DIETARY EFFECT OF MULBERRY LEAF (*Morus alba*) MEAL IN THE PERFORMANCE OF BROILER

A THESIS
BY

MD. RABIUL ISLAM

Registration No. 1005036

Session: 2010-2011

Semester: Summer

MASTER OF SCIENCE (M.S)
IN
POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR,

AUGUST, 2012

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Hajee Mohammad Danesh Science and Technology University,
Dinajpur, in partial fulfillment of the requirements
for the degree of

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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR,

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DEDICATED
TO MY
BELOVED PARENTS

ACKNOWLEDGEMENTS

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, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

The author expresses his deepest sense of gratitude, sincere appreciation and best regards to his benevolent research supervisor Prof. Mst. Afroza Khatun, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for her keen interest, scholastic supervision and guidance, innovative suggestions, constructive criticism, constant encouragement and provisions of facilities and supports during the entire tenure of the research work and thesis preparation.

The author expresses his profound gratefulness, heartfelt respect and immense indebtedness to his co-supervisor Associate Prof. Dr. Tahera Yasmin, Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for her valuable assistance, exclusive suggestions,, helpful criticism, endless inspiration and factual comments throughout the entire period of the research work and during writing up this thesis.

The author expresses his deep sense of appreciation and heartfelt gratitude to Professor Md. Saifur Rahman, Department of Statistics, Hajee Mohammad Danesh Science and Technology University, Dinajpur for helping in statistical data analysis.

The author would like to take the privilege to express his heartfelt indebtedness to all the honorable teachers of Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for their valuable teaching, worthy suggestions and inspiration for improving his knowledge and academic skills in this esteemed discipline during the period of his M.S. study.

The author takes this opportunity to express his sincere appreciation and heartful thanks to Md. Sayed, Lecturer, Department of Biochemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for or helping in statistical data analysis.

The author also acknowledges to the staffs members of Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their cooperation during the research work.

It is great pleasure for the author to express his profound gratitude and deepest appreciation to his beloved father Md. Abdur Rouf Miah and mother Mrs. Golenur Begum who have sacrificed their happiness, ever ending prayers and dedicated efforts to educate the author to this level. The author is also grateful to his younger brother especialy Rifat Haydar and Ahasan Habib who have helped a lot in this study period.

The Author

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SL. NO	ABBREVIATIONS	ELABORATIONS
01.	FCR	Feed Conversion Ratio
02.	MS	Master of Science
03.	nm	Nano Meter
04.	mm	Milli Meter
05.	cm	Centi Meter
06.	MLM	Mulberry Leaf Meal
17.	v/v	Volume/Volume
18.	KOH	Potassium Hydro-oxide
19.	NS	Not Significant
10.	SD	Standard Error
11.	W	Weight
12.	Av.	Average
13.	VLDL	Very Low Density Lipoprotein
14.	IU	International Unit
15.	AOAC	Association of Official Analytical Chemists
16.	UK	United Kingdom
17.	USA	United States of America
18.	DM	Dry matter
19.	K	Potassium
20.	Mg	Magnesium
21.	Mn	Manganese
22.	N	Nitrogen
23.	Na	Sodium
24.	NFE	Nitrogen Free Extract
25.	P	Phosphorus
26.	S	Sulphur
27.	Ca	Calcium

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Poultry is one of the most important sectors of livestock that provides the cheapest animal protein (nutritious egg and meat) for human consumption within the shortest period of time. Poultry production has greatly increased during last one decade in Bangladesh. But, the scarcity and fluctuating quantity and quality of the year-round feed supply and their price is a major constraint to poultry production in developing countries like Bangladesh. By the year, 2020, world population is estimated at 8 billion with most of the population growth coming from the developing countries (Singh and Makkar, 2001). Due to rapid growth of the world population and shrinkage of cultivating land, demand for livestock product is increasing day by day. Future hopes of feeding the millions and safe guarding their food security will depend on better utilization of unconventional feed resources that do not compete with food for human beings. Feed ingredients especially animal protein sources are very expensive and scarce due to high competition among poultry, human and other animals resulting in the escalating cost of these ingredients. The cost of feed constitutes the major proportion of 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 price of conventional protein feeds resources such as groundnut cake, fish meal and soybean meal, is on the high side 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 this elusive feed ingredient.

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, and 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 (2.42-4.71% Ca, 0.23-0.97% P) and

metabolizable energy (1,130-2,240 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 can be a good source of the pigmentation of egg yolk (Sarita *et al.*,2006).

Now a days, most of the people of the world are suffering by 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 provide protein, vitamins, and lipids that contain high levels of cholesterol. Thus, broilers are considered to be a high-cholesterol food. The American Heart Association recommended that cholesterol consumption for each person should be limited to 300 mg per day. People are paying more attention to health and are thus lowering their consumption of high-cholesterol food. Because cholesterol in broiler accounts for greater than 50% of daily intake, the consumption of broiler can hardly increase. Therefore, low-cholesterol broiler meat would not only be beneficial to public health but also bear business advantage. Broiler cholesterol content can be altered by (i) genetic selection such as upward direction method or selection of hens that produce low-cholesterol meat (ii) diet alteration, Mulberry leaf diets may inhibit the synthesis of cholesterol and fatty acids in the liver (Abdonnaser et *al.,* 2007). In addition to this, serum hypocholesterolemic effect was found in poultry feed (Olugbemi et *al.,* 2010). So, Mulberry leaf may be added for investigation. Therefore, present piece of research work was undertaken with the following objectives:

i) To observe the production performance and quality characteristics of broiler.
ii) To produce low cholesterol containing broiler meat.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Mulberry (*Morus spp.)* leaves are the traditional feed for the silk worm moth (*Bombyx mori*). There is evidence that sericulture started about 5,000 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 4,000 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. 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; Bonciarelli; 1980) and in France there was a research project to introduce mulberry in livestock production (Armand, 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 breakthrough 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, fed mulberry leaves to his goats and was impressed by its palatability and by the performance of his animals. He communicated his observations to scientist of the Tropical Agriculture Research and Training Center (Cattle), 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, 1994). 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.

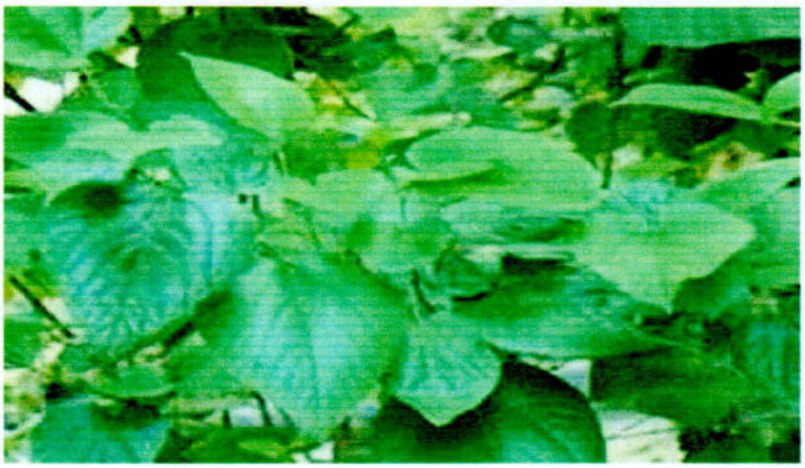

Fig. 2.1: Mulberry tree with leaves

Fig. 2.2: Fresh mulberry leaves

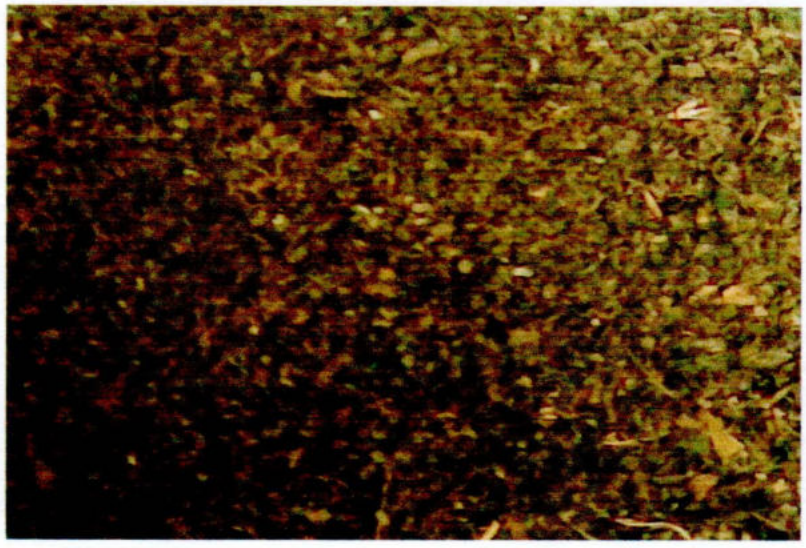

Fig. 2.3: Sun-dried mulberry leaves

Fig. 2.4: Prepared feed (control)

2.1 Nutritive value of mulberry

Al-kirshi *et al.* (2010) found that the mulberry leaves meal 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 matters 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 the dry matter (DM) basis. Benavides (1994) observed that mulberry (*Morus sp.)* is exceptional among woody forages which leaves contain more than 20 % CP.

Makkar *et al.* (1989) observed that mulberry leaf contain 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 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. 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 *et al.* (2006) found 0.83% N, 235 mg/100 g P, 1141 mg/100 g K, 139 mg/100 g Ca, 109 mg/100 g Mg, 60 mg/100 g Na, 4.3 mg/100 g Fe, 0.4 mg/100 g Cu, 4.0 mg/100 g Mn and 3.1 mg/100 g 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 (1994) 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 leaves on DM basis (Machii, 1989)

Nutrient content	Percentage (%)
Dry matter	89.30
Crude protein	29.80
Ether extract	5.57
Crude fiber	11.10
Gross energy, kcal/kg	4220
As	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

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, 1977a
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	González *et al.*, 1998
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 per 100 g

Principle	Nutrient Value
Energy	43 Kcal
Carbohydrates	9.80 g
Protein	1.44 g
Total Fat	0.39 g
Cholesterol	0 mg
Dietary Fiber	1.7 g
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

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

(Machii, 1989)

Table 2.5: The nutritive value of mulberry leaves and shoots of temperate species

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

Harvesting month: September 1997.

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 upto 3% mulberry leaves supplementation. Narayana and Setty (1976) 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 color compared with eggs from commercially reared New Hampshire hens. Simol *et al.* (2009) conducted an experiment and observed that the carcass characteristics and color 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. 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 (1976) found that incorporation of shade dried mulberry leaves at 6% level in layer's mash showed an increase in egg production with desirable yolk color without any adverse effect on body weight and egg quality.

Moringa oleifera has being studied in human nutrition because of its nutritional benefits. However, its hypocholesterolemic potential, as attested to by traditional medical practitioners has not been extensively studied. Due to controversies as to the role of eggs in cardiac related diseases, research has continued to focus on ways in reducing the cholesterol content. The potential of *Moringa oleifera* leaf meal (MOLM) as a hypocholesterolemic agent was therefore investigated using layers fed cassava based diets over a 90 day period. Eighty layers were assigned to four dietary treatments containing MOLM at 0, 5, and 10% (treatments 2, 3 and 4) levels with cassava chip constituting 20% of each diet and a control diet (treatment 1) containing neither cassava nor Moringa. A completely randomized design was employed. The effect of the dietary treatments on serum and yolk cholesterol was determined. Serum cholesterol levels in treatments 2, 3 and 4 declined by 14.2%, 19.8% and 22.0 %, respectively, while yolk cholesterol levels declined by 6.55%, 7.45% and 12.1%, respectively. Results of the study indicate that *Moringa oleifera* possesses hypocholesterolemic properties and its inclusion in layers diets could facilitate reductions in egg cholesterol content (Olugbemi et *al.,* 2010).

In a study, twelve 6 week age chickens were decapitated; liver extracted, sliced and cultured as primary culture. Effects of hydro extract of mulberry leaf on TG secretion and hepatic TG were determined. Data indicated that hydro extract of mulberry leaf extract decreased triglyceride secretion in a dose dependent manner (as much as 82, 76 and 67% in response to 0.075, 0.05 and 0.015% of hydro extract after 48 h incubation, respectively). Moreover, at 0.075 concentrations it decreased TG content as much as 43% after 12 h incubation. Mulberry leaves contain some inhibitory components for accumulation and secretion of TG in chicken hepatocytes (Abdonnaser et *al.,* 2007).

2.4 Mulberry leaves to reduce odour in manure

Kuramoto and Iso (2000) found that ammonium emission from droppings reduced after feeding mulberry leaves to birds whereas others observed that mulberry leaves have an odor 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.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3. Materials

3.1. Feed materials

3.1.1 Source of *Morus alba L.*

Morus alba were collected from local area of Dinajpur district in Bangladesh. The leaves were sundried coarsely powdered manually and then directly mixed with manually prepared diets in appropriate doses (Table 3.1, 3.2 and 3.3).

3.1.2 Sources of other feed ingredients

Sundried and grinded corns, meat meal, bone meal, rice polish, soybean meal, soybean oil and other feed items were collected from local market of Dinajpur, Bangladesh and then directly mixed with manually prepared diets in appropriate doses (Table 3.1, 3.2 and 3.3). Vitamin premix was purchased made by reputed Vet. Medicine Company.

3.1.3 Chemicals/Kit

a) Kits (CRESCENT diagnostic)
b) Jeddah, K.S.A. for the estimation of total cholesterol, HDL-cholesterol and Triglycerides.

3.1.4 Working Instruments

Instruments were used included micro grinder (Tekmar, West German), rotary vacuum evaporator (BUCHI Rota vapor R-114), Vortex mixture (Branson 1210, USA), spectrophotometer (Spectronic® Genesys ™, USA), auto analyzer (ABX pentra 400), electric balance, weigh balance, micropipette, and other conventional laboratory instruments and appliances.

3.1.5 Experimental diets

The experimental diets in mash form and drinking water was provided adlibitum. All diets were formulated manually to meet the nutrient requirements of broiler (NRC, 1994) .The chicks were fed starter diet from 1 to 10 days, grower diet from 11-20 days and a finisher diet from 21 to 42 days old broiler (Tables 3.1, 3.2 and 3.3). Tables (3.1, 3.2 and 3.3) show the ingredient's composition and the chemical composition of the starter, grower and finisher diets respectively.

The experimental diets were designed as

T_0: control.

T_1: 2.5% MLM

T_2: 3.5% MLM

T_3: 4.5% MLM

T_4: MLM Extract

T_5: 0.5% Antibiotic.

Table 3.1: Composition of experimental starter diets fed to broilers

Items	**Dietary level of Mulberry leaf meal (MLM)**					
Feed ingredients (gm/kg feed)	T_0 (control)	T_1 (2.5%MLM)	T_2 (3.5% MLM)	T_3 (4.5% MLM)	T_4 (MLM Extract)	T_5 (0.5% Antibiotic)
Maize	0.52	0.50	0.50	0.50	0.50	0.50
Soybean meal	0.23	0.24	0.23	0.225	0.225	0.225
Rice polish	0.145	0.145	0.14	0.14	0.14	0.14
Soybean Oil	0.005	0.005	0.005	0.005	0.005	0.005
Salt	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Methionine	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Lysine	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Growth promoter	0.005	0.005	0.005	0.005	0.005	0.005
Protein	0.09	0.075	0.08	0.075	0.075	0.08
MLM concentrate	0.00	0.025	0.035	0.045	0.045	0.00
Antibiotic	0.00	0.00	0.00	0.00	0.00	0.00
Vitamin Mineral Premix	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Chemical composition						
ME(kcal/kg)	3085	3120	3100.5	3080	3140	3095
CP(gm/kg)	21.35	21.3	21.4	21.5	21.25	21.45
CF(gm/kg)	3.75	3.77	3.72	3.75	3.77	3.77
Ca(gm/kg)	1.12	1.13	1.12	1.11	1.13	1.12
P(gm/kg)	0.56	0.56	0.56	0.54	0.58	0.56
Methionine(gm/kg)	0.48	0.48	0.48	0.48	0.48	0.48
Lysine(gm/kg)	1.18	1.18	1.19	1.18	1.19	1.19

Added broiler premix (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.

Table 3.2: Composition of the experimental grower diets fed to broilers

Items	**Dietary level of Mulberry leaf meal** (MLM)					
Feed ingredients (gm/kg feed)	T_0 (control)	T_1 (2.5%MLM)	T_2 (3.5% MLM)	T_3 (4.5% MLM)	T_4 (MLM Extract)	T_5 (0.5% Antibiotic)
Maize	0.53	0.52	0.52	0.52	0.525	0.53
Soybean meal	0.22	0.205	0.205	0.215	0.21	0.22
Rice polish	0.145	0.145	0.14	0.13	0.135	0.145
Soybean Oil	0.005	0.005	0.005	0.005	0.005	0.005
Salt	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Methionine	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Lysine	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Growth promoter	0.005	0.005	0.005	0.005	0.005	0.005
Protein	0.09	0.09	0.085	0.075	0.085	0.09
MLM	0.00	0.025	0.035	0.045	0.03	0.00
Antibiotic	0.00	0.00	0.00	0.00	0.00	0.005
Vitamin Mineral Premix	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Chemical composition						
ME(kcal/kg)	3098	3130.5	3110.5	3080	3140	3107.5
CP(gm/kg)	20.6	19.5	21	21.3	19.5	21.3
CF(gm/kg)	3.77	3.78	3.76	3.77	3.78	3.78
Ca(gm/kg)	1.18	1.15	1.16	1.15	1.16	1.13
P(gm/kg)	0.59	0.57	0.58	0.57	0.58	0.57
Methionine(gm/kg)	0.48	0.43	0.43	0.43	0.43	0.48
Lysine(gm/kg)	1.05	1.06	1.05	1.05	1.06	1.05

Added broiler premix (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.

Table 3.3: Composition of the experimental finisher diets fed to broilers

Items	**Dietary level of Mulberry leaf meal** (MLM)					
Feed ingredients (gm/kg feed)	T_0 (control)	T_1 (2.5%MLM)	T_2 (3.5% MLM)	T_3 (4.5% MLM)	T_4 (MLM Extract)	T_5 (0.5% Antibiotic)
Maize	0.55	0.55	0.55	0.55	0.55	0.55
Soybean meal	0.20	0.20	0.20	0.215	0.19	0.205
Rice polish	0.145	0.145	0.125	0.11	0.13	0.135
Soybean Oil	0.005	0.005	0.005	0.005	0.005	0.005
Salt	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Methionine	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Lysine	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
Growth promoter	0.005	0.005	0.005	0.005	0.005	0.005
Protein	0.09	0.09	0.075	0.07	0.085	0.09
MLM concentrate	0.00	0.00	0.035	0.045	0.030	0.00
Antibiotic	0.00	0.00	0.00	0.00	0.00	0.005
Vitamin Mineral Premix	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025
Chemical composition						
ME(kcal/kg)	3115	3130.5	3140.5	3125	3170	3130
CP(gm/kg)	19.5	18.5	18.5	19	18	19.5
CF(gm/kg)	3.72	3.72	3.77	3.65	3.71	3.76
Ca(gm/kg)	1.16	1.08	1.12	1.08	1.08	1.13
P(gm/kg)	0.58	0.52	0.55	0.51	0.52	0.57
Methionine(gm/kg)	0.4	0.4	0.4	0.4	0.4	0.4
Lysine(gm/kg)	1.00	0.99	1.01	1.01	1.01	1.00

Added broiler premix (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.

3.2 Birds management

3.2.1 Birds and experimental design

The experiment was conducted at the poultry shed in Hajee Mohammad Danesh Science & Technology University, Dinajpur. A total 240 one day-old broiler chick (Cobb 500) were purchased from CP Bangladesh.

At first chicks were reared at brooding house to adjust with the environmental condition up to 10 days. After that, chicks were randomly allocated to six dietary treatment groups having 40 birds in each group; each treatment was composed of four replicates with 10 birds in each in a complete randomized design. The birds were housed on floor and routinely managed as any other commercial broiler flock. Heating was provided by a single electric brooder, where the initial temperature was set at 32 °C and decreased by 2°C per week to final temperature of 20°C at the end of experiment. Supplementary heating was provided as required by mobile butane gas heaters besides to electric heater.

3.2.2 Observation of birds

All birds were examined twice daily for any visible physical changes like restlessness, lordosis, abnormal gait, vices and depression as well as changes of feeding style during treatment. All birds were vaccinated against Newcastle and Gumboro as per instruction of the manufacturers.

3.3 The performance trial

During the 42 days experimental period, growth performance was evaluated. Before treatment, body weight was taken for each group of birds. Then body weight and feed consumption were recorded daily and body gain and feed consumption were calculated. Mortality was recorded throughout the study period.

3.3.1 Feed consumption

Feed consumption is the amount of feed consumed every day; it was calculated for each treatment at daily basis. At the end of the week, the residual amount of feed was weight and subtracted from the known weight of feed at the beginning of week.

3.3.2 Body weight gain

Body weight was measured for all birds at the beginning of the experiment, and it was repeated daily at the beginning of the week at the same time. Live weight gain was calculated by subtraction the live weight at the beginning of the week from the live body weight of the next week.

3.3.3 Feed conversion ratio

Feed conversion ratio (FCR) was calculated every week at the amount of feed consumption per unit of body gain (average weekly feed consumption (g)/ average weekly gain (g)).

3.4 Fat estimation

At the end of each experiment a sample of three randomly selected birds from each replicate within a treatment was slaughtered to estimate the fat content. After slaughtering, abdominal fat and subcutaneous fat of each bird was removed and then weighed. Fat contents were then measured as follows:

Fat content (gm) = Abdominal fat + Subcutaneous fat

3.5 Collection of blood serum

Then blood was collected weekly with a syringe and needle directly through wing vein puncture without using any anticoagulant. By the end of each treatment, all the birds were sacrificed.

3.5.1 Separation and storage of blood serum

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

3.5.2 Assay and estimation methods of various lipid parameters of blood samples

3.5.2.1 Estimation of total cholesterol (Tch) from blood plasma using kit method Principle

The determination of serum cholesterol is a major aid in the diagnosis and classification of lipaemias. Normal cholesterol levels are also affected by age, stress. Cholesterol esterase (CHE) catalyses the hydrolysis of cholesterol esters to produce Cholesterol which is oxidized by cholesterol oxidase (CHO) to yield hydrogen peroxide (H_2O_2). In a coupled reaction catalyzed by peroxidase (POD), quinoneimine dye (red) is formed from (H_2O_2), 4-aminoantipyrine (4-AA) and phenol. The absorbance of the dye at 546 nm is proportional to the concentration of cholesterol in the sample.

1. Cholesterol ester + H_2O $\xrightarrow{\text{CHE}}$ Cholesterol + fatty acid

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

3. 2 H_2O_2 + 4-AA + phenol $\xrightarrow{\text{POD}}$ Quinoneimine + 4 H_2O

Reagent Composition:

1. Enzyme reagent

Phosphate buffer (pH 6.5)	30 mmol/L
4-aminoantipyrine	0.25 mmol/L
Phenol	5 mmol/L
Peroxidase	>10 KU/L
Cholesterol esterase	> 350 U/L
Cholesterol oxidase	> 140 U/l

2. Cholesterol standard 200 mg/dl

Assay:

Wavelength : 546, Hg.500 nm

Optical path : 1 cm

Temperature : 37 °C

Measurement : Against reagent blank

Procedure:

	Macro Method		
Pipette into Cuvettes	Blank	Standard	Sample
Test sample(ml)	-	-	0.025
Standard (ml)	-	0.025	-
Distilled Water (ml)	0.025	-	-
Reagent (ml)	2.5	2.5	2.5
It was mixed and incubated for 5 minutes at 37 °C. The absorbance of the sample (As) was measured and the standard (Asd) against the reagent blank within 60 minutes.			

Calculation:

$$\text{Cholesterol concentration (mg/dl)} = \frac{As}{Astd} \times \text{Concentration of standard}$$

3.5.2.2 Estimation of HDL cholesterol from blood plasma using kit method

Principle

Phosphotungstic acid and magnesium ions specifically precipitate low and very low density lipoproteins (LDL and VLDL). After centrifugation the cholesterol content of the high density lipoproteins (HDL) in the supernatant can be determined using CRESCENT DIAGNOSTICS Cholesterol test kit (Cat No. CS 603).

Reagent Composition:

Phosphotungstic acid	0.55 mmol/L
Magnesium ions	25 mmol/L

Reagent preparation:

Macro assay: Reagents are ready for use.

Semi Macro assay: The reagent was pre-diluted with distilled water before use (80 ml of reagent and 20 ml of water).

Also required:

CRESCENT DIAGNOSTICS Cholesterol test kit (Cat No. CS 603).

Procedure:

1. Precipitation

Pipette into centrifuge tube	Semi-Macro
Sample (ml)	0.2
Reagent undiluted (ml)	-
Reagent diluted (ml)	0.5
It was mixed and allowed to stand for 10 minutes. It was centrifuged for 10 minutes at 4000 rmp. The cholesterol content of the HDL supernatant was determined.	

2. Cholesterol assay:

CRESCENT DIAGNOSTICS Cholesterol test

Wavelength : 546 nm

Optical path : 1 cm

Temperature : 37 ºC

Measurement : Against reagent blank

Pipette into cuvettes	Blank	Sample
Distilled water (ml)	0.1	-
HDL supernatant (ml)	-	0.1
Cholesterol reagent (ml)	1.0	1.0
It was mixed and incubated for 10 minutes at 37 ºC. The absorbance of the sample was measured against the reagent blank within 30 minutes (ΔA).		

Calculation:

HDL Cholesterol concentration = ΔA × Factor

Factor:

Semi-macro: 320 mg/dl

Calculation of LDL-Cholesterol:

$$\text{LDL-Cholesterol (mg/dl)} = \text{Total Chol} - \text{HDL Chol} - \frac{\text{Triglycerides}}{5}$$

3.5.2.3 Estimation of triglycerides from blood plasma using kit method

Principle

Lipases catalyze the hydrolysis of triglycerides to yield glycerol and free fatty acids. The glycerol content is determined enzymetically with the Trinder reaction using glycerol kinase (GK), glycerol-3-phosphate oxidase (GPO) and peroxidase (POD). The end product is a quinoneimine dye the concentration of which at 546 nm is directly proportional to the concentration of triglycerides in the sample.

1. Triglyceride + H_2O $\xrightarrow{\text{Lipases}}$ Glycerol + free fatty acids

2. Glycerol + ATP $\xrightarrow{\text{GK}}$ Glycerol-3-phosphate + ADP

3. Glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxyacetone phosphate + H_2O_2

4. 4-Aminoantipyrine + chlorophenol + H_2O_2 $\xrightarrow{\text{POD}}$ quinoneimine dye

Reagent composition:

1. Enzyme concentrate
2. Buffer
3. Triglyceride standard 200 mg/dl

When combined as instructed the working reagent contains the following:

PIPES buffer (pH 7.5)	40 mmol/L
Lipases	150 KU/L
Glycerol kinase (GK)	0.4 KU/L
Glycerol-3-phosphate oxidase (GPO)	1.5 KU/L
Peroxidase (POD)	0.5 KU/L
Magnesium	5.0 mmol/L
Adenosine triphosphate (ATP)	1.0 mmol/L
Chlorophenol	5.0 mmol/L
Aminoantipyrine	0.4 mmol/L
Stabilizers and preservatives	

Reagent preparation:

One part of reagent 1(enzyme concentrate) was diluted with 100 parts reagent 2 (buffer). It was mixed gently and allowed to room temperature before use.

Reagent stability:

Both reagents and standard are stable until the expiry date when stored at 2-8°C. The working reagent is stable for 3 weeks at 2-8°C.

Assay:

Wavelength : 546 nm

Optical path : 1 cm

Temperature : 37 °C

Measurement : Against reagent blank

Procedure:

	Macro Method		
Pipette into Cuvettes	Blank	Standard	Sample
Test sample(ml)	-	-	0.025
Standard (ml)	-	0.025	-
Distilled Water (ml)	0.025	-	-
Reagent (ml)	2.5	2.5	2.5
It was mixed and incubated for 5 minutes at 37 °C. The absorbance of the sample (As) and the standard (Asd) was measured against the reagent blank within 60 minutes.			

Calculation:

$$\text{Triglyceride concentration (mg/dl)} = \frac{As}{Astd} \times \text{Concentration of standard}$$

3.6. Statistical Analysis

The data was analyzed by using the MSTATC program. The values were ranked by Student's-t-test.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

This study was conducted to evaluate the effects of varying doses of Mulberry leaf meal and their extracts supplemented diets on growth rate, changes in body weight, feed intake, fat content, mortality, and serum lipid profile in broiler. The formulated diets were supplemented with 0%, 2.5%, 3.5%, 4.5% MLM and T_4 MLM extract and 0.5% Antibiotic. The birds were fed diets for a period of 6 weeks. Some physical parameters were recorded daily and the chemical parameters were measured also weekly at the end of the feeding trial. All results are expressed as mean ± standard error means. The one way analysis of variance of some values was done followed by Duncan's T-test to evaluate the differences.

4.1 Effects of formulated diets on growth performance of experimental birds

The effect of each formulated diet on growth performances of broiler (Cobb 500) is shown in table 4.1. Body weight in different dietary treatments during experimental periods was found highest in T_4 (1850.0gm) i.e. MLM extract supplemented diet followed by T_3 (1750.0gm), T_2 (1650.0gm), T_1 (1600.0gm), T_0 (1550.0gm), T_5 (1500.50gm) and the lowest weight (1500.50gm) was found with T_5 treatment.

Table 4.1: Growth performance of the birds fed on the experimental diets

Treatments	Average body weight (g)		Average weight gain (g)	Total feed Intake (g)	FCR	Mortality (%)
	Initial	Final				
T_0	242.8	1550.0	1307.20	2350	1.83	5.34
T_1	255.0	1600.0	1500.00	2700	1.80	2.62
T_2	245.0	1650.0	1405.00	2550	1.81	3.24
T_3	260.0	1750.0	1490.0	2500	1.67	4.62
T_4	262.5	1850.0	1587.50	2600	1.63	2.24
T_5	240.5	1500.50	1259.5	2400	1.90	3.72

* FCR = Feed Conversion Ratio

Average body weight gain was found to be the highest in T_4 (1587.5gm) i.e. Extract of MLM compared with other treatments. The results obtained indicated in general that initial body weight of all groups increased. But compared to the control treatment T_0 (242.8gm) and T_5 (240.5gm) the highest weight gain occurred with T_4 (1587.5gm) and T_3 (1500.0gm) where the initial weight of T_4 (262.5gm) and T_3 (260.0gm) respectively.

Data are presented on total feed intake basis in order to observe the trend of feed intake. In T_0 total feed intake was found to be the lowest (2350.0 gm) compared to the other treatments in T_5 (2400.0 gm), T_3 (2500.0gm), T_4 (2600.0gm), T_2 (2550.0gm), and the highest feed intake occurred in T_1 (2700.0gm). Feed intake was higher in mulberry leaf meal (MLM) and their extracts supplemented diet compared to the control diet. But feed intake was not significantly different between the MLM and their extracts. In this experiment variations of feed intake may be due to the different feed ingredients used in diets. Control feed contains the entire ingredient that is responsible for the palatability of the feed. No positive correlation was observed between feed intake and average body weight gain, among the different feed treatments.

Feed conversion ratio (FCR) was lowest in T_4 (1.63) followed by T_3 (1.67), T_1 (1.80), T (1.81), T_0 (1.83) and T_5 (1.90). The variations in FCR among the different fed groups compared to control may be the reason for feed intake. Our findings support the results obtained by other researchers. Improvement of FCR might be due to stimulation of digestive enzymes followed by better digestion and utilization of feed. Moreover, dietary interactions between fat-protein, protein-minerals or minerals-fats may create the differences. Mulberry leaf meal (MLM) possesses potential to improve performance of birds in terms of better FCR.

4.2 Effects of formulated diets on mortality rate

There was no significant effect observed in mortality rate with the supplementation of mulberry leaf meal (MLM) compared to control treatment (Table 4.1). Abu-

Dieyeh and Abu-Darwish (2008) indicated that supplementation with MLM powder and MLM extracts did not affect mortality of broilers. Several studies have shown that MLM possesses anticancer (Abuharfeil *et al.*, 2001), anti-inflammatory (Chai *et al.,* 2005), antioxidative (Katsube *et al.*, 2006) and renal protective (ZOU and CHEN 2003) which improves the immunity of broilers.

4.3 Effects of formulated diets on fat content

The results of the present study showed that the fat content (abdominal+ subcutaneous fat) with the inclusion of 2.5-5.0% MLM diets was found lower in T_2 (69.33gm) followed by T_1 (72.42gm), T_3 (73.00gm), T_4 (75.57gm), T_5 (75.67gm), and T_0 (77.67gm), although the differences were statistically non-significant($P > 0.05$). The results showed that MLM supplemented diets had no significant effect on fat content of broilers.

4.4 Effects of formulated diets on serum lipid profile of broilers

The effects of formulated diets on serum lipids like total cholesterol, HDL-cholesterol, and triglycerides of broilers were shown in Table 4.3. Total cholesterol, HDL-cholesterol and triglyceride of broiler chicks in different dietary treatments during experimental periods were almost statistically similar and the differences were not significant ($P<0.05$) from 10 to 15 days. So, the results clearly showed that MLM supplemented diets up to 3.0% dietary level had no significant effects on total cholesterol, HDL-cholesterol and triglycerides.

15 to 22 days, total cholesterol and HDL-cholesterol were decreased significantly by supplementation of MLM diets in broiler-ration ($P< 0.05$). It is evident from Table: 4.3 that a tendency of gradually reduced total cholesterol was observed in the dietary treatments with inclusion of MLM supplemented diets compared with control treatments. The HDL-cholesterol was gradually decreased with MLM supplemented diets compared with commercial control treatment T_5 (56.33 mg/dl). But triglyceride was almost similar among the treatments and the differences were not significant ($P>0.05$).

At final period of experiment i.e. after 30 days total cholesterol, HDL-cholesterol and triglyceride differences were statistically significant ($P<0.05$). From the table (4.3), it was observed that the values of total cholesterol level in T_3, T_2, T_1, T_4 groups were 130.7, 91.67 mg/dl, 141.7and116.3respectively. All the values were found to be lower than that of control groups T_0 (168.0mg/dl), T_5 (164.3mg/dl) and significantly differing from other groups. In the T_2 and T_4 groups i.e. both 3.5% MLM supplemented and their acetone extract was found total cholesterol content significantly lower than that of control fed groups.

The highest HDL-cholesterol content was found in both T_4 (51.00mg/dl) and T_2 (52.00mg/dl) compared with the control groups T_0 (46.00 mg/dl) in the last week. On the other hand, MLM supplemented diets at different levels caused a significant reduction ($P<0.05$) in the levels of blood triglyceride compared with the control groups.

The data obtained from the serum lipids indicated that the entire blood lipid metabolites (total cholesterol, HDL-cholesterol and triglyceride) tested in broilers blood was significantly improved by all treatments. MLM supplemented diets at different levels caused a significant reduction ($P<0.05$) in the levels of blood (total cholesterol and triglyceride). At the same time, MLM supplemented diets increased the serum HDL level .These results agree with previous reports where dietary supplementation of MLM 3.5% crushed and their acetone extract in broiler chicks was found to cause a significant decrease in the mean values of total cholesterol as compared to control birds. The similar results obtained from El-Dakhakhny *et al.*, (2000) and recommended that black cumin fixed oil could have favorable impact on serum lipid profile by decreasing total cholesterol and triglycerides, while elevating the HDL-cholesterol.

Table 4.2: Fat content and mortality rate of broilers fed different levels of MLM supplemented diets

Items	Dietary level of MLM %						Level of Significance
	T_0 (control)	T_1 (2.5%MLM)	T_2 (3.5% MLM)	T_3 (4.5% MLM)	T_4 (MLM Extract)	T_5 (0.5% Antibiotic)	
Fat content (gm)	$77.67^a \pm 0.50$	$72.42^a \pm 9.86$	$69.33^a \pm 1.52$	$73.00^a \pm 8.32$	$75.57^a \pm 14.97$	$75.67^a \pm 1.52$	NS
*BCR	1.26	1.13	1.23	1.16	1.23	1.29	

Values are expressed as mean ± standard error of at least three replications each of which contains eight birds. Different letters in a row differ statistically significant ($P < 0.05$). Similar letters in a row statistically non- significant ($P > 0.05$).

*BCR: Benefit cost ratio.

Table 4.3: Serum lipid parameters in broilers fed different levels of MLM supplemented diets

Items	Days	Dietary level of MLM						
		T_0 (control)	T_1 (2.5%)	T_2 (3.5%)	T_3 (4.5%)	T_4 (Extract)	T_5 (Antibiotic)	Level Of Significance
Total Cholesterol (Mg/dl)	10-15	$197.0^{a} \pm 14.10$	$182.0^{a} \pm 4.58$	$173.3^{b} \pm 15.69$	$174.0^{a} \pm 9.53$	$181.7^{a} \pm 6.50$	$185.3^{a} \pm 24.00$	NS
	15-22	$182.0^{a} \pm 9.16$	$111.3^{c} \pm 13.65$	$125.3^{b} \pm 4.04$	$120.7^{bc} \pm 11.5$	$120.7^{bc} \pm 5.03$	$171.7^{a} \pm 7.63$	**
	22-42	$168.0^{a} \pm 6.24$	$141.7^{bc} \pm 6.65$	$111.67^{d} \pm 6.42$	$130.7^{c} \pm 30.23$	$116.3^{c} \pm 7.50$	$164.3^{ab} \pm 10.69$	*
HDL-Cholesterol (Mg/dl)	10-15	$43.33^{a} \pm 2.88$	$49.00^{a} \pm 6.08$	$38.33^{a} \pm 4.16$	$48.33^{a} \pm 2.51$	$46.00^{a} \pm 6.08$	$51.33^{a} \pm 11.06$	NS
	15-22	$56.33^{a} \pm 8.14$	$52.33^{ab} \pm 8.73$	$48.00^{ab} \pm 2.64$	$48.67^{ab} \pm 7.02$	$47.67^{ab} \pm 5.03$	$42.67^{a} \pm 2.51$	NS
	22-42	$46.00^{ab} \pm 3.51$	$50.70^{b} \pm 1.52$	$52.00^{a} \pm 3.78$	$48.00^{b} \pm 4.58$	$51.00^{a} \pm 6.65$	45.00 b± 6.24	*
Triglyceride (Mg/dl)	10-15	$82.00^{a} \pm 6.55$	$94.33^{a} \pm 14.64$	$89.00^{a} \pm 2.00$	$74.0^{a} \pm 9.53$	$83.33^{a} \pm 4.50$	$85.3^{a} \pm 24.00$	NS
	15-22	$81.33^{a} \pm 1.52$	$62.33^{a} \pm 5.85$	$73.67^{a} \pm 5.68$	$76.7^{bc} \pm 11.59$	$72.50^{a} \pm 3.60$	$82.0^{a} \pm 9.16$	*
	22-42	$102.0^{c} \pm 3.00$	$77.00^{c} \pm 10.96$	$79.00^{c} \pm 4.58$	$80.7^{c} \pm 30.23$	$82.00^{c} \pm 7.54$	$104.3^{ab} \pm 10.69$	*

Different letters in Values are expressed as mean ± standard error of at least three replications each of which contains eight birds. Different letters in a row differ statistically significant ($P < 0.05$). Similar letters in a row statistically non- significant ($P > 0.05$).

Another findings from Akhtar *et al.*, (2003) supplemented commercial layer-ration with black cumin seeds and observed that serum triglycerides, and total cholesterol contents were reduced, while serum high density lipoprotein cholesterol level was increased.

Moreover, MLM addition had reduced the concentration of serum cholesterol and triglycerides in Pekin ducklings (Mandour *et al.*, 1995. This may probably be due to the possible cholesterol lowering mechanisms of tocopherols explored in number of research investigations i.e. like inhibition of cholesterol oxidation and reduced HMG-CoA-reductase activity (Parker *et al.*, 1993).The mode of action of cholesterol reduction associated with consumption of black cumin fixed and essential oils is multidimensional. Black cumin fixed oil is rich in polyunsaturated fatty acids mainly account for cholesterol lowering potential (Cheikh-Rouhou *et al.*, 2007). Application of grounded *Morus alba* leaves in broilers have been reported to prevent from lipid peroxidation (Sogut *et al.*, 2008).

Although mechanism is not known, the volatile oils of *Morus alba leaves* contain quinines including thymoquinone (TQ) and dithymoquinone, which might be involved in sharp decrease in serum triglyceride content in hens fed *Nigella*-supplemented diet (Swamy and Tan, 2000). Serum triglyceride lowering effect of TQ in rats has recently been reported. The decrease of serum cholesterol and triglycerides by supplementation of *Morus alba* leaves in diets might be associated with the choleretic activity of the seed powder as shown by El-Dhakhny *et al.* (2000). The choleretic function may be due to either by reducing synthesis of triglycerides and cholesterol by hepatocytes or by decreasing its fractional reabsorption from the small intestine. The mechanism by which *Morus alba* leaves decreases cholesterol is difficult to precise however, the contents of secondary metabolites in seeds such as TQ might be involved.

CHAPTER V

SUMMARY AND CONCLUSION

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The objectives of this experiment were to evaluate the varying doses of MLM supplemented diets on broiler chicks. The feeding value of MLM and their extract for broiler (Cobb 500) was evaluated in the poultry shed, Hajee Mohammad Danesh Science and Technology University, Dinajpur. In feeding trial, five (5) diets were prepared including MLM at levels of 0%, 2.5%, 3.5% and 4.5% and 0.5% Antibiotic. Body weight gain and feed consumption were recorded daily basis and serum lipid profiles were measured weekly basis. By the end of each treatment, fat content and intestinal bacteria were recorded. Mortality rate was also recorded throughout the study.

In experimental diets, the growth performances of birds were improved. The experimental birds exhibited progressive weight gain during feeding trail but the rates of gain were different than that of the control groups. Average body weight gain was found to be the highest in treatment T_4 (1850gm) and T_3 (1750gm) compared with T_5 (1500gm) and T_0 (1550gm). Feed intake trend from first day to last day of the experimental period in different treatment groups was recorded and expressed as gm/day. Although the rate of feed intake varied day to day but the feed intake (gm/day) was lowest in T_0 compared to other treatments. This variability in feed intake might be due to different palatability of feeds. Feed conversion ratio (FCR) was lowest in T_4 (1.63) and T_3 (1.67) treatments compared to groups T_5 (1.90) and T_0 (1.83). Mortality rate was highest in control treatment T_0 (5.34%) compared to MLM supplemented diets.

Fat content (abdominal and subcutaneous fats) of broilers in different dietary treatments were almost similar and the differences were statistically non-significant.

Total cholesterol, HDL-Cholesterol and triglycerides were statistically non-significant ($P>0.05$) up to 15 days of experiment. Total cholesterol and HDL-

cholesterol were gradually decreased by MLM supplemented diets but triglyceride was almost similar and statistically non-significant up to 22 days. At the end of experimental period, total cholesterol were higher in control treatments T_0 (168.0mg/dl) and T_5 (164.3mg/dl) compared to MLM supplemented diets and the lowest values were found in both 3.5% MLM powder and their extracts i.e. in T_2 ((91.67mg/dl) and T_4 (116.30 mg/dl). The HDL-cholesterol were highest in both T_2 (52.00mg/dl) and T_4 (51.00mg/dl) compared to control treatments T_0 (46.00mg/dl) and T_5 (43.00mg/dl). The triglycerides were decreased at final period by MLM supplemented diets compared to treatments T_5 (199.0mg/dl) and T_0 (102.0mg/dl) and there was significant differences ($P<.05$) between control and MLM supplemented treatments.

It may be concluded that the MLM supplemented diets had limited effect on mortality rate and had no detrimental effect on fat content. The performances of broiler i.e. final body weight, feed intake, and feed conversion ratio were improved by feeding MLM supplemented diets at different levels. The positive effect of these was on serum lipids. Among the MLM supplemented diets, our findings suggest that supplementation of both 3.5% MLM powder and extracts of MLM has high potential as commercial applications for production of low-cholesterol and healthy broilers. It is recommended to use MLM as feed supplements to broilers in their starter, grower and finisher diets supplementation with the levels (3.5% MLM powder and extracts of MLM) used in this experiment. However, further research is required to assess the mechanisms of serum cholesterol, triglyceride and *E. coli* decreasing function of MLM in broilers.

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