SIGNIFICANT EFFECT OF MULBERRY LEAF (*Morus alba*) MEAL IN THE REDUCTION OF EGG-YOLK CHOLESTEROL

A THESIS
BY

MD. KAMRUZZAMAN

SEMESTER: MARCH-AUGUST/2011
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MASTER OF SCIENCE (M.S)
IN
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DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,
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DEPARTMENT OF DAIRY AND POULTRY SCIENCE
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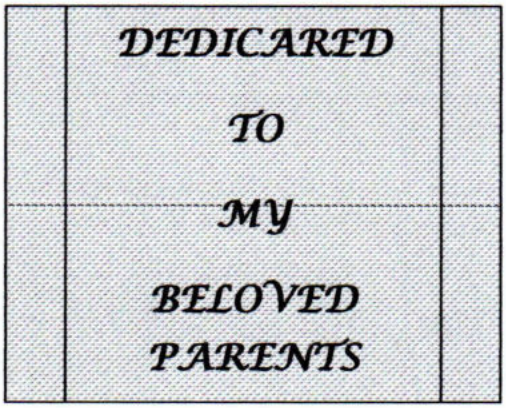
DEDICARED
TO
MY
BELOVED
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All praises are solely for the almighty "ALLAH" whose blessings have enabled the author to complete the research work and to prepare this manuscript for the degree of Master of Science in Poultry Science, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

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ABSTRACT

The objective of this study was to determine the effects of various dietary levels of mulberry (*Morus alba*) leaf meal on production performance, egg qualities and egg yolk cholesterol. The study was conducted at the poultry farm and Dairy and poultry science laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur district. In this study, Forty-eight 30-wk-old laying hens (Hi-sex brown) were divided into 4 dietary groups each with 4 replications (3birds/replication) and offered manually prepared diets supplemented with 0, 3, 6 and 9% mulberry leaf 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, egg mass, 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 9.4, 12.5 and 14.8% with 3, 6 and 9% level of mulberry leaf meal supplementation, respectively. Based on the results, it could be concluded that the supplementation of mulberry leaf meal up to my investigation level (9%) has potentiality in reduction of egg yolk cholesterol.

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ABBREVIATIONS AND SYMBOLS

%	: Percentage
°C	: Degree Celsius
et al.	: and his associates
FCR	: Feed Conversion Ratio
Fig.	: Figure
gm	: Gram
mg	: Milli Gram
mg/dl	: Milli Gram Per Deci Litter
ml	: Milli Litre
MS	: Master of Science
Sec	: Second
nm	: Nano Meter
mm	: Milli Meter
cm	: Centi Meter
MLM	: Mulberry Leaf Meal
T_1	: Treatment-1
T_2	: Treatment-2
T_3	: Treatment-3
T_4	: Treatment-4
v/v	: Volume/Volume
KOH	: Potassium Hydro-oxide
NS	: Not Significant
SD	: Standard Error
HG	: Haugh Unit
W	: Weight
Av.	: Average
VLDL	: Very Low Density Lipoprotein
IU	: International Unit
AOAC	: Association of Official Analytical Chemists
UK	: United Kingdom
USA	: United States of America
Cu	: Cupper

ABBREVIATIONS AND SYMBOLS (CONTD.)

DM	: Dry matter
K	: Potassium
Mg	: Magnesium
Mn	: Manganese
N	: Nitrogen
Na	: Sodium
NFE	: Nitrogen Free Extract
P	: Phosphorus
S	: Sulphur
Ca	: Calcium

CHAPTER I

INTRODUCTION

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INTRODUCTION

Poultry is one of the most important sectors of livestock that provides cheapest animal protein (nutritious egg and meat) for human consumption within shortest period of time. Poultry production has greatly flourished during last three decades in Bangladesh. However, the acute dearth of fluctuating feed supply and their price are the major constraints to poultry production in developing countries like Bangladesh. By the year 2020, world population would be lifted to 8 billions with most of the population growth coming from the developing countries (Singh *et al.*, 2001).With increasing demand for livestock products as a result of rapid growth of population in the world economies and shrinking land area, future hopes of feeding the millions and safeguarding their food security will depend on better utilization of unconventional feed resources that do not compete with food for human beings. Poultry feed ingredients animal protein sources in particular, are very expensive and scarce due to high competition among poultry, human and other animals resulting in the escalating cost of these ingredients. The feed cost usually constitutes the major proportion which ranges between 60-75% of the total cost of poultry production and protein cost account for over 15% of the total feed cost in livestock and poultry farming (Ojewola *et al.*, 2005). The prices of conventional protein source feed ingredients such as groundnut cake; fish meal and soybean meal are always high and cannot permit profit maximization in poultry ventures. In view of this, current research interest in the poultry industry is aimed at finding alternatives to those elusive feed ingredients.

The list of possible feed alternatives includes tree fodder mulberry leaves (*Morus alba)* as a source of dietary protein for commercial livestock and poultry

operations. Mulberry grows well in the tropics and subtropics. It 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).

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

i) to observe whether the MLM has potentiality in reduction of egg yolk cholesterol.

ii) to observe whether the dietary supplementation of MLM up to 9% level causes any alteration of egg quality characteristics and production performance.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Mulberry (*Morus spp.)* leaves have been the traditional feed for the silk worm moth (*Bombyx mori*). There is evidence that sericulture started about 5000 years ago in South China Agricultural University and hence the domestication of mulberry. Mulberry has been selected and improved for leaf quality and yield for a long time. Through silk production projects, mulberry has been taken to countries all over the world and it has now spread from the temperate areas of northwest and central Asia, Europe and North America, through the tropics of Asia, Africa and Latin America, to the southern Africa and South America. There are many mulberry varieties which grow in various environments, from sea level to altitudes of 4000 m (FAO, 1990) and from the humid tropics to semi-arid lands like in the Near East with 250 mm of annual rainfall and southwest of the U.S.A. (Tipton, 1994). Mulberry is also produced under irrigation. Although the majority of silk production projects have had limited duration due to silk processing constraints and limited market opportunities, mulberry trees have remained in most places where they had been introduced.

The main use of mulberry globally is as feed for the silk worm moth. But depending on the location, it is also appreciated for its fruit (consumed fresh, in juice or as preserves), as a delicious vegetable (young leaves and stems), for its medicinal properties in infusions (mulberry leaf tea), for landscaping and as animal feed. In Peru, the multiple uses of mulberry have been recognized (Zepeda, 1991). There are several places where mulberry is utilized traditionally as a feed in mixed forage diets for ruminants like in certain areas of India, China

and Afghanistan. In Italy, there have been several studies on the use of mulberry for dairy cows and other domestic animals (Maymone *et al.*, 1959) and in France there was a research project to introduce mulberry in livestock production (Armand *et al.*, 1995). But it was only in the eighties that specific interest in the intensive cultivation and use of mulberry as animal feed started in Latin America. It is surprising that a plant which has been improved for leaf quality and yield to feed an animal, the silk worm which has high nutritional feed requirements, received limited attention by livestock producers, technicians and researchers.

Like several significant breakthroughs in science and technology, the discovery of the value of mulberry as a high quality feed in Latin America happened serendipitously. A Costa Rican farmer of Chinese origin whose silk project failed by feeding mulberry leaves to his goats. He communicated his observations to scientist of the Tropical Agriculture Research and Training Center who were receptive to the farmer's news and smart enough to include mulberry in their tree fodder evaluations and later in agronomic and animal performance trials (Benavides, 1996). In Africa, the International Centre for Research in Agro forestry (ICRAF) in Kenya and the Livestock Production Research Institute in Tanzania have conducted successful agronomic and animal trials by themselves, apparently without being aware of the interest elsewhere.

Fig. 2.1: Mulberry tree with leaves

Fig. 2.2: Fresh mulberry leaves

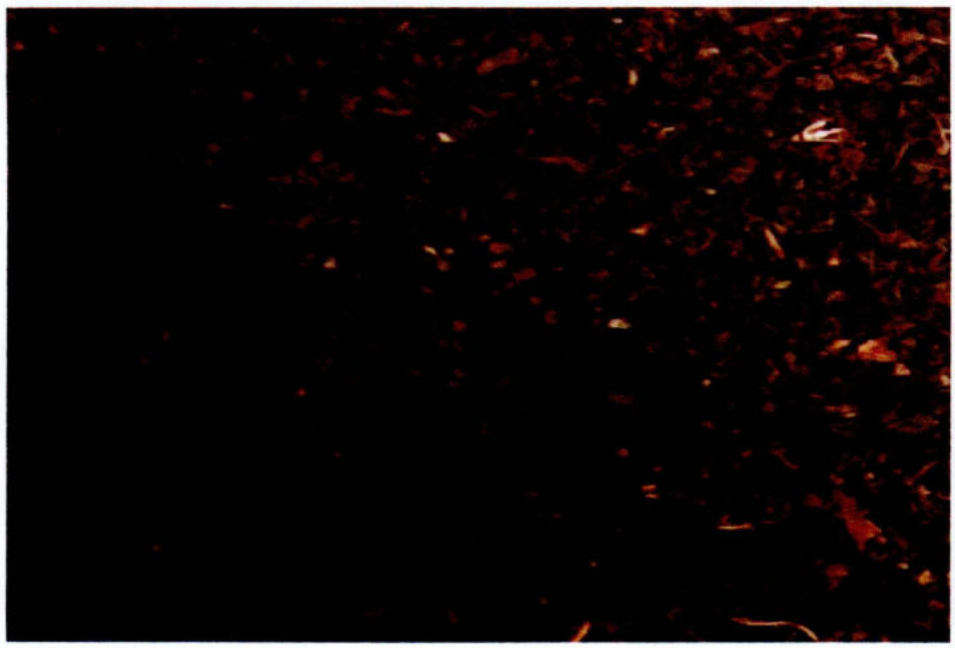

Fig.2.3: Sun-dried mulberry leaves

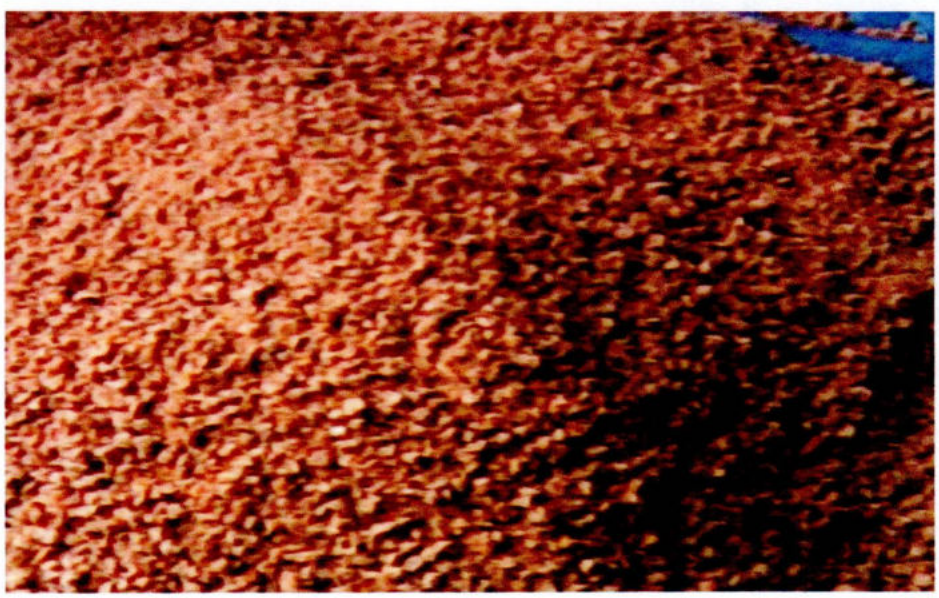

Fig. 2.4: Mixed feed without MLM (control)

2.1 Nutritive value of mulberry

Al-kirshi *et al.* (2010) found that the mulberry leaves contain 89.3% dry matter (DM), 29.8% crude protein (CP) and 4220 kcal/kg gross energy whereas Makkar *et al.* (1989) observed 3750 kcal/kg energy and 15.0-27.6% crude protein (CP).The mulberry leaf has 24-33% dry matter and 18.9-22.3% crude protein depending on the season (Liu *et al.* 2000). Mulberry leaves possess 11.5% CP (Subba Rao *et al.* 1971). Lohan (1980) found 20-23% crude protein, in mulberry on a dry matter (DM) basis. Benavides (1996) observed that mulberry (*Morus sp.)* is exceptional among woody forages which leaves contain more than 20% CP. Makkar *et al.* (1989) observed 2.3-8.0% ether extract (EE), 9.1-15.3% crude fiber (CF), 48.0-49.7% nitrogen free extract (NFE) and 63.3% total carbohydrates, in mulberry leaf on dry matter (DM) basis. Mulberry leaves have 11.1% fat, 32.3% crude fiber, 22.8% NDF, 0.28% ADF (Al-kirshi *et al.* 2010). Liu *et al.* (2000) found 34 -43% neutral detergent fiber (NDF), in mulberry on DM basis. Lohan *et al.* (1979) observed 5-10% hemicelluloses, 19-25% cellulose and 11% lignin in mulberry leaf. Subba Rao *et al.* (1971) found 34.0% CF and 76.5% total carbohydrate in mulberry species. Mulberry leaf has 45.6% neutral detergent fiber (NDF), 35.0% acid detergent fiber (ADF), 8-10% total sugar, 10-40% hemicelluloses, 10% lignin, 21.8% cellulose and 2.7% silica on a dry matter (DM) basis (Lohan, 1980).

Al-kirshi *et al.* (2010) observed that mulberry leaves contain 0.28% Ca, 2.7% P, 11.8% ash. A striking feature of mulberry leaves is the mineral content which contains 25% ash, 1.8-2.4% calcium and 0.14-0.24% phosphorus (Shayo, 1997). The mulberry leaves contain 14.3-22.9% ash, 2.42-4.71% calcium (Ca), 0.23 - 0.97% phosphorus (P), 0.196% sulphur (S), 1.66-3.25% potassium (K), 350 - 840 ppm iron (Fe) on DM basis (Makkar *et al.* 1989). Majumdar *et al.* (1967) reported 0.52-1.25% magnesium, 0.02-0.29% chlorine, 0.18-0.76% sulphur, 0.93-3.19% potassium and 0.13-0.23 % sodium in mulberry leaf

whereas Subba Rao *et al.* (1971) found that mulberry leaves contain 9.3% total ash, 1.6% calcium (Ca) and 0.2% phosphorus (P) on a DM basis. Espinoza *et al.* (1999) observed 1.90-2.87% potassium (K) and 0.47-0.63% magnesium (Mg) in mulberry leaf but young stems contain 1.33-1.53% potassium (K) and 0.26-0.35% magnesium (Mg). Mulberry leaf has 2.4-4.7% calcium (Ca) which could be useful for high yielding ruminants during early stages of lactation (McDowell, 1997). Sezai Ercisli & Emine Orhan (2006) found 0.83% N, 235 mg/100g P, 1141mg/100g K, 139 mg/100g Ca, 109 mg/100g Mg, 60 mg/100g Na, 4.3 mg/100g Fe, 0.4 mg/100g Cu, 4.0 mg/100g Mn and 3.1 mg/100g Zn, respectively in mulberry species. McDowell (1997) found that approximately 22% total N in the form of non-protein nitrogen in young leaves and 14% in mature leaves. The amino acids identified in the leaves are: phenylalanine leucine, valine, tyrosine, proline, alanine, glutamic acid glycine, serine, arginine aspartic acid, cystine, threonine, pipecolic acid and 5-hydroxy pipe colic acid. Mulberry leaf is a good source of essential amino acids especially 1.88% lysine and 2.55% leucine (Al-kirshi *et al.* 2010). Benavides (1996) observed that in-vitro DM digestibility (IVDMD) of mulberry leaves lie between 70-80 percent whereas others reported in-vivo digestibility 79 percent for DM and 89 percent for CP of mulberry leaf (Jegou *et al.* 1991).

Results of chemical composition of mulberry fractions from various authors are presented in the following table-

Table 2.1. Chemical composition of mulberry leaf (%DM), Machii (1989)

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

Table 2.2: Chemical composition (% of dry matter) of mulberry tree

Variety	CP	CF	NDF	ADF	EE	Ash	Ca	P	Reference
Leaf									
Hebba	15.9	12.6			7.1	15.9	2.42	0.24	Narayana & Setty, 1977
Izatnagar	15.0	15.3			7.4	14.3	2.41	0.24	Jayal & Kehar, 1962
Palampur	15.0	11.8			5.1	15.5			Singh *et al.*, 1984
Parbhani	22.1	5.9			3.9	13.4	3.3	1.43	Deshmukh *et al.*, 1993
Kanva-2	16.7	11.3	32.3		3.0	17.3	1.80	0.14	Trigueros & Villalta, 1997
Mpwapwa	18.6		24.6	20.8		14.3			Shayo, 1997
Dominican	20.0			23.1	4.0	4.5	2.70		"
Criolla	19.8						1.90	0.28	Espinoza *et al.*, 1999
Leaf & young stem									
Tigreada	27.6	13.2				10.4		0.20	Gonzalez *et al.*, 2006
Indonesia	24.3	15.3				11.2		0.29	"
Criolla	27.6	16.9				11.8		0.26	"
Acorazonada	25.2	14.1				13.4		0.15	"
Koruso 212	11.0	10.0	22.0	20.6	5.9	13.9	3.13	0.37	Casoli *et al.*, 1986
Koruso 213	8.0	11.8	24.7	24.5	5.3	19.3	4.76	0.37	"
Young stem									
Criolla	11.3						1.33	0.29	Espinoza *et al.*, 1999
Dominican	4.7			48.2	1.7	1.3	1.61		"
Stem									
Dominican	3.8			50.2	1.0	1.8	1.10		"
Mallur	11.5	34.0			2.7	9.32	1.56	0.20	Subba Rao *et al.*, 1971
Bark									
Mpwapwa	7.8		46.8	36.9		6.1			Shayo, 1997
Whole plant									
Dominican	11.3			34.4	1.6	1.9	2.10		"

Table 2.3: Nutritive Value of Mulberry (*Morus nigra .L*) per 100 g

Principle	Nutrient Value
Energy	43 Kcal
Carbohydrates	9.80 gm
Protein	1.44 gm
Total Fat	0.39 gm
Cholesterol	0 mg
Dietary Fiber	1.7 gm
Vitamins	
Folates	6 mcg
Niacin	0.620 mg
Pyridoxine	0.050 mg
Riboflavin	0.101 mg
Vitamin A	25 IU
Vitamin C	36.4 mg
Vitamin E	0.87 mg
Vitamin K	7.8 mcg
Electrolytes	
Sodium	10 mg
Potassium	194 mg
Minerals	
Calcium	39 mg
Copper	60 mcg
Iron	1.85 mg
Magnesium	18 mg
Selenium	0.6 mcg
Zinc	0.12 mg
Phyto-nutrients	
Carotene--ß	9 mcg
Carotene, α	12 mcg
Lutein-zeaxanthin	136 mcg

(Source: USDA National Nutrient data base)

Table 2.4: Average amino acid composition and N content of mulberry leaf (Machii, 1989)

Compound	Mulberry Content (mg/g DM)
Non essential amino acids	108.93
Essential amino acids (EAA):	
Lysine	12.33
Methionine	2.99
Threonine	10.52
Valine	12.83
Isoleucine	10.04
Leucine	19.45
Tyrosine	7.40
Phenylalanine	12.26
Histidine	4.61
Nitrogen (%)	4.36

Table 2.5: The nutritive value of mulberry leaf and shoots of temperate species (Srivastava R.P. 2006)

Fraction	CP (%)	ADF (%)	NDF (%)	Ash (%)
Mulberry				
Leaves	25.8	21.0	31.6	11.8
Shoots	12.1	45.6	60.5	8.8
Sward	20.4	27.9	53.5	11.6

2.2 Mulberry as livestock feed

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.

2.3 Mulberry as poultry feed

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 3% mulberry leaves supplementation. Narayana and Setty (1977) indicated that incorporation of 6 percent 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 percent 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 3 percent, 6 percent and 9 percent of mulberry leaf. Yatabe and Iso (1999)

reported that egg quality was significantly lowered when feeding 15 percent 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 (Machii, 1990). Narayana and Setty (1977) found that incorporation of shade dried mulberry leaves at 6% 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 percentage after supplementation of up to 2% mulberry leaves but, the blood cholesterol and triglyceride were found to decrease.

2.4 Mulberry leaves to reduce odour in manure

Sudo *et al.* (2000) found that ammonium emission from droppings reduced after feeding mulberry leaves to birds whereas others observed that mulberry leaves have an odour reduction effect in manure (Suda, 1999).

2.5 Mulberry as rabbit feed

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

2.6 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 *et 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).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

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 district. In an 8-week experiment period, 48 Hi-sex brown laying hens (age 30 weeks) were assigned to four dietary treatments with four replication of three (3) 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 leaves powder and test feed

Mulberry 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 0 (control), 3, 6 and 9% sun-dried mulberry leaf meal. Feed and water were provided *adlibitum*. The chemical composition of experimental diets is shown in the Table 3.1.

Fig. 3.1: Laying cage with experimental birds.

Table 3.1: Chemical composition of experimental diets

Feed ingredients	Dietary level of mulberry leaf meal(MLM)			
	T_1(Kg) (0%)	T_2(Kg) (3 %)	T_3(Kg) (6 %)	T_4(Kg) (9 %)
Maize	53	53	51	51
Soybean meal	22.6	21.1	20.1	20.1
Rice polish	11.5	10	10	9
Meat & bone meal	4	4	4	2
Oyster shell	7.8	7.8	7.8	7.8
DCP	0.75	0.75	0.78	0.75
Mulberry leaves	0	3	6	9
Salt	0.35	0.35	0.35	0.35
Vitamin-mineral premix*	*	*	*	*
Calculated composition:				
ME (Kcal/Kg)	2727.9	2742.3	2693.9	2698.5
CP (%)	17.77	17.53	17.06	16.69
CF (%)	3.28	3.52	3.05	3.20
Ca (%)	3.51	3.45	3.6	3.49
P (%)	0.45	0.50	0.70	0.46
Lysine (%)	0.94	0.96	0.90	0.85
Methionine (%)	0.28	0.32	0.34	0.35

*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 k_3: 800 mg; vitamin B_1: 600 mg; vitamin B_2: 2 mg; vitamin B_3: 12 mg; vitamin B_5: 3.2 mg; vitamin B_6: 1.8 mg; vitamin B_9: 2 mg; vitamin B_{12}: 0.004 mg; Co: 0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; I: 0.6 mg; Mn: 19.2 mg; Zn: 16 mg; Se: 0.48 mg; DL – Methionine: 20 mg; L- lysine:12 mg.

3.3 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. The 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.

3.4 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.

3.4.1 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-

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

3.4.2 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-

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

3.4.3 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-

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

3.4.4 Haugh unit determination

The haugh unit was calculated for each egg from the weight and albumin height using the formula suggested by Haugh (1937).

$$HU = 100 \text{ Log } (H + 7.57 - 1.7\ W^{0.37})$$

Where, HU = Haugh unit
H = Height of thick albumin
W = Egg Weight (gm)

3.4.5 Shell thickness

After removing of shell membrane, shell thickness (mm) was measured by screw gauge.

3.4.6 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 petridish and the raw yolk weight was taken. The albumin was also transferred to a previously weighed petridish 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 petridish. 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.

3.5 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.

3.5.1 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 stoppered 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.

e) 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.

3.5.2 Procedure for cholesterol determination

The eggs were hard cooked to facilitate the separation of yolk and albumin. Cooked eggs were broken and the yolks were separated gently and weighted one gram (1gm) of yolk sample. The weighted yolk sample was taken in a centrifuge tube and sonnicated with fifteen milliliters (15 ml) of chloroform: methanol solution (2:1v/v) solvent mixture and it was kept overnight for complete extraction of lipid. The extracted was filtered into a forty milliliters centrifuge tube and the residue was re-extracted with chloroform: methanol solution (2:1v/v). The two filtrates were combined and evaporated under vacuum. Five milliliters ethanol was added to the solid portion contained in the tube and mixed well and 0.3 ml of 33% KOH was added to it. The tube was shaken well and then incubated in a water bath at 37°C-40°C for 55 minutes. After cooling to room temperature, ten milliliters (10 ml) of petroleum ether was added followed by five milliliters (5 ml) deionized water and the contents of the tube were mixed thoroughly. Petroleum ether aliquot (one milliliter) in duplicate was collected from the clear supernatant petroleum ether layer and was taken in glass tube. Other steps were similar to that of preparation of standard. Result was calculated from standard curve (Fig.3.2).

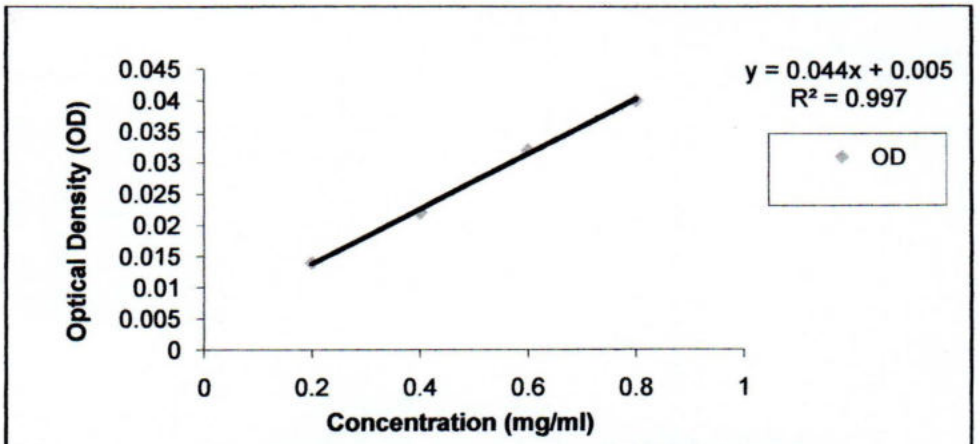

Fig 3.2. Standard curve of Cholesterol

3.5.3 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-Burchard reagent while pippeting 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 colour in each tube was read at regular interval of one minute against the blank in a spectrophotometer set at 620 nm.

3.6 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 Mstatc and SPSS Program.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Laying performances

4.1.1 Egg production

The hen day egg production observed in different dietary treatments was almost similar and the differences were statistically non-significant (P> 0.05) (Table 4.1). Result indicates that the feeding of mulberry leaf meal up to 9 percent in the diet of laying hen has no detrimental effect on egg production. Feeding of mulberry leaf meal in up to 6 percent levels showed slightly higher egg production whereas the production was slightly decreased when the birds received 9 percent mulberry leaf meal 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 9% mulberry leaf meal (Suda, 1999).

4.1.2 Egg weight

The egg weights in different dietary treatments during experimental periods were statistically insignificant (P> 0.05) (Table 4.1). These results indicate that inclusion of mulberry leaf meal up to 9 percent 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 egg size after the birds exposed to 15 percent mulberry leaf meal in the diet.

4.1.3 Egg mass output

The results of the present study showed that the egg mass output (gm/hen/day) with the inclusion of 3-6% mulberry leaf meal was improved but slightly decreased with 9% mulberry leaf meal, although the differences were non-significant($P > 0.05$). The results are agreement with the report of Tateno *et al.* (1999) and Sudo *et al.* (2000).

4.1.4 Body weight

Body weight in different dietary treatments during experimental periods was almost similar and the differences were not significant ($P > 0.05$) (Table 4.1).These results indicate that inclusion up to 9 percent mulberry leaf meal had no adverse effect on body weight. However, the body weight slightly improved in the dietary treatment at 6 percent mulberry leaf meal in comparison to T_1 (control). This is in agreement with the results of Machii (2000) who observed there was no adverse effect of mulberry leaf meal on body weight when mulberry leaves were given as part of the diet to domestic fowl.

4.1.5 Feed intake

Feed intake of laying hens in different dietary treatments during experimental periods was almost statistically similar and the differences were non-significant ($P > 0.05$) (Table 4.1). So, the result clearly showed that mulberry leaf meal up to 9 percent dietary level had no detrimental effect on feed consumption. Similar results have been observed by Lokaewmanee *et al.* (2009) who found that there was no adverse effect in feed intake compared to control. But, Ravindran *et al.* (1986), Limcangco-Lopez (1989), Udedibie and Opara (1998), Odunsi (2003) and Akande *et al.* (2007) reported a reduction in feed intake with increased dietary leaf meals in the diets for broilers and laying

hens. A decrease in feed intake for increased levels of mulberry leaf may be due to bulkiness and unpalatable taste which may affect the appetite of the birds.

4.1.6 Feed conversion ratio

Feed conversion ratio in different dietary treatments at 3, 6 and 9 percent level was almost similar and the differences were non-significant (P> 0.05(Table 4.1)).The results indicate that there was no detrimental effect on feed conversion ratio after feeding up to 9 percent level of mulberry leaf meal. This is in agreement with the results of Machii (2000) who observed there was no adverse effect of mulberry leaf meal on feed conversion ratio (FCR) when mulberry leaves were given as part of the diet to domestic fowl.

4.2 External and Internal egg quality

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). These results indicate that feeding mulberry leaf meal up to 9 percent level had no adverse effect on external and internal qualities of eggs. However, egg shell weight (gm) decreased slightly after supplementation of 3-9% mulberry leaf meal. Egg shell thickness slightly improved at the level of 6 percent mulberry leaf meal. Albumin weight decreased in the dietary treatments 3 and 6% but a little bit increased in dietary treatment 9% 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 decreased after the inclusion of 3-9% mulberry leaf meal but yolk index slightly improved at 6 percent level of mulberry leaf meal. Haugh unit decreased at 6 percent levels but, improved at 9 percent mulberry leaf meal. Similar results have been obtained by Tateno *et al.* (1999) and Sudo *et al.* (2000) who did not found any

significant differences in the external and internal qualities of eggs up to 9 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 leaf meal in layer-ration ($P< 0.05$). It is evident from Table 4.2 that a tendency of reduced egg yolk cholesterol was observed in the dietary treatments with inclusion of 3-9% mulberry leaf meal. However, the highest level of cholesterol was 11.6 mg/gm at 3% level and lowest level was 10.9 mg/gm of egg-yolk at 9 percent level of mulberry leaf meal whereas cholesterol of "control egg" ranged from as low as 10 mg/gm of yolk to as high as 18 mg/gm of yolk (USDA, 2008). During experimental periods egg yolk cholesterol was reduced to 0.8 mg/gm, 1.2 mg/gm and1.5 mg/gm after dietary supplementation of 3-9% of mulberry leaf meal as compared to control. Thus, the result of current study clearly showed that mulberry leaf meal at 3, 6 and 9 percent 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 4.1: Effect of mulberry leaf meal (*Morus alba*) on laying performance

Parameters	Mulberry leaf supplementation (%)				Level of significance
	T_1 0% (Control)	T_2 3 % MLM	T_3 6 % MLM	T_4 9 % MLM	
Body weight (gm)	1745 ± 23.4	1730 ± 18.3	1754 ± 15.8	1742 ± 20.1	NS
Egg production (%)	88.48 ± 0.70	88.53± 0.67	88.78 ± 0.59	88.32 ± 0.45	NS
Feed intake (gm/hen/d)	112.8 ± 2.45	112.3 ± 2.08	111.8 ± 2.03	112.2 ± 2.31	NS
Egg weight (gm/egg)	63.40 ± 0.53	63.33 ± 0.51	62.84 ± 0.44	63.36 ± 0.41	NS
Egg mass (gm/hen/d)	56.84 ± 1.72	56.92 ± 2.10	56.86 ± 1.56	56.31 ± 1.60	NS
Feed conversion ratio (gm feed /gm egg)	1.98 ± 0.07	1.97 ± 0.05	1.95 ± 0.04	1.96 ± 0.08	NS

Values are expressed as mean ± standard error of means. NS: Statistically not significant ($P > 0.05$). Means represent four replicates, three birds per replicate.

MLM= Mulberry Leaf Meal.

Table 4.2: Effect of mulberry leaf meal (*Morus alba*) on quality characteristics of egg

Parameters	Mulberry leaf supplementation (%)				Level of signific-ance
	T_1 0% (Control)	T_2 3 % MLM	T_3 6 % MLM	T_4 9 % MLM	
Egg Shell weight (gm/egg)	6.56 ± 0.09	6.41 ± 0.12	6.53 ± 0.09	6.50 ± 0.11	NS
Shape index (%)	80.04 ± 0.67	80.54 ± 0.86	81.59 ± 0.64	79.93 ± 0.83	NS
Shell thickness (mm)	0.40 ± 0.02	0.40 ± 0.02	0.41 ± 0.00	0.40 ± 0.01	NS
Albumin weight (gm/egg)	37.06 ± 0.77	36.87 ± 0.67	36.87 ± 0.70	37.86 ± 0.67	NS
Albumin index (%)	8.35 ± 0.28	8.40 ± 0.32	8.55 ± 0.38	8.40 ± 0.30	NS
Yolk weight (gm/egg)	17.24 ± 0.09	17.09 ± 0.08	17.12 ± 0.1	17.15 ± 0.11	NS
Yolk index (%)	42.7 ± 0.42	42.5 ± 0.36	42.9 ± 0.39	41.3 ± 0.35	NS
Yolk Cholesterol (mg/gm)	12.8 ± 0.28^{a}	11.6 ± 0.29^{b}	11.2 ± 0.32^{b}	10.9 ± 0.28^{c}	*
Haugh unit (%)	89.07 ± 2.33	88.56 ± 1.92	88.85 ± 2.12	89.19 ± 2.30	NS

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. MLM= Mulberry Leaf Meal.

From the above discussion, it is said that egg-yolk cholesterol was decreased significantly without affecting egg qualities with increased level of mulberry leaf meal supplementation. 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 V

SUMMARY

AND

CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Mulberry 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 leaf for laying hen (Hi-sex brown) was evaluated at Hajee Mohammad Danesh Science and Technology University poultry farm, Dinajpur district. In feeding trial, four (4) diets were prepared including mulberry leaf at levels of 0 (control), 3, 6 and 9% in the diet by replacing soybean meal, maize and rice polish. 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 9% level 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 (g/day) was maximum in control group (112.8g) followed by (112.3 g) at 3% mulberry leaf meal, (111.8 g) at 6% level and (112.2 g) at 9% level. In all test groups food consumption was almost similar to control group (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 (FCR) was the highest in control group (1.98) compared with other group. The FCR values were found to be almost the same with diet at 0% (1.98), 3% (1.97), 6% (1.95) and 9% (1.96) mulberry leaf meal. Egg production was maximum in diet

at 6% level of mulberry leaf meal but values were almost same in the diet at 0 percent (88.48%), 3 percent (88.53%), 6 percent (88.78%) and 9 percent (88.32%). Data obtained on egg weight expressed as maximum level in control group (63.40 gm) than the other feeds fed group but almost similar to diet with 3% (63.33 gm), 6% (62.84 gm) and 9% (63.36 gm) level of mulberry leaf meal. Egg mass were statistically similar in all groups which expressed as diet with 0% (56.84 gm/h/d), 3% (56.92 gm/h/d), 6% (56.86 gm/h/d) and 9% (56.31gm/h/d) level of mulberry leaf meal. Data obtained on egg shell weight were almost similar at 0% (6.56 g), 3% (6.41 g), 6% (6.53 g) and 9% (6.50 g) level of mulberry leaf meal in the diets. Shape index were found to be highest at diet with 6percent (81.59%) level of mulberry leaf meal but almost same to all other feed groups at 0 percent (80.04%), 3 percent (80.54%) and 9 percent (79.93%). Shell thicknesses were indifferent with diet at 0% (0.40 mm), 3% (0.40 mm), 6% (0.41 mm) and 9% (0.40 mm) mulberry leaf meal. Data obtained on albumin index exhibited maximum level in diet with 6% (8.55 percent) than the other feeds fed group but almost similar to diet with 0% (8.35 percent), 3% (8.40 percent) and 9% (8.40 percent) level of mulberry leaf meal. The yolk index values were found to be almost the same with diet at 0 percent (42.7%), 3 percent (42.5%), 6 percent (42.9%) and 9 percent (41.3%) mulberry leaf meal. Haugh unit values were indifferent with diet at 0% (89.07 percent), 3% (88.56 percent), 6% (88.85 percent) and 9% (89.19 percent) mulberry leaf meal. Data obtained on total cholesterol exhibited a higher level in control group (12.8 mg/gm) than the other foods fed group. On the other hand, the total cholesterol level was significantly lower in diet at 9% mulberry leaf meal (10.9 mg/gm) than control group. The best reduced egg yolk cholesterol was obtained with diets at 6% (11.2 mg/gm) and 9% (10.9 mg/gm) levels of mulberry leaf meal. Egg yolk cholesterol was reduced 9.4% at 3% level, 12.5% at 6% level and 14.8 at 9% level of mulberry leaf meal supplementation.

Based on the results of present study it may be concluded that mulberry leaf is a good source of protein and it has 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. The results of the study suggest that supplementation of mulberry leaf meal (*Morus alba*) up to my investigation level (9%) in diets has high potential as commercial applications for production of low-cholesterol containing eggs and healthy birds. Therefore, 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.

CHAPTER VI

REFERENCES

CHAPTER VI

REFERENCES

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CHAPTER VII

APPENDICES

APPENDICES

Appendix Table 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	2.00	0.02	0.35	0.24	0.12	0.18	0.07
Rice polish	88.1	3090[a]	16.4	14.8	10.5	0.27 [a]	0.14 [a]	0.57 [a]	0.22 [a]	0.21 [a]	0.13 [a]
Soybean meal	89.0	2426	42.0	3.50	6.50	0.25	0.20	2.70	0.60	0.62	0.58
Bone & Meat meal	93.0	2536	50.0	8.50	2.80	9.20	4.70	2.60	0.67	0.33	0.26
Oyster shell	99.0	-	-	-	-	35.0 [d]	0.018 [d]	-	-	-	-
Mulberry leaf	94.0	3200[c]	21.6	7.40	12.2	3.5 [c]	0.24	0.35 [c]	0.78 [c]	0.11	0.09

a = NRC, 1994
c = Lohan, 1979 and Makkar, 1989
d = Singh and Panda, 1992

Appendix Table-5: Food intake trend over the experimental period

Diets	1st week (g)	2nd week (g)	3rd week (g)	4th week (g)	5th week (g)	6th week (g)	7th week (g)	8th week (g)
Control diet	791	800	755	780	812	805	788	790
Diet with 3% MLM	760	777	792	803	770	791	807	745
Diet with 6% MLM	784	750	780	789	754	765	793	800
Diet with 9% MLM	780	798	775	760	748	790	813	755

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

Chemicals	**Instruments**
Chloroform	Water bath
Methanol	Vortex Mixture
Potassium Hydro-oxide (KOH)	Incubator
Acetic Anhydride	Sonnicator
Conc. Sulphuric acid (H_2SO_4)	Hot air oven
Glacial acetic acid	Vacuum evaporator
Ethanol	Centrifuge machine
Distilled water	Spectrophotometer
Deonized water	Centrifuge tube
Petroleum ether	Routine laboratory articles

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

Feed ingredients	Price (TK/ Kg)
Wheat	20
Maize	18
Rice polish	15
Meat and bone meal	60
Soybean meal	34
Oyster shell	8
Fish meal	60
Vitamin-mineral premix	120

Appendix Table-2: Weekly average temperature of the experimental house during the experimental period

Weeks	Average temperature (^{o}F)
30	87.5
31	89.2
32	89.0
33	85.6
34	84.9
35	89.4
36	88.3
37	87.5
38	85.8