduction of egg yolk cholesterol.
2) to find out a suitable levels of combination before NLM and MLM for the improvement of production performance and egg quality characteristics including that of yolk cholesterol.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Cholesterol

Cholesterol is a waxy, fat-like substance that's found in all cells of the body. Our body needs some cholesterol to make hormones, vitamin D, and substances that help you digest foods. Our body makes all the cholesterol it needs. However, cholesterol also is found in some of the foods we eat. Cholesterol travels through your bloodstream in small packages called lipoproteins (lip-o-PRO-teens). These packages are made of fat (lipid) on the inside and proteins on the outside. Two kinds of lipoproteins carry cholesterol throughout your body: low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Having healthy levels of both types of lipoproteins is important. LDL cholesterol sometimes is called 'bad' cholesterol. A high LDL level leads to a buildup of cholesterol in your arteries (Arteries are blood vessels that carry blood from your heart to your body). HDL cholesterol sometimes is called 'good' cholesterol. This is because it carries cholesterol from other parts of your body back to your liver. Our liver removes the cholesterol from your body (www.nhlbi.nih.gov/health/health-topics/topics/hbc).

2.1.1 High blood cholesterol

High blood cholesterol is a condition in which you have too much cholesterol in your blood. By itself, the condition usually has no signs or symptoms. Thus, many people don't know that their cholesterol levels are too high. People who have high blood cholesterol have a greater chance of getting coronary heart disease, also called coronary artery disease (In this article, the term 'heart disease' refers to coronary heart disease). The higher

the level of LDL cholesterol in your blood, the GREATER your chance is of getting heart disease. The higher the level of HDL cholesterol in your blood, the LOWER your chance is of getting heart disease. Coronary heart disease is a condition in which plaque (plak) builds up inside the coronary (heart) arteries. Plaque is made up of cholesterol, fat, calcium, and other substances found in the blood. When plaque builds up in the arteries, the condition is called atherosclerosis (ATH-er-o-skler-O-sis). Atherosclerosis Figure A shows the location of the heart in the body. Figure B shows a normal coronary artery with normal blood flow. The inset image shows a cross-section of a normal coronary artery. Figure C shows a coronary artery narrowed by plaque. The buildup of plaque limits the flow of oxygen-rich blood through the artery. The inset image shows a cross-section of the plaque-narrowed artery. Over time, plaque hardens and narrows your coronary arteries. This limits the flow of oxygen-rich blood to the heart. Eventually, an area of plaque can rupture (break open). This causes a blood clot to form on the surface of the plaque. If the clot becomes large enough, it can mostly or completely block blood flow through a coronary artery. If the flow of oxygen-rich blood to your heart muscle is reduced or blocked, angina (an-JI-nuh or AN-juh-nuh) or a heart attack may occur. Angina is chest pain or discomfort. It may feel like pressure or squeezing in your chest. The pain also may occur in your shoulders, arms, neck, jaw, or back. Angina pain may even feel like indigestion. A heart attack occurs if the flow of oxygen-rich blood to a section of heart muscle is cut off. If blood flow isn’t restored quickly, the section of heart muscle begins to die. Without quick treatment, a heart attack can lead to serious problems or death. Plaque also can build up in other arteries in your body, such as the arteries that bring oxygen-rich blood to your brain and limbs. This can lead to problems such as carotid artery disease, stroke, and peripheral artery disease.

2.1.2 Cholesterol and human health

High cholesterol levels in the diet have been linked with increased incidence of atherosclerosis Friedman *et al.* (1968). Concern about the relation-ship between dietary fat and the development of atherosclerosis has led to publication of a number of reports encouraging changes in the human diet. These have included recommendations for the reduction in total fat content in the ratio of saturated to unsaturated fatty acids and in intake to total cholesterol Lo less than 300 mg/day (Brown, 1990; Cannon, 1990). The mysteries of the fatty protein, Cholesterol. Essential to the production of hormone and cell membranes, have become an obsession with many people today, especially those whose sedentary life styles make heart disease a distinct possibility. The role of high Density Lipoprotein (HDL) is to carry fat to the liver and that of Low Density Lipoprotein (LDL) is to move fat around the blood stream but can also build up a residue on the walls of blood vessels and eventually block- them. People without enough HDL have a hi-risk of heart disease even if their combined cholesterol is low (Anonymous, 1994). The evidence correlating plasma cholesterol levels with coronary heart disease was established from early observations that cholesterol is a major component of the atherosclerotic plaque. In 1985, the recommendation of a consensus conference in America was ‘All American (except children under 2 years of aye) be advised to adopt a diet that reduces total dietary fat intake from the current level of 40% of total calories to 30% of total calories, reduces saturated fat intake to less than 10% of total calories and reduces daily cholesterol intake to 200 to 250 mg or less’. Naber (1976) and Noble (1987) stated that the reason for particular concern about eggs has a questionable scientific basis for the presence of substantial amount of cholesterol.

2.1.3 The problems due to cholesterol

2.1.4 Cholesterol and coronary heart disease

Cholesterol is a sterol required by the body for a number of functions including the maintenance of cell-membrane 'flexibility and permeability and the production of sex hormones' cortical, vitamin-D and bile salts. The human body can synthesize cholesterol but it is also found in some foods, particular in meals, poultry. Dairy products and eggs. Dietary cholesterol can elevate levels of blood cholesterol, however. It is increasingly accepted that saturated fats and trans-fats can have a greater impact than dietary cholesterol in raising blood cholesterol level generally and LDL cholesterol specifically. This is important because a high level of cholesterol (in particular LDL) in the blood is a major risk factor for CHD, which in turn can lead to a heart attack. As eggs are a rich source of dietary cholesterol and experimental evidence showed that dietary cholesterol increased serum cholesterol, the public were cautioned about eating eggs because of concerns about the associated risk of C11D. However, the correlation between diet-in, fat and cholesterol and plasma lipid concentrations had bee. The subject of many contradictory views and studies over the years. Epidemiology studies such as those undertaken Hu *et al.* (1999) to examine the effect of egg consumption on the risk of cardiovascular disease. Concluded that the consumption of up to one egg per day was unlikely to have a significant effect on the risk of CHD or stroke among healthy men or women. The authors did however note that there was a tendency for egg consumption to be associated with an increased risk of CHD if the participant was diabetic and suggested that this aspect should be considered further. In contrast a meta-analysis conducted by weggemans *et al.* (2001) on data covering the period 1974-1999 led to the conclusion that dietary cholesterol did raise the ratio of total to HDL cholesterol and hence the

advice to limit cholesterol intake by reducing the consumption of eggs and other cholesterol rich foods may, therefore still be valid. Similar findings, albeit only for women (there was no correlation for men), have been reported recently by Nakamura *et al.* (2004). In contrast Song and Kerver (2000) concluded that dietary cholesterol was not related to serum cholesterol concentration and furthermore that consumers of more than four eggs/week had a significantly lower mean serum cholesterol concentration compared with those who reported eating less than or equal one egg per week.

2.1.5 Cholesterol, animal fat, and heart diseases

Numerous types of heart diseases contribute to the death toll of few million persons in the1P world every year-, among them, hypertension, cerebra-vascular disease (stroke), congestive heart failure and atherosclerosis are common. Much attention has been given to the role of animal fats in atherosclerosis, a type of disease wherein a build-up of soft, amorphous lipids and connective tissue develops on the wails of the arteries of the heart. When these deposits become sufficiently large, clots may form and subsequently decrease the diameter of the arterial lumen. In some cases blood flow is greatly impaired resulting a heart attack. Research indicated that individuals with high serum cholesterol levels had a higher rate of atherosclerosis than people with normal levels. Increased serum cholesterol levels can be induced in. susceptible individuals when animal fats which are highly saturated anti foods high in cholesterol as in eggs are consumed. Thus, the hypothesis that cholesterol is responsible for heart disease becomes accepted by many as fact. In recent years, research has clearly indicated that this position is entirely too simplistic. For example, studies have shown that certain African tribes whose diets consist almost entirely of animal products do not have elevated serum cholesterol levels. It is fact that

dietary fat is implicated in atherosclerosis, but must be realized from, further research that it is not the sole cause, rather, a number of factors enter into the cause of heart disease, many of which are more important that, cholesterol ; among them, stress, heredity, hypertension, diabetes mellitus, smoking, lack of exercise, and obesity. When the heart disease is correlated with the consumption of animal products one must also consider the benefits against the hazards. Countries with the highest life expectancies (70 to 72 years) such as, Sweden, Norway, Denmark, Japan, Israel and Switzerland are noted for their high egg production and per capita egg consumption. The nutrients supplied by eggs and meat provide well balanced nutrition, hence poultry products must not be eliminated from the diet. Rather, a well-planned diet, along, with exercise and a minimum of stress provides the best prevention against heart disease. Bangladesh is a developing country. A great majority of her people suffers from protein deficiencies and per capita consumption of egg is very low. When we compare egg consumption in between rural and urban people, the urban people consume more eggs and they are the consumes of most of the eggs produced in this country. In fact, a part of entire is facing many health problems and such as blood pressure, heart disease etcwhich may be related to dietary cholesterol intake. Even the people who do not have any vascular disease or heart problem are showing a tendency to cut own egg intake in fear of any future problem.

2.2 How can neem and mulberry leaf help to reduce cholesterol

Cholesterol alone is often a chemical compound which the entire body obviously delivers as a combo of unwanted fat and steroids. It is crucial as it can be a making block for hormones like as testosterone and estrogen not to mention for cell membranes. Almost all of the cholesterol, about 80% is created through the liver but 20% of it arises from our diet which happens to be in which most of the troubles start off. We get cholesterol from food this

kind of as poultry, fish, and meat and dairy, some of which may be really substantial in cholesterol. Most periods the liver is in a position to regulate the cholesterol amounts while in the physique but when too much is brought in by food it would make it more difficult to secrete and can result in what exactly is referred to as large cholesterol. There are two sorts of cholesterol; the LDL or 'bad' cholesterol and also the HDL or 'good' cholesterol. Any time you have too much poor cholesterol it prospects to a risk of coronary heart condition which consequently sales opportunities to heart attacks and stroke. The best way to complete this can be to help keep your pounds at a standard range, eat food items with less cholesterol, working out often and following an general healthier way of living. Prescription drugs also bring down cholesterol but you end up remaining on them your overall daily life which may cause side effects. Treating Superior Cholesterol with Neem Leaf Extract Scientific tests have proven that Neem leaf extract can reduce the poor cholesterol and entire cholesterol amounts greatly. The fact is, it has been proven to lessen it as much as 30% making it a really healthful and natural substitute to prescription prescription drugs. Neem leaf extract in Neem educate which may be utilized each day to aid maintain cholesterol at wholesome stages. Or for those who frequently have decrease cholesterol will be able to basically drink a glass of Neem tea or consider a Neem capsule after a especially fatty meal. This will likely enable to retain a balanced life-style and hold blood cholesterol below manage.

2.3 Egg yolk composition

Hen egg yolk is a complex mixture of different micro particles held in suspension. The solids, content of yolk is about 50%. Proteins and lipids are the major constituents of yolk accounting 15.7-16.6% and 32-35%, respectively (Powrie and Nakai. 1985). The yolk fraction contains and approximately, 66% triglycerol. 28% phospholipids, 5% cholesterol and

minor amounts of other lipids. It was estimated that the composition of yolk phospholipids is 73% pliosphatidvi choline (PC) 15.5% phosphatidylethan colamine (PC), 5.8% lyso phosphatidyl choline (LPC)2.5% shingomyelin, 2.1% lyso-phosphatidyl ethanolamine (LPE), 0.9% plasmalogen, and 0.6% inositol phospholipids. Egg yolk is a homogeneously emulsified fluid (Juneja, 1997). When diluted with water or saline it can be separated by centrifugation into plasma (the supernatant) and granule (the precipitate). The granule consists mainly of high density lipoprotein (HDL), and phosvitin. The major component of plasma is low-density lipoprotein (LDL) accounting for 65% of the total egg yolk protein and livetin, which accounts for 30% of the plasma protein. The livetin fraction consists of α- and β and livetins in egg yolk, HDL consists of α- and β lipovitellins, and exists as a complex containing about 10% phosphorus. About 80% of the phosphorus in yolk exists in phosvitin. It has been show that LDL composes 7major polypeptide ranging from 19-225kDa and some minor polypeptides by SDS-PAGE analysis (Mine, 1998).

2.4 Effect of egg-yolk cholesterol on human health

Hassel bring *et al.* (2011) observed and said that eating eggs regularly may increase risk for high blood cholesterol. One egg with its yolk contains about 213 mg of cholesterol. The National Cholesterol Education Program recommends limiting your cholesterol intake to no more than 200 mg per day. For most people, it’s okay to eat more than 200 mg of cholesterol per day once in a while. But it’s not a good idea to do this several times a week. A new study finds that people susceptible to blood-cholesterol spikes after eating eggs manage this extra cholesterol in a way that limits damage to their hearts. Adults are continually bombarded with messages about how eating foods rich in cholesterol can elevate an individual’s risks of atherosclerosis and heart attacks. Cholesterol moves through blood within

capsule like structures known as lipoproteins. Ingestion of several eggs a day does tend to increase blood concentrations of cholesterol, particularly the amount circulating in low-density lipoproteins (LDLs) the so called bad cholesterol. However, the new study showed, eating eggs can also increase the amount of cholesterol in high-density lipoproteins (HDLs) the good cholesterol. However, the new study showed that when people ate three or more eggs per day their bodies made bigger LDL- and HDL-lipoprotein particles than when they ate no eggs. That's important because other recent studies have suggested that larger LDLs are less likely than small ones to enter artery walls and contribute their cholesterol load to artery-clogging plaque. Similarly, larger HDLs are more robust than smaller ones at hauling cholesterol out of the bloodstream and, ultimately, out of the body. Stamler *el al.* (1998) investigated the real life effects of eggs in a large population of nearly 6,000 vegetarians and 5,000 non-vegetarians over a period of 13 years. Within this group of nearly 11,000 people, those eating eggs more than 6 times a week had a 2.47 times greater risk of dying of heart disease than those eating less than one egg a week. They also found a dietary reduction in cholesterol intake of 430 mg/dl (same as 2 eggs) was associated with a 43% reduction in long-term risk of coronary heart disease, a 25% reduction of risk of death from all causes, and 3 years longer life expectancy. In addition to heart disease, a higher cholesterol intake is also associated with more risk for strokes, blood clots, high blood pressure, and cancers of the breast, prostate, colon, lung, and brain. Rochford (1960) said that eggs are filled with too much protein, cholesterol, calories, fat, bacteria, and environmental chemical contamination to be consumed with any frequency, with any expectation of health. Egg protein is a common source of allergy in infants, children and adults, producing problems from hives to asthma. Eggs are high in fat which promotes obesity and type-2 diabetes. Fats and cholesterol in eggs promote the formation of cholesterol gallstones

and gallbladder attacks. Egg-borne infections caused by the salmonella bacteria can give rise to cramps, diarrhea, nausea, and vomiting, chills, fever and/or headache food poisoning called salmonellosis. The cholesterol in eggs is high; the amount of cholesterol from eggs and other foods that actually affects your blood cholesterol levels is different for everyone. The American Heart Association recommends that people with healthy levels of LDL cholesterol should consume no more than 300 mg of cholesterol per day, while those with problematic LDL levels should stay below 200 mg of cholesterol. Since one whole egg contains about 210 mg of cholesterol, limiting consumption is a good idea for people with high cholesterol. Egg cholesterol is located in the yolk.

Janine Baer (2011) found more than half the calories in eggs come from the fat in the yolk; a large (50 gram) chicken egg contains approximately 5 grams of fat. People on a low-cholesterol diet may need to reduce egg consumption; however, only 27% of the fat in egg is saturated fat that contains LDL cholesterol. The egg white consists primarily of water (87%) and protein (13%) and contains no cholesterol and little, if any, fat. There is debate over whether egg yolk presents a health risk. Some research suggests dietary cholesterol increases the ratio of total to HDL cholesterol and, therefore, adversely affects the body's cholesterol profile; whereas other studies show that moderate consumption of eggs, up to one a day, does not appear to increase heart disease risk in healthy individuals. Eggs are both bad and good for cholesterol. The egg yolks are definitely not good and may raise cholesterol levels. The egg whites are considered safe. However, if looking at the eggs and cholesterol levels, research has found that the egg whites contain a substance that counteracts the harmful effects of the egg yolk on your cholesterol level, to a big extent anyway. So eating a whole egg appears to be somewhat safe, even for someone on a low cholesterol diet.

Many health professionals, including myself, consider two eggs a week to be relatively safe to eat consider the eggs as unlikely to cause any serious effect on ones blood cholesterol levels. However, because of the eggs high cholesterol and saturated fat, we still look at the whole topic of eggs and cholesterol with some trepidation, which may turn out to be justified by later research. Also, some recipes require the egg yolk be used with the egg whites left out, such as when making certain cakes. In this scenario, the eggs high cholesterol may well end up killing you in the long run. The following facts and figures on eggs and cholesterol levels should help you monitor the cholesterol and saturated fat you are adding into your diet and recipes (Weggemans *et al.*, 2000).

2.5 Chemical composition of neem leaves

Table 2.1 Shows the chemical composition of neem leaves as reported by various researchers

Chemical components (%)	Vietmeyer (1992)	Bais *et al.* (2002)	Chaudhary (1999)	Sonaiya & Olori (1989)	Esonu *et al.* (2006)	Laboni and Choudhury (2007)
Dry matter	40.6	45.0	30.1	-	92.4	-
Crude Protein	17.5	18.7	16.0	17.5	20.7	18.2
Crude fiber	15.3	24.6	15.4	12.3	16.6	26.3
Ether extract	2.5	2.4	3.8	4.2	4.1	6.5
Nitrogen free extract	56.4	-	49.6	-	43.9	39.1

It can be concluded from the above table that variations in CP, CF, EE and NI-E concentration of neem leaves ranged from 16-21, 12-26, 1.4-6.5 & 50-56% respectively. The greatest variation was for CF, which probably resulted from variation in the maturity of leaves of lives plants of different age groups.

Table 2.2 Chemical composition of mulberry leaf (% DM), (Gonzalez *et al*., 2006)

Nutrient content	
Dry matter %	89.30
Crude protein %	29.80
Ether extract %	5.57
Crude fiber %	11.10
Gross energy kcal/kg	4220
Ash %	11.8
Neutral detergent fiber %	35.80
Acid detergent fiber %	28.00
Hemicelluloses %	7.80
Calcium %	2.73
Phosphorus %	0.28

2.6 Medicinal properties of neem leaves in human

Hao F *et al*., (2014) reported that neem component have anticancer function. Use of small amount of neem leaf extracts may protect the liver from damage when toxic agents were used to induce hepatocellular necrosis (Chattopadhay *et al.*, 1994). Udeinya (1994) reported that neem leaves may be used to prevent the adhesion of cancer cell to other cells in the body. Sing *et al.* (1979) reported that neem may be used in skin problems to

prevent infection. According to his report, neem reduced pain, kills bacteria that can cause infection, stimulates the local immune system and promotes rapid healing with reduced scarring. Viral hepatitis is a deadly disease with no effective remedy. Indian tests indicate that as much as 80% of file test cases showed significant improvement when treated with neem leaf (Wagh, 1988). Alain *et al.* (1989) concluded that neem leaves were effective in treating and preventing diabetes. Chattopadhay *et al.* (1992) reported that neem leaf extracts reduced cholesterol lncls significantly. He also reported that alcoholic extract of neem leaves reduced serum cholesterol by about .30% beginning two hours after administration and kept the level low for an additional four hours until that ended.

Upadhay *et al.,* (1993) reported that the neem leaf extracts and neern, bark extracts significantly reduced the P-24 viral proteins and induced in vitro production of IL-1 infection. Caldwell (1994) observed that neems have the ability to enhance the cell mediated vaginal lubricant before intercourse to protect the diseases due to vaginal contraction.

2.7 Medicinal properties of neem and mulberry leaves in livestock feeding

Mulberry leaves (*Morus alba*) are growing under varied climatic conditions, ranging from temperate to tropical, all over the world. The biomass yield of fresh leaves is often in the order of 25-30 tonnes/ha/year with a cutting interval of about 9-10 weeks, while leaves have a high protein content (18 to 25% in DM) and high (75 to 85%) in vivo DM digestibility (Ba *et al*., 2005). Therefore mulberry leaves have a high potential as a protein-rich forage supplement to be used in feed for monogastrics, ruminants and rabbits (Benavides 2000). Since mulberry leaves are rich in nitrogen, sulphur and minerals (Singh and Makkar 2002) they have the potential to be

used as a supplementary feed for improving livestock productivity. lictrosernoli *et al.* (1999) reported that the oral administration of neem *(Azadirachta indica)* leaves has an anti parasitic effect on grazing cattle. They found that the addition of neem leaves to the nutritional blocks reduced the number of parasite egg per gram of faces of grazing cattle. Firclin (2002) studied the effect of fresh neem leaves on sheep. He reported that fresh neem leaves significantly reduced the number of *Haemonchus controtus* in the abomasum of the treated sheep. Arunachal *et al.* (2002) conducted an experiment with 75 lambs naturally infected with gastrointestinal ysteines. They divided the animals into five groups and gave aqueous extracts of neem leaves, seeds and bark or standard dose of praziplus (G1V). GIV was used as control. The observed that anthelmentic efficacy of neem extracts and praziplus 53, 49 and 87 % respectively. Koley *et al.* (1994) tested mature, green leaves of *Azadirachta indica* (neem) for its antiinflamatory activity. They found significant and dose dependent anti inflamatory activities in rats and mice. They also found that doses which were sufficient to produce an anti-inflammatory, analgesic or antipyretic action had no ulcer genic effect on the gastric mucosa of rats. Pari *et al.* (2001) reported that ant hyperglycemic effect of diamed a ystei formulation composed of the aqueous extract of three medicinal plants. *Azadirachta indica, Cassia auriculata, Mondica charantia in rats with* alloxan induced diabetes. They also reported that diamed also prevented a decrease in body weight.

Subba Rao *et al.* (1971) found that mulberry leaf stalks and their residues after silkworm feeding are generally fed to cattle and gained weight whereas Jayal and Kehar (1962) observed that Mulberry was used to replace grain-based concentrates in lactating cows with excellent results. They also suggested that *M. indica* leaves could be used as supplements for lower

quality forages based on the high digestibility values of them. Rojas and Benavides (1994) observed that milk production of goats increased due to feeding of mulberry leaves. Trigueros and Villalta (1997) conducted an experiment on growing pigs in which a commercial concentrate was replaced by up to 20% by mulberry leaf but the best level of substitution was 15%. Vu Chi Cuong *et al.* (2005) used mulberry leaves to replace cottonseed for finishing steers and concluded that inclusion of mulberry leaves in the diet improved growth rate and feed conversion ratio. The mulberry leaves is used as a feed for ruminants which has been investigated extensively in both beef and dairy cattle with excellent results (Benavides *et al.*, 2002). They also observed that no difference in milk yield and quality among groups of grazing dairy cattle supplemented with either 100% concentrate, 60% concentrate with 40% mulberry or 25% concentrate with 75% mulberry supplements. Boschini (2002) reported that mulberry *(Morus SPP-)* appears to be an exceptional forage for the ruminant due to its high yield of biomass, high protein content of leaves and high DM digestibility as well as high palatability. Miller *et al.* (2005) conducted an experiment and concluded that mulberry leaf meal can be used effectively as a substitute for commercial grain concentrate in the diet of growing goats. Ly *et al.* (2001) found that mulberry leaves had potential as an alternative protein source for pigs. Jayal and Kehar (1962) conducted a feeding experiment on sheep and showed that the mulberry leaves are highly palatable.

2.8 Medicinal properties of neem and mulberry leaves in poultry

Odoh and Bratte *et al.* (2015) found that feeding of neem leaf meal to laying birds without deleterious effects on their blood constituents, serum biochemistry and benefit of reducing possible risks of infection from pathogenic bacteria. Olayinka Pius et al. (2012) found that feeding neem leaf have a dietary effect on growth performance. Sadekar *et al.* (1998)

found that feeding neem leaves to immunosuppressed broilers increased their humoral and cell mediated immune responses. They also suggested that neem leaves may be useful for treatment of immunos up press viral diseases, such as Infectious Bursal Disease (IBD) and Newcastle disease in birds.

Al-kirshi *et al.* (2010) conducted an experiment and observed that feeding mulberry leaf meal (MLM) reduced the feed intake, egg production, egg weight and egg mass, but feed conversion ratio was not affected. Shell weight and yolk weight were decreased, but shell thickness and albumen weight were not affected, Haugh units increased as the level of mulberry leaf meal increased. They also found that feeding mulberry leaf meal improved the yolk color whereas Lokaewmanee *et al.* (2009) conducted an experiment on the effects of dietary mulberry leaves on egg yolk color and egg quality. They observed that there were no adverse effects in feed intake, body weight gain, egg production rate, egg weight, yolk weight, albumin weight, shell thickness and Haugh unit in dietary mulberry leaves groups, compared to control. But, yolk color is increased up to 2.5% mulberry leaves supplementation. Narayana and Setty (1977) indicated that incorporation of 7.5% shade-dried mulberry leaves in poultry feed increased egg production. Uchino *et al.* (1988) found that New Hampshire hens raised in a mulberry garden produced eggs with a greater proportion of yolk and higher Haugh unit and yolk colour compared with eggs from commercially reared New Hampshire hens. Simol *et al.* (2009) conducted an experiment and observed that the carcass characteristics and colour of internal organs were apparently better for the mulberry leaf supplemented diet compared to the control diet. Panja (2004) conducted an experiment to determine the quantity of estrogen like compound in mulberry leaves and also studied on supplement in broiler feed for broiler production

performance. They found that the broilers with 5% mulberry leaves supplementation showed the better performance. Sudo *et al.* (2000) gave several reports on the utilization of mulberry leaves for poultry production. They observed that egg quality (e.g. egg weight and egg production ratio) was almost the same after feeding 2.5, 5, 7.5 and 10% of mulberry leaf. Yatabe and Iso (1999) reported that egg quality was significantly lowered when feeding 15% of mulberry leaves. They also observed that yolk was more yellow due to beta-carotene of mulberry leaves. Mulberry leaf has a role in reducing human blood pressure and there was significant difference in the cholesterol content of egg yolk feeding mulberry leaves at 2% level (Machu, 1990). Narayana and Setty (1977) found that incorporation of shade dried mulberry leaves at 7.5% level in layer's mash showed an increase in egg production with desirable yolk colour without any adverse effect on body weight and egg quality.

Park *et al.* (2010) conducted an experiment to investigate the effects of dietary supplementation of mulberry leaves on performance and blood characteristics of chickens. They found better weight gain and feed conversion. They also observed that total cholesterol and triglyceride were significantly decreased by the supplementation of mulberry leaves at 2% level compared to the control whereas Paichok Panja (2003) observed non-significant result of feed intake, weight gain, feed efficiency, carcass weight and dressing %age after supplementation of up to 2% mulberry leaves but, the blood cholesterol and triglyceride were found to decrease.

2.9 Medicinal properties of neem and mulberry leaves in rabbit

Ifeanyi Charles Okoli with Michael Uwaezuoke Iloeje (2010) reported that the Neem leaf meal have a significant effect on blood serum cholesterol and serum alkaline phosphatase concentrations. Ifeanyi Charles Ogbuewu

Ifeanyichukwu princewill (2008) Neem leaf meal increase the breeding performance in rabbits. Khosla *et al.* (2000) studied the hypoglycemic effects of neem *(Azadirachta indica) ndica)* leaf extract and seed oil in normal as well as in diabetic rabbit. They suggested that *Azadirachia indica* could he beneficial in diabetes ysteine in controlling blood sugar or might also be helpful in preventing or delaying onset of the diseases.

Abron Toure (2010) conducted an experiment and observed that blood glucose level of rabbit reduced at 5% level of mulberry leaves supplementation.

2.10 Medicinal properties of neem leaves in rats

Voravuth Somsak *et al.* (2015) reported that the plant can be recommended for use since it possessed a high protective effect against malaris and can renal damage in mice. Koley *et al.* (1994) tested mature, green leaves of *Azadirachta indica* (neem) for its anti inflamatory activity. They found significant and dose dependent anti inflamatory activities in rats and mice. They also found that doses which were sufficient to produce an anti inflamatory, analgesic or antipyretic action had no side effect.

CHAPTER 3

MATERIALS AND METHODS

This study was conducted at the Poultry Farm and Dairy and Poultry science laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. In an 8 weeks experiment period, 60 Hi-sex brown laying hens (age 60 weeks) were assigned to four dietary treatments with four replication of four (4) birds in each.

3.1 Preparation of birds

The experimental birds were housed in cages. Each compartment of the poultry cages has the dimensions of 35 cm length, 20cm breath and 37cm height where two birds were kept in each compartment. The cages and the poultry house were disinfected and fumigated properly before placing the birds.

3.2 Preparation of mulberry and neem leaves powder

Mulberry and Neem leaves were collected from the local area of Dinajpur district. The leaves were initially cut into small pieces and then sun-dried for about fifteen (15) days. The sun-dried mulberry leaves were milled into a powder. The diets were formulated to as per recommendation of the National Research Council (NRC, 1994) to satisfy the nutrients requirement of the laying hens. Diets were supplied with T_0: (control), T_1: (NLM 1% and MLM 2.5%), T_2 : (NLM 1% and MLM 5%), T_3 : (NLM 1% and MLM 7.5%), T_4 : (NLM 1% and MLM 10%). Feed and water were provided adlibitum.

3.3 Experimental diets

The experimental diets in mash form and drinking water were provided adlibitum. All diets were formulated manually to meet nutrient requirements as per recommendation of NRC. The chemical composition of experimental diets is shown in the Table 3.1.

Table 3.1 Chemical composition of experimental diets

Feed ingredients	Dietary level of mulberry and neem leaf meal				
	T_0(Kg)	T_1(Kg)	T_2(Kg)	T_3(Kg)	T_4(Kg)
Maize	55.5	53.5	52	51	47
Soybean meal	22	20	19	19	19
Rice polish	7	7	7	6.5	6.5
Meat & bone meal	7	7	7	6.5	6.5
Lime stone	8	8	8	8	8
DCP	0.75	0.75	0.78	0.75	0.75
Neem leaves	0	1	1	1	1
Mulberry leaves	0	2.5	5	7.5	10
Salt	0.5	0.5	0.5	0.5	0.5
Vitamin-mineral premix*					
Calculated composition:					
ME (Kcal/Kg)	2727.9	2752.10	2762	2774.35	2767.90
CP (%)	17.77	17.53	17.06	16.69	16.23
CF (%)	3.28	3.52	3.05	3.20	3.12
Ca (%)	3.51	3.45	3.6	3.49	3.10
P (%)	0.45	0.50	0.70	0.46	0.53
Lysine (%)	0.94	0.96	0.90	0.85	0.82
Methionine (%)	0.28	0.32	0.34	0.35	0.38

*Added vitamin-mineral premix (Rena-Layer; Renata Animal Health Ltd.) @ 250 g per 100 kg which contained: vitamin A: 4800 IU; vitamin D: 960 IU; vitamin E: 9.2 mg; vitamin $_{k3:}$ 800 mg; vitamin B_1: 600 mg; vitamin B2: 2 mg; vitamin B3: 12 mg; vitamin B_5: 3.2 mg; vitamin B6: 1.8 mg; vitamin B9: 2 mg; vitamin $_{B12:}$ 0.004 mg; Co: 0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; 1: 0.6 mg; Mn: 19.2 mg; Zn: 16 mg; Se: 0.48 mg; DL - Methionine: 20 mg; L- lysine: 12 mg.

The experimental diets were designed as

Here,

T_0 (control),

T_1 (NLM 1% and MLM 2.5%),

T_2 (NLM 1% and MLM 5%),

T_3 (NLM 1% and MLM 7.5%),

T_4 (NLM 1% and MLM 10%)

3.4 Collection, processing and storage of neem leaf meal

The fresh green neem leaves were harvested from neem trees grown at HSTU, Dinajpur. The green leaves were cleaned and then sun-dried on a polyethylene sheet. The leaves were made free of sterns and ground properly by hands. The neem leaf meal (NLM) so prepared were stored in polyethylene bag and preserved in the feed storage room until used for diet formulation. Proper care was taken in the feed storage room to avoid spoilage of NLM.

3.5 Data collection

During the experimental period, eggs were collected and weighed daily. Data on feed intake were collected weekly. Initial and final body weights of birds were taken. Eggs used in the experiment were collected per hen on day zero (0) and after 15 days interval up to two months. Egg production recorded daily but external and internal quality characteristics of eggs were determined bi-weekly followed by regular collection.

3.6 Observation of internal and external egg qualities

Egg qualities were measured from those eggs laid by birds of different diets group. Measured egg qualities were egg weight, shape index, shell dry weight, shell thickness, albumin index, fresh albumin weight, yolk index, fresh yolk weight and Haugh unit. For quality determination, egg weight was recorded by an electric weighing balance. The length of egg was measured by a slide calipers. The width was also estimated by a slide calipers. The eggs were then carefully broken down on a glass plate (40 x 20 cm) to determine the internal egg qualities.

3.6.1 Egg shape index determination

The shape index calculated for each egg from the width and length of the eggs using the formula derived by Reddy et al. (1979). The formula used for calculating the shape index is given below-

$$\text{Egg shape index} = \frac{\text{Av. Weight of egg}}{\text{Av. Length of egg}} \times 100$$

3.6.2 Albumin index determination

The albumin index was determined by dividing the height of thick albumin by the width of thick albumin (Heiman and Carver, 1936). The albumin index was then calculated by the following formula-

$$\text{Albumin index} = \frac{\text{Av. height of albumin}}{\text{Av. Diameter of albumin}}$$

3.6.3 Yolk index determination

The yolk index was calculated as the ratio of yolk height to yolk width without removing the yolk from the albumin (Wesley and Staldelman, 1959). The yolk index was calculated by the following formula-

$$\text{Yolk index} = \frac{\text{Av. height of yolk}}{\text{Av. Diameter of yolk}}$$

3.6.4 Weight of different egg components

The method outlined by Chowdhury (1988) was followed for partitioning different egg components. At first, egg was broken on glass plate. Then the yolk was separated carefully from albumin with the help of a spatula and transferred to a previously weighed Petridis and the raw yolk weight was taken. The albumin was al-so transferred to a previously weighed Petridis by a spatula and weighed. Precautions were taken· at all stages to avoid rupture of yolk.

The shells of the broken eggs were rinsed and washed thoroughly in tap water keeping the membranes intake. The washed shells with membranes were immersed in a beaker of water for removal of the shell membranes. The shell and shell membranes were oven dried separately at 105°C over night keeping them in a glass Petridis. On the following day, oven dried shell and shell membranes were cooled in room temperature. Weight of shell and shell membranes were taken. Finally, the following calculations were made for different components suggested by Chowdhury (1988).

1. Fresh yolk weight:

   (Weight of yolk + weight of petridish) - Weight of petridish.

2. Fresh albumin weight:

   (Weight of wet albumin + weight of petridish) - Weight of petridish.

3. Shell dry weight:

   (Weight of dried shell + weight of blotting paper) - Weight of blotting paper.

3.7 Determination of cholesterol of egg yolk

Cholesterol of egg yolk was determined in accordance with the method suggested by Lieberman-Burchard (1952) with little modification.

Figures 1: Neem leaf (*Azadirachta indica*)

Figures 2: Mulberry leaf (*Morus alba*)

Figures 3 : Sun drying of mulberry leaves

Figures 4 : Feeding to the laying birds

3.7.1 Preparation of solution and reagent

a) Chloroform-methanol solution

   Chloroform was mixed with methanol at ratio of 2:1 (v/v).

b) Potassium hydroxide (KOH)-33%

   Ten grams of potassium hydroxide (KOH) pellets were dissolved in twenty milliliter of distilled water to make 33% solution.

c) Petroleum ether (Prepared)

d) Modified Liebermann-Burchard reagent

Twenty volumes of acetic anhydride was chilled at temperature below 5°C in a stopper glass container and one volume of concentrated sulfuric acid was added. The well shaken mixture was kept at 0°C. Finally ten milliliters (10 ml) of glacial acetic acid was added and then was shaken properly. The mixture was kept at 0°C for 9 minutes, The reagent was allowed to warm at room temperature and thereafter used in the experiment within 1 hour.

Standard cholesterol (0.4 mg/ml)-Stock solution

One hundred milligrams (100 mgs) of cholesterol was dissolved in two hundred fifty milliliters (250 ml) of ethanol to make standard solution.

3.7.2 Preparation of standards

Standards were prepared for inclusion with series of determination. This was most conveniently done alone with samples. Five milliliters (5 ml) standard cholesterol solutions (0.4 mg/ml) were taken in a centrifuge tube and 0.30 ml of 33% KOH was added to it. The tube was then incubated for 55 minutes at 37°C-40°C. Ten milliliters (10 ml) of petroleum ether was added followed by five milliliters deionized water and mixed thoroughly.

Aliquots of 1, 2, 3 and 4 milliliters from the petroleum ether layer taken into tubes and evaporated to dryness to provide standard equivalent to 0.2, 0.4, 0.6 and 0.8 mg of cholesterol respectively. The tubes containing the dry cholesterol residue of sample and standards were arranged in such a way that one set of standard tubes appeared at the beginning and another set at the end the series. Clear empty tube was kept in the beginning as the blank. The tubes were kept in a water bath at 25°C. Six milliliters (6 ml) of Liebermann-Burchard reagent was added to the blank tube first and then at regular intervals of 1 minute to the sample's and standards tubes. The entire surface of the tubes was washed down with the Liebermann-Burcbard reagent while pipetting and the tubes were shaken and returned to the water bath maintained at 25°C in a dark chamber. The reading was taken at 30 minutes after the addition of Liebermann-Burchard reagent. The intensity of the color in each tube was read at regular interval of one minute against the blank in a spectrophotometer set at 620 nm.

3.8 Statistical analyses

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 Range Test (Duncan, 1955). All analyses were performed by M-STAT and SPSS Program.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Laying performances

The performance of laying hens fed neem 1% and Mulberry Leaf meal up to 10% are discussed under the following sub -heading.

4.1.1 Egg production

The hen day egg production observed in different dietary treatments fed NLM and MLM was not similar and the differences were statistically significant (Table 4.1). Result indicates that the feeding of NLM 1% and MLM up to 10% in the diet of laying hen has detrimental effect on egg production. Feeding of MLM up to 5 percent with NLM 1% levels showed slightly higher egg production whereas the production was slightly decreased when the birds received 1% NLM with 10% MLM in the diet. These results are closed with the previous report of Lokaewmanee *et al.* (2009), however slightly differed from the observations of Ravindran *et al.* (1986), who found decreased egg production with the increased of the mulberry leaf meal. Similarly, egg production of White Leghorn birds was not different from the control groups by feeding up to 10 percent mulberry leaf meal (Soda, 1999).

On the other hand Gowda Sk *et al.* (1998) and Mj Akpan *et al.* (2010), feeding NLM up to 10% get decreased egg production. Linus and Lawrence *et al*. (2015) reported higher egg production by feeding NLM up to 7.5% in diet.

Table – 4.1 Performance of laying hens fed different levels of NLM MLM meal on the basis of total and digestible protein.

Variables	Periods	Dietary treatments					LSD values & level of significant
		T_0	T_1	T_2	T_3	T_4	
% Hen-day egg production	1st - 8th weeks	83.50 ± 0.065	85.77 ± 0.069	84.25 ± 0.064	79.25 ± 0.058	78.25 ± 0.057	*
Feed consumption (g/bird/day)	1st - 28 day	109.79 ± 2.03	113.50 ± 2.65	111.71 ± 1.98	110.71 ± 2.33	111.43 ± 2.31	NS
	2nd -28 day	113.03 ± 2.23	114.21 ± 3.12	113.64 ± 2.21	111.82 ± 2.45	113.25 ± 2.42	NS
Feed conversion ratio (gm feed / bd weight)	1st - 28 day	1.70 ± 1.90	1.72 ± 1.75	1.70± 1.825	1.67± 133	1.70± 2.02	NS
	2nd - 28 day	1.65 ± 2.10	1.57 ± 1.92	1.63± 1.93	1.65± 1.42	1.67± 1.65	NS
Body weight and body weight gain (g)	1st -28 day	1.789 ± .039	1.848 ± .049	1.842 ± .030	1.858 ± .037	1.837 ± .079	NS
	2nd - 28 day	1.912 ± .047	2.043 ± .080	1.958 ± .045	1.895 ± .064	1.900 ± .093	NS
	Weight gain	0.123	0.195	0.116	0.037	0.063	NS
Livability (%)	56 days	100	100	100	100	100	NS

NS = Non significant

* = Significant at the 0.05 % level ** = Significant at the 0.01% level

4.1.2 Feed consumption

Supplementation of NLM and MLM in the diet show (Table - 4.1) the similar feed consumption ($p<0.01$). Birds of control group consumed 110.10 gm per day, whereas group T_1 (113 gm) was highest. Group T_2 and T_4 showed same feed consumption 112.68 gm and 112.30 gm respectively.

4.1.3 Body weight

Body weight in different dietary treatments before experimental periods was almost similar and the differences were not significant ($p> 0.05$) (Table 4.1) neem leaf have growth performance in poultry. Most of the bird had improved body weight after the dietary treatment by NLM with MLM. After complete the experiment, the result shows the body weight gain non-significantly level which is the similar report as Olayinka Pius *et al.* (2012). Where he get a no significant ($p<0.05$) effect on growth performance by feeding NML in broiler.

4.1.4 Body weight gain

Birds on fed T_1 group gained the highest (195gm) body weight and that of control group was (123gm) body weight. In spite of little increases in body weight in T_2, T_3 and T_4 level, no significant effect of MLM and NLM on body weight was appeared.

4.1.5 Livability

No bird was died during the entire experimental period, suggesting that the inclusion of NLM / MLM alone or their combination up to the levels tested in present study had no detrimental effect.

Table- 4.2 External egg quality characteristics of laying hens fed different level of NLM and MLM on the basis of total and digestible protein.

Variables	Periods	Dietary treatments					LSD values & level of significant
		T_0	T_1	T_2	T_3	T_4	
Weight of egg (gm/ egg)	4th week	68.38 ± 2.318	70.06 ± 3.601	67.18 ± 0.660	66.67 ± 0.845	69.20 ± 4.884	**
	8th week	68.86 ± 7.215	63.84 ± 0.581	66.66 ± 0.569	66.89 ± 1.150	60.50 ± 1.646	NS
Length of egg (mm)	4th week	58.70 ± .264	59.88 ± .508	59.60 ± .513	59.43 ± .501	60.23 ± 2.258	NS
	8th week	62.13 ± 2.539	57.51 ± 1.340	59.31 ±.316	59.50 ± 1.148	58.27 ± 1.307	NS
Width of egg (mm)	4th week	46.10 ± 1.167	45.75 ± .621	48.73 ± 3.007	44.88 ± .376	45.77 ± 1.289	NS
	8th week	45.87 ± .983	44.30 ± .217	44.77 ± .185	44.40 ± .929	43.48 ± .422	NS
Shell dry wt	4th week	6.12 ± .164	6.52 ± .182	6.85 ± .065	6.67 ± .189	6.54 ± .042	NS
(gm/egg)	8th week	6.67 ± 0.189	7.06 ± 0.044	6.51 ± 0.164	6.54 ± 0.037	6.71 ± 0.265	NS
Egg Shape Index (%)	8th week	78.54 ± 0.67	76.40 ± 0.76	81.76 ± 0.83	75.52 ± 0.72	75.99 ± 0.82	NS

NS = Non significant,

* = Significant at the 0.05% level

4.2 Egg weight

The egg weight in different dietary treatments during experimental periods were statistically non significant (Table 4.2). These results indicate that inclusion of NLM 1% with MLM 10% in the diet of laying hens has no inimical effect on egg size. However, feeding mulberry leaf meal in with higher dietary levels showed a tendency to reduce egg weight. The results are consistent with the report of Tateno *et al.* (1999) and Sudo, *et al.* (2000). Both of these researchers found non-significant difference in size after the birds exposed to 15 percent mulberry leaf meal in the diet. Where Mj Akpan *et al.* (2010) get increasing egg weight by feeding neem leaf meal. Linus and Lawrence *et al*. (2015) reported that feeding up to 7.5 % NLM show the higher egg, yolk weight compared the control.

Table - 4.3 Internal egg quality characteristics of laying hens fed different level of NLM and MLM on the basis of total and digestible protein.

Variables	Periods	Dietary treatments					LSD values & level of significant
		T_0	T_1	T_2	T_3	T_4	
Height of thick albumin (mm)	4th week	7.31 ± .253	6.93 ± .825	6.62 ± .195	6.19 ± .397	6.45 ± .693	*
	8th week	6.79 ± 1.211	8.26 ± .396	6.98 ± .361	7.70 ± .424	6.10 ± .367	NS
Diameter of albumin (mm)	4th week	97.18 ± 1.806	103.30 ± 5.953	102.58 ± 4.399	110.45 ± 3.847	102.43 ± 4.474	NS
	8th week	91.47 ± 6.143	89.82 ± 1.093	92.83 ± 1.841	92.29 ± 1.076	91.83 ± 5.074	*
Height of yolk (mm)	4th week	17.91 ± .299	17.52 ± .329	16.66 ± .208	16.87 ± .370	17.41 ± .604	NS
	8th week	17.12 ± .663	16.68 ± .153	17.19 ± .499	15.46 ± .628	16.55 ± .455	NS
Width of yolk (mm)	4th week	41.16 ± .841	41.87 ± .484	41.56 ± .428	43.05 ± .954	43.50 ± .857	NS
	8th week	37.43 ± 4.649	37.60 ± .360	39.55 ± .930	40.95 ± 1.797	40.60 ± .519	*
Fresh yolk wt. (gm/egg)	4th week	18.38 ± .947	17.21 ± .474	17.76 ± .728	17.99 ± 1.082	17.43 ± .612	*
	8th week	20.83 ± 2.463	15.46 ± .597	17.87 ± .174	18.01 ± 1.165	16.06 ± .886	NS
Fresh albumin wt (gm/egg)	4th week	39.61 ± 3.156	41.33 ± 1.787	39.24 ± 1.031	38.33 ± 1.304	39.49 ± 1.347	*
	8th week	38.64 ± 4.524	39.09 ± .651	39.78 ± 1.479	39.07 ± 1.894	35.08 ± .750	NS
Albumin index (%)	8th week	7.52 ± 0.38	6.71 ± 0.28	6.45 ± 0.32	5.60 ± 0.35	6.29 ± 0.42	NS
Yolk index (%)	8th week	45.73 ± 0.41	44.36 ± 0.36	43.46 ± 0.38	37.75 ± 0.42	40.76 ± 0.41	NS

NS = Non significant,

* = Significant at the 0.05% level

4.2.1 External and internal egg quality characteristics

It was observed that the shape index, shell thickness, albumin weight, albumin index, yolk weight, yolk index and Haugh of the eggs laid by hens fed different diets were almost similar during experimental periods and the differences were non-significant ($p > 0.05$) (Table 4.2 and Table 4.3 These results indicate that feeding mulberry and neem leaf meal up to 10:1 % level had no adverse effect on external and internal qualities of eggs. However, egg shell weight (gm) increase slightly after supplementation of 2.5:1 mulberry and neem leaf meal. Egg shell thickness slightly improved at the level of 2.5 and 10 % mulberry leaf meal. Albumin weight decreased in the dietary treatments 5 and 7.5% but a little bit increased in dietary treatment 10% from those of control groups. Albumin index improved slightly after inclusion of mulberry leaf meal in comparison to control. Moreover, Yolk weight and yolk index slightly increased after the inclusion of 2.5 to 10% mulberry leaf meal but al weight are lower than control. Similar results have been obtained by Tateno *et al.* (1999), Sudo *et al.* (2000) and Mj Akpan *et al.* (2010) who did not found any significant differences in the external and internal qualities of eggs up to 10 percent level of mulberry leaf meal.

4.3 Egg-yolk cholesterol

This study showed that egg-yolk cholesterol was decreased significantly by supplementation of mulberry and neem leaf meal in layer-ration ($p < 0.05$). It is evident from Table 4.4 that a tendency of reduced egg yolk cholesterol was observed in the dietary treatments with inclusion of 1% neem leaf meal and 2.5 to 10% mulberry leaf meal. However, the highest level of cholesterol was 18.94 mg/gm at 2.5 % level and lowest level was 16.71 mg/gm of egg-yolk at 10 % level of mulberry leaf meal whereas cholesterol of "control egg" ranged from as low as 19.38 mg/gm of yolk to as high as 18 mg/gm of yolk (USDA, 2008). During experimental periods egg yolk cholesterol was reduced to 0.07 mg/gm to

2.67 mg/gm after dietary supplementation of 2.5 - 10% of mulberry leaf with 1% neem leaf meal as compared to control. Thus, the result of current study clearly showed that mulberry leaf meal at 2.5, 5, 7.5 and 10% dietary level had beneficial effect in reduction of egg yolk cholesterol. The similar results obtained from (Machii, 1990) who found reduced egg-yolk cholesterol at 2% level of mulberry leaf meal. Liver is the organ that regulates the deposition of lipids and phospholipids in egg-yolk (Bell and' Freeman, 1971). Since liver and serum cholesterol are decreased by supplementation of mulberry leaf meal which may leads decreased egg-yolk cholesterol. Thus, the decrease in egg-yolk cholesterol by dietary mulberry leaf meal supplementation may be due to a lesser deposition of cholesterol by liver in egg-yolk during yolk synthesis.

Table 4.4 : Effect of NLM and MLM in egg yolk Cholesterol

Week	Neem and Mulberry leaf supplementation (%)					Sign.
	T_0	T_1	T_2	T_3	T_4	
4^{th} week (mg/gm)	19.38^{a} ±.48636	19.31^{a} ±.50207	18.98^{a} ±.21957	19.29^{a} ±.34588	19.10^{a} ±.38459	NS
8^{th} week (mg/gm)	18.98^{a} ±.37302	18.94^{a}±.25115	18.63^{ab}±.10039	17.83^{b}±.33045	16.71^{c}±.08660	**

NS = Non significant,

** = Significant at the 0.01% level

Values are expressed as mean ± standard error of means. a, b, c means within row with different superscripts are statistically different ($p<0.05$). NS: Statistically not significant ($p> 0.05$). * Statistically significant ($p<0.05$).Means represents four replicates, three birds per replicate. NLM= Neem Leaf Meal, MLM= Mulberry Leaf Meal.

From the above discussion, it is said that there is no significantly difference at 4^{th} week and the cholesterol level significantly decreased at 8^{th} week without affecting egg qualities with increased level of neem and mulberry leaf meal supplementation for long time. Neem and mulberry leaves contain phytosterol that is responsible for lower absorption of cholesterol from the intestine resulting lower deposition of cholesterol in egg-yolk. As a result, cholesterol of egg-yolk was reduced.

CHAPTER 5

SUMMARY AND CONCLUSION

Mulberry leaves and neem leaves were collected from local area of Dinajpur district to observe its effect on reduction of egg yolk cholesterol and any alteration of egg quality characteristics and production performance. The feeding value of mulberry and neem leaf for laying hen (Hi-sex brown) was evaluated at Hajee Mohammad Danesh Science and Technology University poultry farm, Dinajpur district. In feeding trial, five (5) diets were prepared including combined of Neem leaf at levels of T_0 : (control), T_1: (NLM 1% and MLM 2.5%) , T_2 : (NLM 1% and MLM 5%), T_3 : (NLM 1% and MLM 7.5%), T_4 : (NLM 1% and MLM 10%) by replacing soybean meal, maize, rice polish, meat and bone meal. In experimental diets, laying performance, external and internal quality characteristics of eggs in different dietary treatments were almost similar and the differences were statistically non-significant except egg yolk cholesterol. Egg yolk cholesterol decreased with increased level of mulberry leaf meal up to 10% level with 1% neem leaf meal which I investigated.

Food consumption for the entire experimental period in different treatment groups was recorded and expressed as g/day. Although the rate of food intake varied from day to day but the total food intake (gm/day) was maximum in control group (110.10) followed by T_1 (113.86gm), T_2 (112.68gm), T_3 (111.26gm) and T_4 (112.30gm) respectively. In all test groups food consumption was almost similar to control (112.3 g). Data obtained on final average body weight indicated that there was no positive correlation between body weight and food consumption. Food conservation ratio (F.C.R.) was the highest in control (1.68) compared with other groups. The FCR values were found to be almost the same in different diets at T_0 (1.67), T_1 (1.65), T_2 (1.67),

T_3 (1.66) and T_4 (1.69) neem and mulberry leaf meal. Egg production was maximum in diet at 5% level of mulberry leaf meal but values were almost same in the diet at T_0 (83.50%), T_1 (85.77%), T_2 (84.25%), T_3 (79.25%) and T_4 (78.25%). Data obtained on egg weight expressed as maximum level in control group T_0 (65.23 gm) than the other feeds fed group but almost similar to diet with T_1 (66.21 gm), T_2 (65.27 gm), T_3 (65.01 gm) and T_4 (64.23gm) level of neem and mulberry leaf meal. Data obtained on egg shell weight were almost similar at T_0 (6.67 g), T_1 (7.06 g), T_2 (6.51 g), T_3 (6.54g) and T_4 (6.571 g) level of neem and mulberry leaf meal in the diets. Shape index were found to be highest at diet with T_2 (81.76%) level of mulberry leaf meal but almost same to all other feed groups at T_0 (78.54%), T_1 (76.40%), T_3 (75.52%) and T_4 (75.99%). Data obtained on albumin index exhibited maximum level in diet with T_0 (7.52%) than the other feeds fed group but almost similar to diet with T_1 (6.71%), T_2 (6.45%) T_3 (5.60%) and T_4 (6.29%) level of neem and mulberry leaf meal. The yolk index values were found maximum level in the diet T_0 (45.73%) than the other feeds T_1 (44.36%), T_2 (43.46%), T_3 (37.75%) and T_4 (40.76%) level of neem and mulberry leaf meal.. Data obtained on total cholesterol exhibited a higher level in control group (18.98 mg/gm) than the other foods fed group. On the other hand, the total cholesterol level was significantly lower in diet at 10% mulberry leaf meal (16.71 mg/gm) than control group. The best reduced egg yolk cholesterol was obtained with diets at T_4 (16.71mg/gm) than control group. Whereas Other foods fed group was T_1 (18.94 mg/gm), T_2 (18.63 mg/gm), T_3 (17.83 mg/gm) by neem and mulberry leaf meal supplementation.

Based on the results of present study it may be concluded that mulberry and neem leaf are the good sources of protein, which have significant effect on the reduction of egg yolk cholesterol of laying hens without affecting the bird's feed intake, body weight and egg quality characteristics. Results suggest that the supplementation of neem leaf meal 1% with mulberry leaf meal *(Morus alba)*

up to the tested level (10%) in diets has high potential as commercial applications for production of low-cholesterol containing eggs and healthy birds. Therefore, neem and mulberry leaf meal can be used along with the other conventional feed ingredients. However, further study is to be needed to understand the active principle (s) of cholesterol lowering and other beneficial effects of mulberry leaf meal observed in this experiment prior to practical use it as unconventional feed of poultry.

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APPENDICES

Appendix- 1: Chemical composition of feed ingredients used for formulation of experimental diets

Ingredients	DM %	ME (Kcal/kg)	CP %	EE %	CF %	Ca %	TP %	Lys %	Meth %	Cyst. %	Tryp. %
Maize	90.0	3400	10.0	3.50	2.0	0.02	0.35	0.24	0.12	0.18	0.07
Rice polish	88.1	3090a	16.4	14.8	10.5	0.27a	0.14a	0.57a	0.22a	0.21a	0.13a
Soyabean meal	89.0	2426	42.0	3.50	6.5	0.25	0.20	2.70	0.60	0.62	0.58
Bone & meat meal	93.0	2536	50.0	8.50	2.8	9.20	4.70	2.60	0.67	0.33	0.26
Oyster shell	99.0	-	-	-	-	35.0d	0.018	-	-	-	-
Mulberry leaf	94.0	3200c	21.6	7.40	12.2	3.5c	0.24	0.35c	0.78c	0.11	0.09
Neem leaf	89.30	4300	17.48	2.46	15.27	1.2	-	0.89	0.51	-	-

a = NRC, 1994

c = Lohan, 1979 and Makkar, 1989

d = Singh and Panda, 1992

Appendix- 2: Food intake trend over the experimental period.

Diets	1st week (gm)	2nd week (gm)	3rd week (gm)	4th week (gm)	5th week (gm)	6th week (gm)	7th week (gm)	8th week (gm)
Control diet	755	765	758	768	775	772	785	788
Diet with 1% NLM and 2.5% MLM	795	800	798	785	803	810	795	790
Diet with 1% NLM and 5% MLM	778	785	775	790	792	798	800	792
Diet with 1% NLM and 7.5% MLM	765	772	778	785	772	782	787	790
Diet with 1% NLM and 10% MLM	770	782	778	790	798	800	795	778

Appendix- 3: Chemicals and instruments used in egg - yolk cholesterol determination.

Chemicals	Instruments
Chloroform	Water bath
Methenol	Vortex Mixture
Potassium Hydro - oxide (KOH)	Incubator
Acetic Anhydride	Sonnicator
Conc. Sulphuric acid (H2SO4)	Hot air oven
Glacial acetic acid	Vacuum evaporator
Ethanol	Centrifuge machine
Distilled water	Centrifuge tube
Deonized water	Spectrophotometer
Petroleum ether	Routine laboratory articles

Appendix- 4: Price of feed ingredients in experimental diet.

Feed ingredients	Prise (Tk/Kg)
Wheat	20
Maize	16
Rice polish	15
Meet and bone meal	60
Soybean meal	34
Oyster shell	8
Fish meal	60
Vitamin-mineral premix	120

Appendix- 5: Weekly average temperature of the experimental house during the experimental period.

Weeks	Average temperature (^{o} F)
60	87.5
61	89.0
62	89.2
63	88.4
64	87.8
65	86.5
66	85.7
67	86.2