# EFFECT OF GUAVA LEAF MEAL ON PRODUCTION PERFORMANCE AND ANTIBACTERIAL SENSITIVITY IN COMMERCIAL BROILER



A Thesis
By

**DR. MD. ZAMINUR RAHMAN**

REGISTRATION NO. 1005114

SESSION: 2010-2011

SEMESTER: SEPTEMBER-FEBRUARY, 2011

MASTER OF SCIENCE (MS)

IN

POULTRY SCIENCE

**DEPARTMENT OF DAIRY AND POULTRY SCIENCE**

**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY**
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Submitted to the Department of Dairy and poultry Science, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur in partial fulfilment of the requirements for the degree of

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***Approved as to style and content by:***

**(Prof. Dr. Mst. Afroza Khatun)**
Professor
Research Supervisor

**(Dr. Tahera Yesmin)**
Associate Professor
Research Co-supervisor

**(Prof. Dr. Mst. Afroza Khatun)**
Chairman of the Examination Committee

And
Chairman, Department of Dairy and Poultry Science

**DEPARTMENT OF DAIRY AND POULTRY SCIENCE**

**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY**
**DINAJPUR**

FEBRUARY, 2013

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. Dr. 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, as well as thesis preparation.*

*The author expresses his profound gratefulness, heartfelt respect and immense indebtedness to his co-supervisor, Dr. Tahera Yeasmin, Associate Professor, 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 to inspire for improving his knowledge and academic skills in this esteemed discipline during the period of his study.*

*The author takes this opportunity to express his sincere appreciation and heartful thanks to Md. Kamruzzaman and Md. Robiul Islam, former MS student of the*

*Department of Dairy and Poultry Science Hajee Mohammad Danesh Science and Technology University, Dinajpur for their helpful co-operation.*

*The author also acknowledges to the staff members of the 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. Habibur Rahman and mother Mst. Shayara 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 friend especially Gonopoty Roy and younger brother Rifat, Hayder, Ahsan Habib and Mamun Bhai who have helped a lot during the study period.*

*The Author*

## ABSTRACT

This study was conducted at the poultry shed and poultry laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. The study was carried out in order to apply the possibility of treated guava leaf meal in broiler diets to determine the effects of it's at various levels of dietary treatment on production and quality characteristics of broiler. However, another investigation was also conducted to identify the antibacterial effects of guava leaf meal on broiler production. For this 180 day old broiler chicks were taken and divided into four treatments, each with three replications (15 birds/ replication) at the age of four or day and then offered manually prepared diets supplemented with 0%, 2.5%, 3.5 %, 4.5 % guava leaf meal after treating by means of some physical and chemical processes. Live weight, mortality rate, feed conversion ratio, internal fat content were calculated from the regular collecting data. At the same time antibacterial sensitivity test was also evaluated. The result showed that, feed intake, body weight gain and feed conversion ratio at different dietary treatments were almost similar and the differences were statistically non-significant except fat content, mortality rate and antimicrobial sensitivity. Fat content and mortality rate were decreased with increased level of guava leaf meal up to 4.5% level. However guava leaf extract had significant effect on antibacterial activity basically higher against *E. coli* followed by *streptococcus* sp. and *staphylococcus* sp. and was significant at 5 % level of significance. Based on the results of present study it may be concluded that guava leaf is a good source of nutrients and it has significant effect on fat content of broiler, mortality rate and antimicrobial sensitivity without affecting the bird's feed intake, body weight and feed conversion ratio. The results of the study suggest that supplementation of guava leaf meal (*Psidium guajava*) up to 4.5% level in diets has high potential as commercial applications for production performance of broiler. Therefore, guava leaf meal could be used along with other conventional feed ingredients. However, further study is to be needed to understand the active principle(s) of antimicrobial sensitivity and other beneficial effects of guava leaf meal observed in this experiment prior to practical use it as unconventional feed of poultry.



Key words: Guava leaf, Broiler, FCR, Antimicrobial sensitivity.

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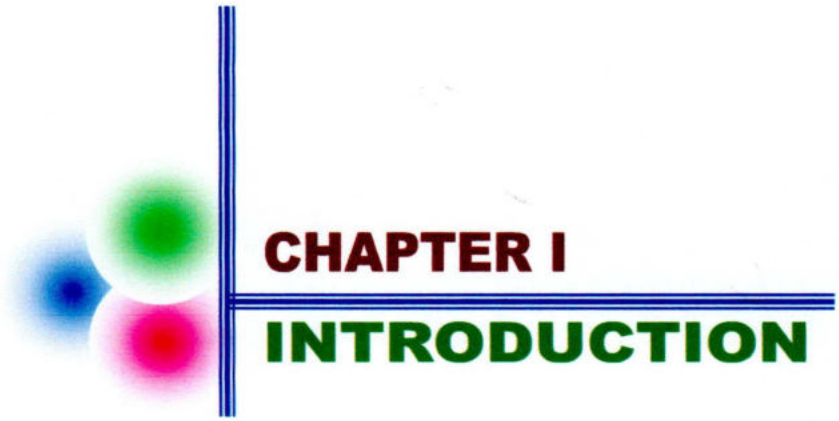
# LIST OF ABBREVIATIONS

| ABBREVIATIONS | ELABORATIONS |
|---|---|
| FCR | Feed Conversion Ratio |
| MS | Master of Science |
| nm | Nano Meter |
| mm | Milli Meter |
| cm | Centi Meter |
| GLM | Guava Leaf Meal |
| v/v | Volume/Volume |
| KOH | Potassium Hydro-oxide |
| NS | Not Significant |
| SD | Standard Error |
| W | Weight |
| Av. | Average |
| IU | International Unit |
| AOAC | Association of Official Analytical Chemists |
| UK | United Kingdom |
| USA | United States of America |
| 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

# CHAPTER I

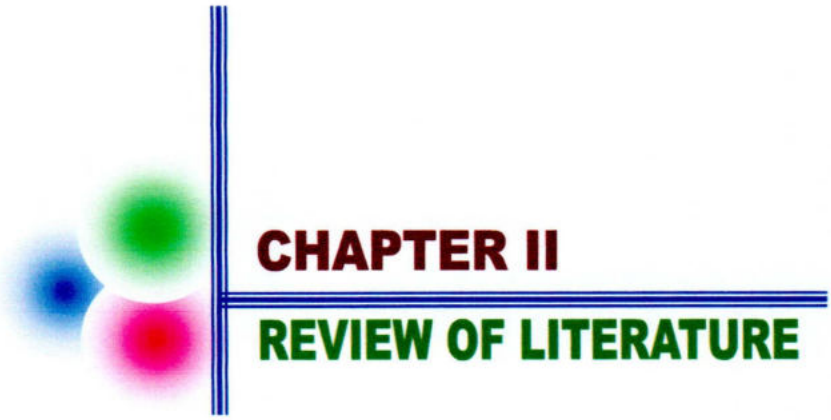
## INTRODUCTION

The biggest impediments to livestock production in developing Bangladesh are the high cost of feed ingredients. Unfortunately, nearly all sources of agricultural leaf and plant protein posses associated high fiber and anti-nutritional factors which must be eliminated by special processing techniques to make them of maximum nutritional value. Water soaking, autoclaving, cooking in boiling water, steaming, radiation and treatment with acid or alkaline considered among the most common processing procedures being in use to improve the nutritive value as reported by many investigators (Abiola and Adekunle, 2002; González-Alvarado *et al*., 2007 and Garcia *et al*., 2008). A great quantity of guava leaf meal (pulp and peel) is produced as a waste of canning industry in Egypt and yet was not fully evaluated as a feedstuff for poultry. Aly *et al.* (1981 found that guava leaf contained 8.9% oil. Gas-liquid chromatographic analysis of the methyl esters for the fatty acid of the oil revealed the presence of twelve fatty acids. The protein content of guava leaf was 9.73% on dry weight consumed fresh and also processed (beverages, syrup, ice cream, and jams). Pulp and peel fractions were tested, and both showed high content of dietary fiber (48.55-49.42%) and extractable polyphenols (2.62-7.79%). These results indicate that guava could be a suitable source of natural antioxidants. Peel and pulp could also be used to obtain antioxidant dietary fiber. To explore the possibility of incorporation guava leaf in broiler diets, different treatments e.g. (boiling, autoclaving, boiling in alkaline solution or in acid solution) on the nutritional value of guava leaf was studied basis. Qualitative and quantitative analysis revealed the presence of fifteen amino acids and the major amino acid constituted about 67% of the total amino acid percent in protein of guava leaf. Guava is a tropical fruit, widely consumed fresh and also processed (beverages, syrup, ice cream, and jams). To explore the possibility of incorporation guava leaf in broiler diets, different treatments e.g. (boiling, autoclaving, boiling in alkaline solution or in acid solution) on the nutritional value of guava leaf was studied. Again guava is commonly known as the poor man's apple of the tropics has a long history of traditional use, much of which is being validated by scientific research. Guava is rich in tannins, phenols, flavanoids, essential oils, lectins, vitamins, fatty acids etc. Much of the guava's medicinal activity is attributed to these flavanoids. The flavanoids have demonstrated anti-bacterial activity. A decoction of bark of leaves or a

flower infusion is used tropically for wounds, ulcers and skin sores. Flowers are also mashed and applied to painful eye conditions such as sun strain, conjunctivitis or eye injuries. Commercially the fruit is consumed fresh or used in the making of Jams, Jellies, paste. Guava leaves are in the "Ducth-Pharmacopoea" for the treatment of Diarrhea. In the present work an assessment of the antibacterial potential of leaves of guava has been carried out. Traditionally, usage of plants in curing illness has deep roots in man's history (Grabley and Thiericke., 1999; Aibinu *et al.,* 2007). Plants are used in treating malaria, diarrhoea, burns, gonorrhoea, stomach disorders and other infectious diseases. Tremendous efforts of scientists have been employed in establishing plants with promising antimicrobial activity and yielding fruitful results (Adedayo *et al.,* 2001; Ndukwe *et al.,* 2005 and Aibinu *et al.,* 2007). The plants are easily available and accessible in this part of the world and cheaper than the conventional drugs. The disease condition omphalitis is technically defined as an infectious but non-contagious disease that is characterized by infected yolk sac often accompanied by unhealed navel (Jordon and Pattison., 1999). In every flock, there is usually increased mortality between 3-4 days of age due often to navel-yolk sac infection or omphalitis, commonly associated with Escherichia coli infections or other bacterial contamination. Because the navel is still open when the chick is hatching, or when tissue is stock in the navel after closing; it is very easy for bacteria to enter the body cavity, so infecting both the navel and the yolk sac. This is to say that navel yolk sac infection is one of the causes of high mortality during the early days of young chicks (Jordon and Pattison, 1999). Due to the significant number of birds claimed by omphalitis, the tragedy is called naval-yolk sac mortality. Bacterial pressure is the only determining factor through navel deformity and the chick's ability to use its natural defense mechanisms are also critical observed by Meijerhof *et al.,*(2005). Common bacteria involved are *E. coli*, *Entrococcus* spp. *Salmonella* spp. and *Pseudomonas* spp.. These bacteria can cause generalized septicemia and result in high mortality. Affected birds will show depreciation, drooping of the head and hurdling near the heat source. The navel may be inflamed and when fail to close produce a wet spot abdomen. Treatment of this condition is not specific and no specific antibiotic for the treatment. The antibiotic used is based on the prevalent bacteria type involved but of probably little value (Koteeswaran *et al.,* 2004). It may not always be appropriate to be treated as if there is niggling mortality. Treatment result in infected yolk sac retention and this can lead to uneven mass in the flock (Jordon and Pattison., 1999). The escalating problems of antibacterial resistance shown by several species of bacteria to most of the

antibiotics used today has made it mandatory to search for newer drugs that are effective, affordable, acceptable and available. Many research groups and organizations in many countries under take multidisciplinary research on local medicinal plants with a view of revealing that plants are potential source of drugs (Sofowora, 1993). Although, traditional medicine has been in practice as far back as 1500BC and many plants have been used to cure certain diseases, there may not be scientific data to confirm their efficacy (Sofowora, 1993). Guava is a common shade tree or shrubs in the tropics and has a long history of traditional medicinal uses, which led modern day researchers to study its extracts ((Jordon *et al.,* 2003). Extracts of roots, bark and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothaches, coughs, sore throat, inflamed gums and a number of other conditions (Morton, 1981). It has been documented to have pronounced antibacterial, antiamoebic and antispasmodic activity. Its bark and leaf extracts have been shown to have in vitro toxic action against numerous bacteria (Geidam *et al.,* 2007). Therefore the present piece of research work was undertaken with the following objectives:

1. To observe the effect of guava leaf on production performance and quality characteristics of broiler.
2. To know the antibacterial effect of guava leaf on broiler.



# CHAPTER II

# REVIEW OF LITERATURE

# CHAPTER II

# REVIEW OF LITERATURE

## 2.1 Nutritive value of guava leaf

Guava commonly known for its food and nutritional values throughout the world. It is a low evergreen tree or shrub 6 to 25 feet high, with wide-spreading branches and square, downy twigs, is a native of tropical America. It is a common vegetation cover by roads and in waste places in Hawaii. Guava is a tropical and semitropical plant. It is well known in the islands for its edible fruit. It is common in the backyards. The branches are crooked, bringing opposite leaves. The flowers are white, in curved petals, 2 or 3 in the leaf axils; they are fragrant, with four to six petals and yellow anthers. The fruit is small, 3 to 6 cm long, pear-shaped, reddish-yellow when ripe. The plant is readily available in Bangladesh and Asia Pacific area as backyard nature within the reach of the local populace. Guava is a large tropical evergreen shrub or small shade tree. The medicinal properties of guava fruit, leaf and other parts of the plant are also well known in traditional system of medicine. Since, each part of guava tree possesses economic value; it is grown on commercial scale. Guava plant is considerable process has been achieved regarding the biological activity and medicinal application of guava and the fruit considered as poor man apple of tropics. The guava plant parts are used for the treatment of various disease and important medicinal values. In the present review, nutritional value of guava fruit and medicinal properties its various parts have been discussed to provide collective information on its multipurpose commercial values.

Guavas are rich in dietary fiber, vitamin A and C, folic acid, and the dietary minerals, potassium, copper and manganese. Having a generally broad, low-calorie profile of essential nutrients, a single common guava (*P. guajava*) fruit contains about four times the amount of vitamin C as an orange. However, nutrient content varies across guava cultivars. Although the strawberry guava (*P. littorale* var. *cattleianum*) has about 25% of the amount found in more common varieties, its total vitamin C content in one serving (90 mg) still provides 100% of the Dietary Reference Intake for adult males.' Thai maroon' guavas, a red apple guava cultivar, rich in carotenoids and polyphenols. Guavas contain both carotenoids and polyphenols like (+)- gallocatechin, guaijaverin, leucocyanidin and amritoside – the major classes of antioxidant pigments – giving them

relatively high potential antioxidant value among plant foods. As these pigments produce the fruit skin and flesh color, guavas that are red-orange have more pigment content as polyphenol, carotenoid and pro-vitamin A, retinoid sources than yellow-green ones.

Figure 1. Guava leaf

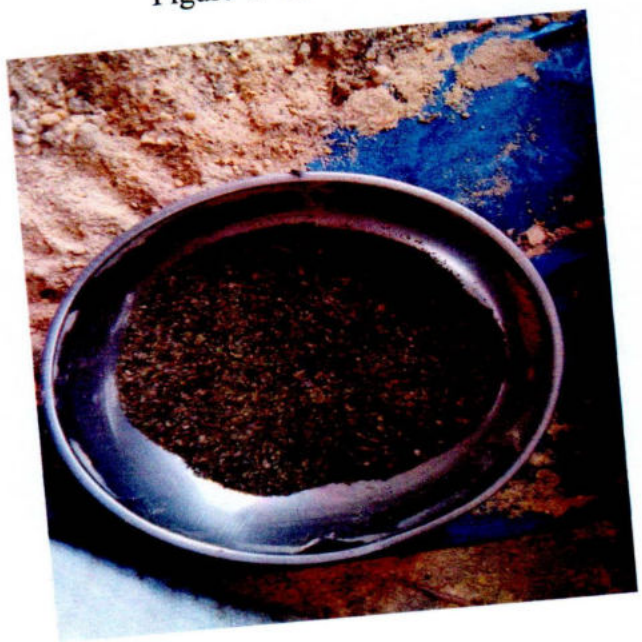


Figure 2. Guava dry leaves

Figure 3. Guava leaves

Figure 4. Guava tree with fruits

**Table 2.1 Nutritive value of guava (*Psidium guajava*) fresh leaves per 100 g**

| Principle | Nutrient Value |
|---|---|
| Energy | 68 Kcal |
| Carbohydrates | 14.3 g |
| Protein | 2.55 g |
| Total Fat | 0.95 g |
| Cholesterol | 0 mg |
| Dietary Fiber | 5.4 g |
| **Vitamins** | |
| Folates | 49 µg |
| Niacin | 1.084 mg |
| Pantothenic acid | 0.451 mg |
| Pyridoxine | 0.110 mg |
| Riboflavin | 0.040 mg |
| Thiamin | 0.067 mg |
| Vitamin A | 624 IU |
| Vitamin C | 228 mg |
| Vitamin E | 0.73 mg |
| Vitamin K | 2.6 µg |
| **Electrolytes** | |
| Sodium | 2 mg |
| Potassium | 417 mg |
| **Minerals** | |
| Calcium | 18 mg |
| Copper | 0.230 mg |
| Magnesium | 22 mg |
| Manganese | 0.150 mg |
| Phosphorus | 11 mg |
| Selenium | 0.6 mcg |
| Zinc | 0.23 mg |
| **Phyto-nutrients** | |
| Carotene-ß | 374 µg |
| Crypto-xanthin-ß | 0 µg |
| Lycopene | 5204 µg |

(Source: USDA National Nutrient data base)

## 2.2 Antimicrobial activity of guava leaf

Iwu (1993) showed that the extract of guava and its leaves have antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Shigella dysenteria*. Another paper showed the effectiveness of the leaf extract against *Staphylococcus aureus* (Gnan and Demello, 1999). It was shown to antibacterial in another study and in addition to *Staphylococcus aureus* was also useful against *Streptococcus* sp (Pranee, 1999). A strong antimicrobial action of guava leaves on Gram-positive and Gram-negative organisms has been reported (*Sarcina lutea* and *Staphylococcus aureus*) and also noted action on *Mycobacterium phlei*. Oliver-Bever (1986) reported that the flavone derivatives inhibit the growth of *Staph aureus*. Arima *et al.* (2002) observed that four antibacterial compounds were isolated from leaves of guava, flavonoid glycosides, morin-3-O-α-L-lyxopyranoside and morin-3-O-alpha-L arabopyranoside, and two known flavonoids; guaijavarin and quercetin. Bark tincture exhibited higher efficacy in controlling the mycelial growth of dermatophytes than the leatincture. The tincture showed fungicidal property in different concentrations but exhibited only fungistatic property in case of *C. albicans* (Dutta *et al.*, 2000).Another paper showed good effect with the methanolic extract (Rabe and Staden, 1997).A leaf extract enters into a Nigerian remedy for skin infections, and examination has shown a positive action on Gram-positive microbial organisms, but no action on Gram-negative organisms, nor any antifungal action. Three antibacterial substances have been detected in the leaves which are derivatives of quercetine. As in the bark barpolyphenols and many other substances are present (Burkill, 1997).

## 2.3 Hypocholesterolemic and medicinal effect of guava leaf

In recent years, the number of people with metabolic syndrome has continued to rise because of changing eating habits, and accompanying hepatic steatosis patients have also increased. This study examined the effect of guava leaf extract on liver fat accumulation using SHRSP.Z-Leprfa / IzmDmcr rats (SHRSP/ZF), which are a metabolic syndrome model animal.

# CHAPTER III

# MATERIALS AND METHODS

# CHAPTER III

# MATERIALS AND METHODS

## 3 Materials

### 3.1 Feed materials

#### 3.1.1 Source of *Psidium guajava*

Guava leaf was collected from different places of Dinajpur district of Bangladesh. The leaves were coarsely powdered after treating by means of physical and chemical processes. Then it was directly mixed with manually prepared diets in appropriate doses.

#### 3.1.2 Treatment of guava leaf as feed samples

At first guava leaf was boiled in water for one hour, boiled in alkaline solution 0.1 N for one hour. Then alkali treated guava leaf was boiled in acid solution 0.1 N for one hour, and autoclaved for 20 minutes at 15 IP pressure. Chemical analysis was conducted on both raw and treated guava leaves. Treated guava leaves were dried at 80°C in an electric oven and grind in hummer mill then samples were taken for determination of chemical composition according to AOAC (1990)

#### 3.1.3 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 (Appendix I, Tables 3.4 and 3.5). Basically Tables (3.3, 3.4 and 3.5) show the composition and the chemical composition of the starter, grower and finisher rations, respectively. The experimental diets were designed as-

$T_1$ : control

$T_2$ : control+ 2.5% guava leaves as mash form

$T_3$ : control+ 3.5% guava leaves as mash form

$T_4$ : control + 4.5% guava leaves as mash form

**Table 3.3 Composition of the experimental starter diets fed to broilers**

| Feed ingredients | Dietary level of guava leaves | | | |
|---|---|---|---|---|
| | $T_1$ (0%) | $T_2$ (2.5 %) | $T_3$ (3.5 % ) | $T_4$ (4.5 % ) |
| Maize | 49.60 | 49.60 | 49.20 | 49.00 |
| Soybean meal | 26.90 | 25.04 | 24.85 | 24.50 |
| Rice polish | 10.90 | 10.77 | 10.70 | 10.00 |
| Meat & bone meal | 8.00 | 8.00 | 8.00 | 8.00 |
| DCP | 0.71 | 0.70 | 0.70 | 0.70 |
| Soybean oil | 3.50 | 3.00 | 2.73 | 2.88 |
| Guava leaves | 0.00 | 2.50 | 3.50 | 4.50 |
| Salt | 0.27 | 0.27 | 0.30 | 0.30 |
| Vitamin-mineral premix* | 0.12 | 0.12 | 0.12 | 0.12 |
| **Elements** | **Calculated composition** | | | |
| ME (Kcal/Kg) | 3084 | 3106.5 | 3124.5 | 3133 |
| CP (%) | 21.40 | 21.35 | 21.30 | 21.28 |
| CF (%) | 3.77 | 3.71 | 3.78 | 3.78 |
| Ca (%) | 1.16 | 1.12 | 1.12 | 1.13 |
| P (%) | 0.54 | 0.54 | 0.55 | 0.55 |
| Lysine (%) | 1.19 | 1.19 | 1.18 | 1.18 |
| Methionine (%) | 0.48 | 0.48 | 0.48 | 0.48 |

Broiler premix was added @ 120 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 3.4 Composition of the experimental grower diets fed to broilers**

| Feed ingredients | Dietary level of guava leaves | | | |
|---|---|---|---|---|
| | $T_1$ (0%) | $T_2$ (2.5 %) | $T_3$ (3.5 % ) | $T_4$ (4.5 % ) |
| Maize | 52.00 | 52.00 | 52.00 | 52.00 |
| Soybean meal | 22.78 | 22.13 | 21.13 | 20.13 |
| Rice polish | 12.70 | 11.70 | 11.70 | 11.70 |
| Meat & bone meal | 8.00 | 7.00 | 7.00 | 7.00 |
| Soybean oil | 3.50 | 3.70 | 3.70 | 3.70 |
| DCP | 0.75 | 0.70 | 0.70 | 0.70 |
| Guava leaves | 0.00 | 2.50 | 3.50 | 4.50 |
| Salt | 0.27 | 0.27 | 0.27 | 0.27 |
| Vitamin-mineral premix* | * | * | * | * |
| **Elements** | **Calculated composition** | | | |
| ME (Kcal/Kg) | 3120 | 3142.5 | 3130.5 | 3169 |
| CP (%) | 18.85 | 18.76 | 18.69 | 18.85 |
| CF (%) | 3.69 | 3.70 | 3.71 | 3.69 |
| Ca (%) | 1.06 | 1.07 | 1.07 | 1.06 |
| P (%) | 0.51 | 0.50 | 0.52 | 0.51 |
| Lysine (%) | 1.01 | 1.00 | 1.00 | 1.01 |
| Methionine (%) | 0.40 | 0.40 | 0.40 | 0.40 |

Broiler premix was added @ 120 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 3.5 Composition of the experimental finisher diets fed to broilers**

| Feed ingredients | Dietary level of guava leaves | | | |
|---|---|---|---|---|
| | $T_1$ (0%) | $T_2$ (2.5 %) | $T_3$ (3.5 % ) | $T_4$ (4.5 % ) |
| Maize | 55.30 | 55.00 | 55.00 | 55.00 |
| Soybean meal | 22.53 | 21.83 | 21.00 | 20.33 |
| Rice polish | 10.70 | 10.70 | 10.70 | 10.70 |
| Meat & bone meal | 7.00 | 6.00 | 6.00 | 6.00 |
| DCP | 0.70 | 0.70 | 0.70 | 0.70 |
| Soybean oil | 3.50 | 3.00 | 2.74 | 2.50 |
| Guava leaves | 0.00 | 2.50 | 3.50 | 4.50 |
| Salt | 0.27 | 0.27 | 0.27 | 0.27 |
| Vitamin-mineral premix* | * | * | * | * |
| **Elements** | **Chemical composition** | | | |
| ME (Kcal/Kg) | 3120 | 3142.5 | 3130.5 | 3169 |
| CP (%) | 18.85 | 18.76 | 18.69 | 18.85 |
| CF (%) | 3.69 | 3.70 | 3.71 | 3.69 |
| Ca (%) | 1.06 | 1.07 | 1.07 | 1.06 |
| P (%) | 0.51 | 0.50 | 0.52 | 0.51 |
| Lysine (%) | 1.01 | 1.00 | 1.00 | 1.01 |
| Methionine (%) | 0.40 | 0.40 | 0.40 | 0.40 |

Broiler premix was added @ 120 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 open sided poultry shed in Hajee Mohammad Danesh Science and Technology University, Dinajpur. A total 180 day-old broiler chick (Cobb 500) were purchased from CP Bangladesh Ltd. At first chicks were reared at brooding house to adjust with the environmental condition up to 10 days. After 10 days chicks were randomly assigned to their treatments and was divided into four dietary treatment groups composed of 45 chicks in each; each treatment was composed of three replications with 15 birds in each in a complete randomized design (CRD).

#### 3.2.2 Brooding of baby chicks

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 37° C and decreased by 1° C per day to final temperature of 28° C at the end of experiment. Supplementary heating was provided as required by mobile butane gas heaters besides to electricity heater.

Figure 5. Brooding of chicks

### 3.2.3 Feeding and watering

During brooding period, linear feeder and round plastic drinker were used. After that linear feeder was replaced by round plastic drinker. Feed and fresh water were offered to the bird manually according to experimental schedule. One round plastic feeder and drinker were provided for seven birds.

### 3.2.4 Lighting

During the experimental period, all birds were exposed to continuous lighting of 23 hours and one hour dark period per day. Electric bulb of 20 lux was the source of light.

### 3.2.5 Vaccination

All birds were vaccinated against Newcastle disease at day one and boostering by day 21. Against Gumboro disease the birds were vaccinated firstly at day seven and boostering at day 14.

### 3.2.6 Medication

At very first week Gluco- C was used @ 50gm/liter water. Water solublable vitamin Rena WS @ 1gm/liter and normal saline also provided for first 3 days of brooding.

### 3.2.7 Sanitation

Strict sanitary measures were taken during the experimental period. Disinfectant was used to disinfect the shed premises regularly. Foot bath was present at the entrance of the shed area. Fumigation procedure was ensured before the birds arrival. Routine biosecurity programme was properly maintained as strict manner.

### 3.2.8 Observation of birds

All the birds were examined twice daily for any visible physical changes like restlessness, lordosis, abnormal gait, vices and depression as well as feeding style during study period.

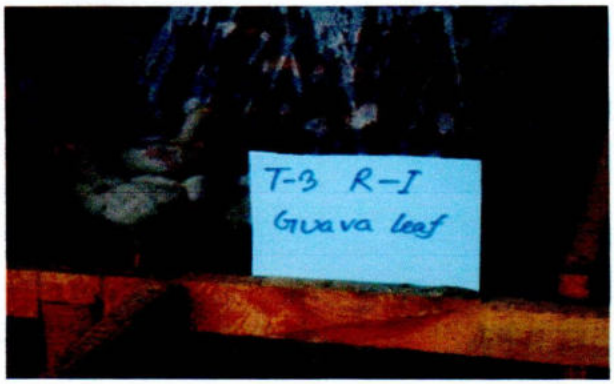



Figure 6. Treatment of chicks by guava leaf

### 3.3 The performance trial

During the 42 days of 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 conversion were then 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. The product was divided by the total number of birds.

Feed Intake =Feed intake in replication/No. of birds in replication.

#### 3.3.2 Body weight gain (kg)

Body weight was measured for all birds at the beginning of the experiment, and it was repeated weekly, 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.

Body weight gain (kg) = Final weight – Initial weight.

Figure 7. Weighing of chicks

### 3.3.3 Feed conversion ratio

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

FCR = Feed intake(kg)/Weight gain(kg)

### 3.3.4 Evaluation of carcass characteristics

Dressing percentage is based on the relationship between the dressed carcass weight and live animal weight after things like the skin and internal organs have been removed. Dressing percentage can be calculated by taking weight of the carcass divided by weight of live animal. Experimental birds were slaughtered after 42 days of feeding trial to assess the carcass quality. Four birds were taken to measure the selected quality. Before slaughtering the birds were kept in fasting condition for 24 hours. Just before slaughtering the birds were weighted. Birds were slaughtered according to halal method. Data were recorded in terms of live weight, breast meat weight, thigh bone weight, thigh meat weight, drumstic meat weight, drumstick bone weight, skin weight, abdominal fat weight, digestive tract weight, liver weight, gizzard weight, shank weight, heart weight, head weight, neck weight, spleen weight and wing weight.

$$\text{Dressing percentage} = \frac{\text{Weight of the carcass}}{\text{Weight of live animal}} \times 100$$


Figure 8. Dressed broiler

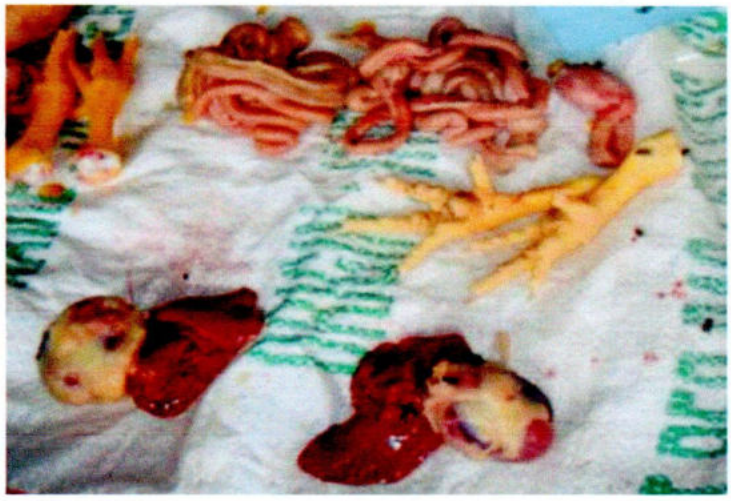


Figure 9. Offal's of broiler

### 3.4 Determination of Anti-bacterial activity of guava leaf extract

#### 3.4.1 Collection of samples

Swaps were taken using sterile swap sticks from the naval of day-old chicks. These were inoculated into the plates containing MacConkey and blood agars. Using the half and quarter plate streaking method, respectively.

#### 3.4.2 Culturing and identification of the organisms

The inoculated plates were incubated immediately for 24 h at 37°C. The growth was then identified using colonial appearance, gram stain, examination of the organisms under microscopes. Sub-culture was done by using different media for confirmation of the organisms earlier identified. All media used were prepared according to manufacturer's instructions.

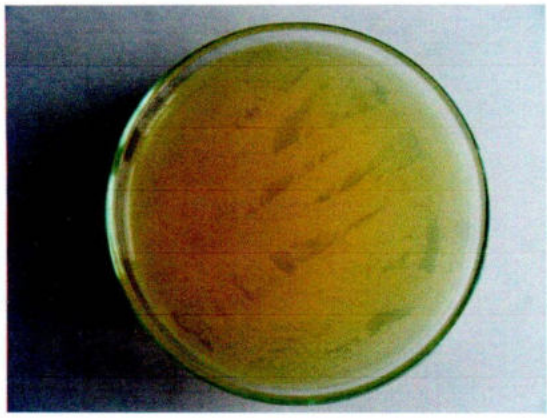


Figure 10. Collection of organisms from SS agar media

### 3.4.3 Determination of Anti-bacterial properties

#### 3.4.3.1 Bacterial isolates

The bacteria were isolated by special media culture; E.g MacConkey for *E. coli.* The bacterial isolates from the naval of day-old-chicks were used for determining the anti bacterial properties of guava leaf extract. The isolates were propagated and stored on nutrient agar plates. All the isolates were maintained on nutrient agar plate at 4°C and sub-cultured in nutrient broth at 37°C for 8 hours prior to antimicrobial testing. One milliliter of the broth culture was then used to flood the agar plates.

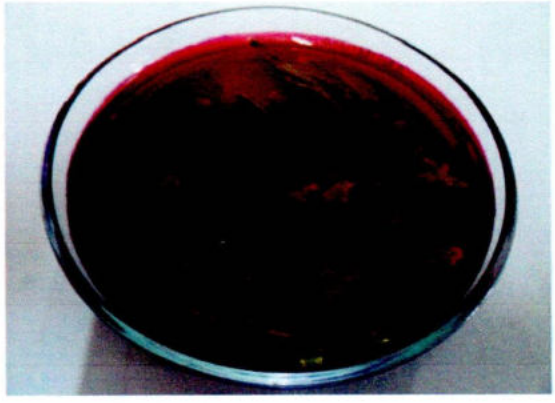


Figure 11. Collection of organisms from EMB agar media

### 3.4.3.2 Concentration of extracts

Stock solutions of the extract were prepared by dissolving known weight of the extract in known volume of distilled water 0.01, 0.02 and 0.04 g of the extracts were dissolved in 1 ml of distilled water to afford 100, 200 and 400 mg/ml of the extract, respectively. Standard antibacterial agent oxytetracycline (Renamycin -500mg, renata animal health. Bangladesh) at a concentration of 10 mg /ml was also used on all the bacteria and the zones of inhibition compared with those of the plant extract.

### 3.4.3.3 Antibacterial sensitivity testing

Bauer-Kirby disc diffusion method as described by Bauer *et al.* (1966) was used to determine the antibacterial activity. Discs containing different concentrations of dissolved extract were prepared. Sterilized filter papers (Whatman No. 1, 6 mm in diameter) soaked in beakers containing different concentrations (100, 200 and 400 mg/ ml) of the extract. Overnight cultures of each bacterial isolate was spread on the surface of dried nutrient agar plates. The plates were incubated at 37°C for 30 min before the discs were applied aseptically. The treated plates were incubated at 37°C for 48 hours. The same procedure was carried out with the oxytetracycline (10 mg/ ml) as standard antibiotic. Plates without the antibiotic or extract discs were set up as control experiment. The zones of inhibition above 6 mm diameter of each isolate were used as measure of susceptibility to the extracts and were compared to that of the standard antibiotic.

### 3.4.3.4 Determination of minimum inhibitory concentration (MIC) of the extracts

The MIC was determined using the method described by Greenwood (1989). For each extract three sterile test tubes were arranged in a test tube rack in a row for each organisms and 0.5 ml of sterile nutrient broth was pipetted into each tube. Half a millimeter of the crude extract containing 100 mg/ml was pipetted into tube one to obtain a concentration of 50 mg /ml. There after there was a serial dilution of the extract to obtain concentrations of 25, 12.5, 6.25 and 3.13 mg /ml, respectively. 0.5 ml of the test organism was pipetted into each test tube and incubated at 37°C for 24 hours. The MIC was recorded as the least concentration of plant extract that completely inhibit the growth of the test organism.

### 3.5 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. All analyses were performed by Mstatc and SPSS Program.

# CHAPTER IV

# RESULTS AND DISCUSSION

# CHAPTER IV

## RESULTS AND DISCUSSION

This study was conducted to evaluate the effects of varying doses of guava leaves and their extracts supplemented diets on growth rate, changes in body weight, feed intake, fat content, and mortality of broiler. The formulated diets were supplemented with 2.5%, 3.5% and 4.5% guava leaf respectively. 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 to Duncan's T-test to evaluate the differences. Antibacterial activity of GLM was measured under the methods of placing leaf extract and antibiotic disc on agar plate and minimum inhibitory concentration (MIC) was measured by broth dilution method.

### 4.1 Effect of guava leaf meal on body weight gain

Body weight gain in different dietary treatments during experimental periods was almost similar and the differences were not significant ($P > 0.05$) (Table 4.6). These results indicate that inclusion up to 4.5 percent guava leaf meal had no adverse effect on body weight gain. This result similar with El-Deek *et al.* (2009). They had found that the final results of broiler body weight and body weight gain at 8 wks of age showed no significant differences as the result of feeding 2 or 4% levels of guava by-products, raw or treated, in comparison with the control. However, a noticeable, but not significant increase in body weight or body weight gain of broiler fed diets with 2 or 4% levels of guava by-product regardless of the processing. Moreover, feeding with the higher levels of raw or treated samples 6 to 8% showed slightly reduction of broiler body weight and body weight gain, but not significantly. They also added that this observation could be due to the presence of higher amount of fiber compared to the other treatments.

### 4.2 Effect of guava leaf meal on feed intake

Feed intake of broilers in different dietary treatments during experimental periods was almost statistically similar and the differences were non-significant ($P > 0.05$) (Table 4.6). So, the result clearly showed that guava leaf meal up to 4.5 percent dietary level

had no detrimental effect on feed consumption. The result supported by Abiola and Adekunle (2002). They had found that high fiber diets increased feed intake. If the chickens fed diet include the autoclaved sample and sample treated with alkaline will consume more digestible fiber than those fed the raw or the other ones. Gadalla (1983) arrived to similar finding with autoclaving apricot kernel meal. Abiola *et al.*, (2002) reported that the alkali treatment of melon husk increased the feed intake with increase in the level of alkali treatment of melon husk in the diet.

**Table 4.6 Performance of the broiler chickens fed the experimental diets**

| Parameters | Guava leaf supplementation | | | | Level of significance |
|---|---|---|---|---|---|
| | $T_1$ (0%) Control | $T_2$ (2.5 %) | $T_3$ (3.5 %) | $T_4$ (4.5 %) | |
| Initial body weight (g) | 41.0± 6.4 | 39.9± 6.7 | 42.9± 6.7 | 38.5± 6.25 | NS |
| Final body weight | 2164 ± 55.3 | 2064± 56.0 | 2095 ± 56.5 | 2026± 54.9 | NS |
| Weight gain (g) | 2113 ± 56.2 | 2013 ± 56.6 | 2044 ± 56.5 | 1975 ± 56.4 | NS |
| Feed intake (gm/d) | 3950 ± 57.8 | 3893 ± 57.2 | 4021 ± 57.3 | 3960 ± 57.4 | NS |
| Feed conversion ratio (gm feed / gm gain) | 1.88 ± 0.07 | 1.95 ± 0.05 | 1.95 ± 0.04 | 2.00 ± 0.08 | NS |

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

### 4.3 Effect of guava leaf meal on feed conversion ratio

Feed conversion ratio in different dietary treatments at 2.5, 3.5 and 4.5 percent level was almost similar and the differences were non-significant (P> 0.05) (Table 4.6)).The results indicate that there was no detrimental effect on feed conversion ratio after feeding up to 4.5 percent level of guava leaf meal. Similar result was found by El-Deek *et al.* (2009). They reported that the broiler given diet with 2 or 4% guava by-products utilized their diets more efficiently than those fed on diets with 6 or 8% during the finishing period. However, the processing technique of guava by-products had no significant effect on the Feed Conversion Ratio of the broiler chickens during the finishing period, regardless of the levels of incorporation into there diets. Also, the interaction results between processing technique and levels of incorporation of guava by-products had no significant differences in FCR.

### 4.4 Effect of guava leaf meal on fat content

This study showed that fat content of broiler was decreased significantly by supplementation of guava leaf meal in broiler-ration ($P< 0.05$). It is evident from Table 4.7 that the tendency of reduced fat content was observed in the dietary treatments with inclusion of 2.5-4.5% guava leaf meal. However, the highest level of fat content was 78.0g at control and lowest level was 68.6g at 4.5 percent level of guava leaf meal. Although this result is dissimilar with El-Deek *et al.* (2009), because they had found that the abdominal fat weight showed no significant differences in the relative weight for the broiler received 2, 4 or 6% raw or treated by-products. But, broiler receiving 8% raw or treated guava by-product have significantly less abdominal fat than any other dietary level or the control. Also, dietary treatments had a significant effect on relative abdominal fat weight, regardless to the level of inclusion. The application of Duncan's test indicated that relative weight of abdominal fat of broiler given diets with raw or treated with alkaline or acid were not significantly different while the relative weight of abdominal fat of broiler given boiled sample was significantly higher than the values for other treated diets.

**Table 4.7 Fat content and mortality percentage of the broiler chickens fed the experimental diets**

| Parameters | Guava leaf supplementation | | | | Level of significance |
|---|---|---|---|---|---|
| | $T_1$ (0%) Control | $T_2$ (2.5 %) | $T_3$ (3.5 %) | $T_4$ (4.5 %) | |
| Fat content (g) | $78.0\pm 3.32^b$ | $77.56\pm 1.87^b$ | $72.45\pm 4.08^a$ | $68.6\pm 3.45^a$ | * |
| Mortality (%) | $3.56 \pm 1.66^b$ | $3.00\pm 2.21^b$ | $2.87 \pm 1.08^a$ | $2.50\pm 1.90^a$ | * |

Values are expressed as mean ± standard error of means. a, b Means within row with different superscripts are statistically different ($P <0.05$). * Statistically significant ($P <0.05$). Means represents three replicates, fifteen birds per replication.

### 4.5 Effect of guava leaf meal on mortality rate

In this experiment, the experimental diets produced a decrease mortality rate in comparison to control. The reduced mortality rate was obtained at 4.5% level of guava leaf meal supplementation. The result remarkably differ with the El-Deek *et al.* (2009). They had found that the processing technique of guava leaf had no effect on mortality rate, regardless of the inclusion levels. However, when the percentages of guava by-products inclusion in the diets increased to 6 and 8%, a significant increase in mortality rate was evident, regardless of the processing employed.

### 4.6 Effect of guava leaf meal on carcass quality of broiler

Effects of guava leaf meal on the carcass characteristics of broiler chickens are given in Table 4.8. The data on slaughter of broiler chicks fed experimental diets are represented in g/100g (%) of live weight. No significant ($p < 0.05$) effect was observed for carcass weight and weights of internal organs of broilers fed experimental rations except the digestive tract weight and head weight. Guava leaf meal has an effect on digestive tract weight. Birds of higher body weight gain have the smaller digestive tract. The breast meat weight higher in $T_1$ (16.96±1.740)g and lower in $T_4$ (14.51± 0.573)g. However, higher weight of heart weight, head weight, neck weight and spleen weight were found in control group when compared with broilers in groups fed different level of guava leaf meal. Thigh bone weight and gizzard weight are almost same in $T_1$ and $T_4$ and the values are (1.80-+0.270); (1.80±0.230), (2.96±0.046) and (2.94±0.045) respectively. Average liver weight of different treatment groups are (2.30±0.41), (2.22±0.011), (2.25±0.035), (2.14±0.052) and (2.53±0.227) g/100g of live weight for $T_1$, $T_2$, $T_3$ and $T_4$ respectively. Highest gizzard weight found in both $T_1$ and is $T_4$ 2.95 g/ 100g live weight.

Ekenyem *et al.* (2006) used *Ipomoea asarqblia* leaf meal and found liver weight 2.125 1.900, 1.513, 1.435g and gizzard weight 2.850, 2.100, 1.675 and 1.475 g for 0% IALM, 5% IALM , 10% IALM, 15% IALM, respectively.

### 4.7 Effect of guava leaf meal on dressing percentage of broiler

Dressing percentage of different treatment group is presented in the Table 4.8. The highest dressing percentage (60.42±1.151)g is found in $T_1$ 0% GLM and the lower value (57.00±0.736)g found in $T_4$ at 4.5% GLM. Ekenyeni *et al.* (2006) used *Ipomoea asarqblia* leaf meal (IALM) and found dressing percentage 63.63, 63.41, 63.3and 62.30g using 0% IALM, 5% IALM, 10% IALM, and 15% IALM, respectively.

**Table 4.8 Effect of guava leaf on carcass quality of broiler**

| Slaughtering data | $T_1$ Mean ± SE | $T_2$ Mean ± SE | $T_3$ Mean ± SE | $T_4$ Mean ± SE | P value | Significant |
|---|---|---|---|---|---|---|
| Breast weight | 16.96±1.740 | 16.20±0.839 | 15.52±1.336 | 14.51± 0.573 | 0.306 | NS |
| Thigh bone weight | 1.80±0.270 | 1.56±0.026 | 1.49±0.226 | 1.80±0.230 | 0.578 | NS |
| Thigh meat weight | 8.64±0.016 | 8.22±0.241 | 8.46±0.785 | 8.17±0.075 | 0.358 | NS |
| Drumstick meat weight | 2.80±0.577 | 3.34±1.062 | 2.19±0.045 | 2.62±0.033 | 0.723 | NS |
| Skin weight | 7.05±0.521 | 7.18±0.455 | 7.41±0.560 | 6.88±0.288 | 0.942 | NS |
| Abdominal fat weight | 1.56±0.477 | 1.35±0.003 | 1.98±0.052 | 2.08±0.084 | 0.361 | NS |
| Digestive tract weight | $8.35^{b}$±0.214 | $7.56^{a}$±0.018 | $7.41^{a}$±0.051 | $9.54^{c}$±0.049 | 0.015 | * |
| liver weight | 2.30±0.41 | 2.22±0.011 | 2.25±0.035 | 2.14±0.052 | 0.452 | NS |
| Gizzard weight | 2.96±0.046 | 2.81±0.002 | 2.85±0.021 | 2.94±0.045 | 0.310 | NS |
| Shank weight | 4.92±0.145 | 4.62±0.321 | 4.65±0.086 | 4.03±0.032 | 0.548 | NS |
| Heart weight | 0.64±0.125 | 0.56±0.035 | 0.62±0.006 | 0.49±0.044 | 0.451 | NS |
| Head weight | $0.64^{b}$±0.075 | $0.56^{b}$±0.058 | $0.63^{b}$±0.002 | $0.48^{a}$±0.014 | 0.007 | ** |
| Neck weight | 3.09±0.055 | 2.55±0.123 | 2.93±0.152 | 3.03±0.048 | 0.249 | NS |
| Spleen weight | 0.27±0.060 | 0.14±0.005 | 0.19±0.001 | 0.12±0.000 | 0.157 | NS |
| Wing weight | 6.94±0.080 | 6.69±0.075 | 6.82±0.468 | 6.97±0.141 | 0.668 | NS |
| Dressing% | $60.42^{b}$±1.151 | $58.51^{c}$±0.094 | $58.00^{b}$±1.390 | $57.00^{b}$±0.736 | 0.024 | * |

Here, $T_1$ indicates broiler without guava leaf; $T_2$ indicates broiler fed with 2.5% guava leaf; T3 indicates broiler fed with 3.5% guava leaf; T4 indicates broiler fed with 4.5% guava leaf

* indicates significant; when $p<0.01$ at 5% level of significance ** indicates more significant; when $p<0.01$ at 1% level of significance.
NS indicates non-significant; the mean value duffers significantly ($p<0.05$). SE = Standard Error

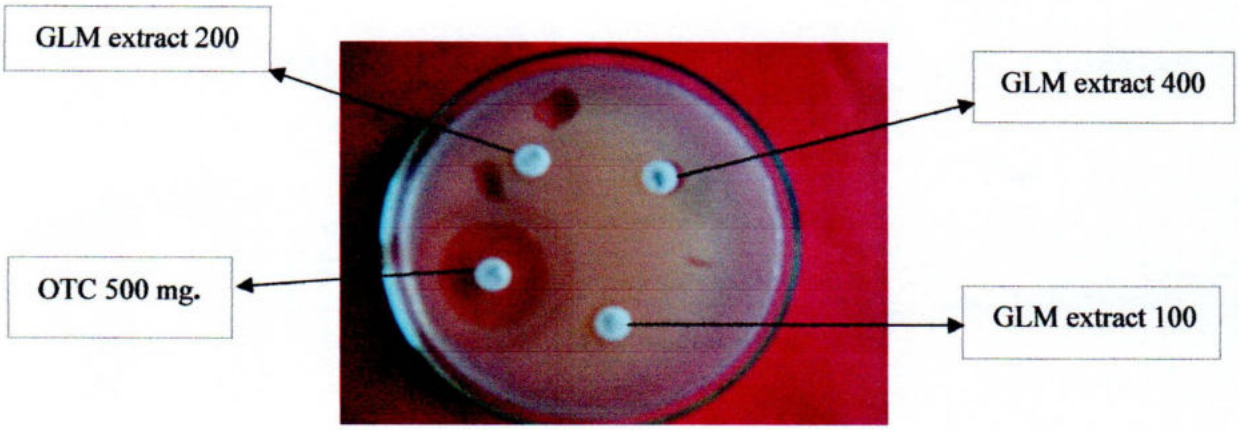
### 4.8 Antimicrobial activity of guava leaf

Two hundred and fifty grams of the dried powder leaves of guava was exhaustively extracted with 1.5 L of distilled water in a reflux apparatus and then concentrated to yield 70.8 g of the crude extracted that is 28.3% w/w with respect to the dried powdered extract. Three different bacterial organisms were isolated from the 60 swabs taken from the navel of broilers day-old-chicks. The isolated organisms include; *Staphylococcus* sp., *E. coli* *Streptococcus* sp. All these three organisms were isolated from broiler day-old-chicks while only four of the organisms (*Staphylococcus* sp., *Streptococcus* sp., *E. coli*.) were isolated from the broiler day-old-chicks (Table 4.9). The effect of the three different concentrations of the extract on the bacteria isolated is presented in Table 10. The extract showed concentration dependent antibacterial activity against *E. coli*, *Streptococcus* sp., *Staphylococcus* sp.

**Table 4.9 Bacterial organisms isolated from the navels of day-old-chicks**

| Bacterial isolates | Broilers |
|---|---|
| *E. coli* | + |
| *Streptococcus* sp | + |
| *Staphylococcus* sp | + |

+:= Presence,




Antibiotic sensitivity

Figure 12: Legends: GLM extract 400, GLM extract 200 and GLM extract 100, OTC 500 mg

**Table 4.10 Antibacterial activity of guava aqueous leaf on organisms isolated from the navel of day-old chicks**

| Bacterial isolates | Concentration of the Extract(mg $ml^{-1}$) | Zones of Inhibition (mm) |
|---|---|---|
| *E. coli* | 400 | 25 |
| | 200 | 18 |
| | 100 | 16 |
| *Streptococcus* sp | 400 | 20 |
| | 200 | 16 |
| | 100 | 13 |
| *Staphylococcus* sp | 400 | 25 |
| | 200 | 20 |
| | 100 | 18 |

**Table 4.11 The minimum inhibition concentration of guava aqueous leaf extract against some of the isolated bacteria**

| | Concentration of the Extract (mg $ml^{-1}$) | | | | |
|---|---|---|---|---|---|
| Bacterial isolates | 50 | 25 | 15.5 | 6.25 | 3.13 |
| *E. coli* | -ve | -ve | -ve | +ve | +ve |
| *Streptococcus* sp | -ve | -ve | +ve | +ve | +ve |
| *Staphylococcus* sp | -ve | -ve | +ve | +ve | +ve |

+ve= with bacterial growth, -ve = without bacterial growth.

A study of the antibacterial effect of guava aqueous leaf extract on bacterial organisms isolated from the navel of day old chicks was carried out. The result of the study showed that guava leaf extract have concentration dependant inhibitory effect on the growth of *E. coli*, *Staphylococcus* sp., *Streptococcus* sp. isolated from the navel of day-old chicks (Table-4.12). Similar results on growth inhibition were obtained by Gnan and Demello (1999), when testing the effect of the extract on *Staphylococcus aureus* by using guava leaf water extract. Iwu (1993) reported antibacterial effect guava leaf extract against *E. coli*, *Staphylococcus aureus, Streptococcus.* All the bacteria inhibited by the leaf extract have been incriminated in omphalitis as shown by Jordon and Pattison (1999).

The susceptibility test of the extract (400 mg $mL^{-1}$) against most of the organisms screened indicated that *E. coli* exhibited the highest inhibition zone of 25 mm which could be compared favourably with 30 mm of Oxytetracycline (20 mg $mL^{-1}$). The activity of the extract against *E. coli* is important since many avian pathogenic *E. coli* strains have been reported to be resistant to common antibacterial agents used in poultry production (Ewers *et al.*, 2003).The minimum inhibitory concentration against the susceptible organisms indicated that *E. coli.* had the lowest, suggesting that the extract can be a potential antibacterial agent if the active compound responsible is isolated.

Phytochemical evaluation of the leaf has shown the presence of flavonoids, tannins, saponins, Phenols lectins, triterpenes and carotenoids (Geidam *et al.*, 2007). These compounds are known to be biologically active. The antimicrobial activity of the leaf extracts demonstrated can be attributed to the presence of flavonoids (Ali and Shamsuzzaman, 1996). Similarly, Berdy *et al.* (1981) demonstrated that the antibacterial effect could also be due to guajaverine and psydiolic acid, which are also present in the leaf. Flavonoids derivatives have been found to inhibit the growth of *Staphylococcus*

*aureus* at the dilution of 1: 10,000 (Ali *et al.*, 1996). This is medically important in the treatment of inflamed tissues and lectins in guava were shown to bind to *E. coli* preventing its adhesion to the intestinal wall and thus preventing infection (Berdy *et al.*, 1981). Therefore, the activity of the extract against the isolated organisms in this study could be linked to the aforementioned reports. These effects can explain the long history of guava use in traditional medicine as a cure for many bacterial diseases. In conclusion, this study has provided a basis for the use of *Psidium guajava* in the treatment of yolk sac infection caused by *E. coli*, *Staphylococcus* sp. and *Streptococcus* and either primarily or in combination. However, it is necessary to further investigate the *in vivo* antibacterial activities of the extracts in chicks.

# CHAPTER V

# SUMMARY AND CONCLUSION

# CHAPTER V

## SUMMARY AND CONCLUSION

The objectives of this experiment were to evaluate the varying doses of guava leaves supplemented diets on broiler chicks. The feeding value of guava leaf meal and antibacterial effect of their acetone extract on broiler (Cobb 500) was evaluated in the poultry shed , Hajee Mohammad Danesh Science and Technology University, Dinajpur. In feeding trial, four (4) diets were prepared including guava leaf meal at levels of 0% (control), 2.5%, 3.5% and 4.5%. Body weight gain and feed consumption were recorded weekly basis. By the end of each treatment, lives weight. Body weight gain, feed intake, feed consumption and fat content were recorded. Mortality rate was also recorded throughout the study.

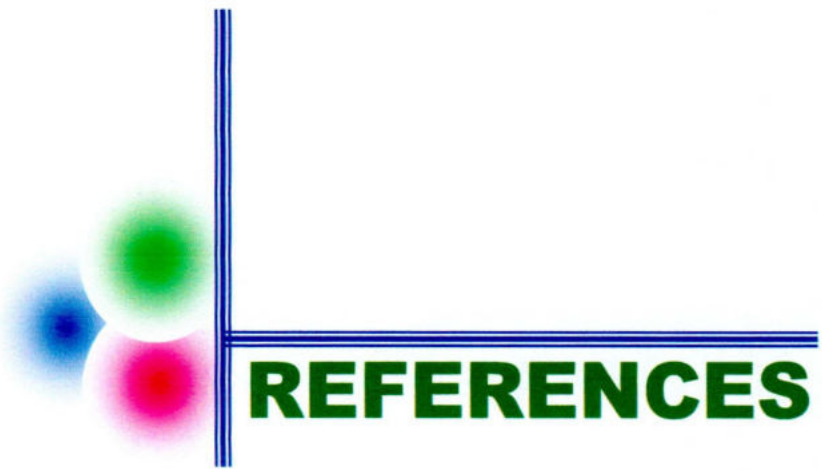
By using experimental diets feed intake, body weight gain and feed conversion ratio of different dietary treatments were almost similar and the differences were statistically non-significant (showed in Table 4.6) except fat content, mortality rate and antimicrobial sensitivity. Fat content and mortality rate were decreased with increased level of guava leaf meal up to 4.5% level (showed in Table 4.7) which I investigated. Antimicrobial sensitivity was significant at 5 % level of significance (showed Table 4.10 and 4.11).

Feed 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 feed intake (g/day) was maximum at 3.5% level of guava leaf meal (4021g) followed by (3950 g) in control group, (3893 g) at 2.5% level and (3960 g) at 4.5% level. In all test groups feed consumption was almost similar to control group (3950 g). Data obtained on final average body weight indicated that there was no positive correlation between body weight and feed consumption. Feed conservation ratio (FCR) was the highest at 4.5% level of guava leaf meal (2.00) compared with other group. The FCR values were found to be almost the same with diet at 0% (1.88), 2.5% (1.95), 3.5% (1.95) and 4.5 %( 2.00) guava leaf meal.

Mortality rate was decreased after inclusion of guava leaf meal in broiler diet. In this experiment lowest mortality (2.50%) was observed at 4.5% level of guava leaf meal in comparison to others groups. Mortality rate was (3.56%) in control group, (3.00%) at

2.5% level and (2.87%) at 3.5% level of guava leaf meal. Fat content was also reduced due to inclusion of guava leaf meal .The highest fat content was observed in control group (78.0g) and the lowest level at 4.5% level of guava leaf meal (68.6g).

Guava leaf meal have not any remarkable effect on the carcass characteristics of broiler chickens. Based on the results of present study it may be concluded that guava leaf is a good source of nutrients and it has significant effect on fat content of broiler, mortality rate and antimicrobial sensitivity without affecting the bird's feed intake, body weight and feed conversion ratio. The results of the study suggest that supplementation of guava leaf meal (*Psidium guajava*) up to my investigation level at 4.5% in diets has high potential as commercial applications for production performance of broiler. Therefore, guava 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 antimicrobial sensitivity and other beneficial effects of guava leaf meal observed in this experiment prior to practical use it as unconventional feed of poultry.



# REFERENCES

## REFERENCES


A. O. A. C. 1990. Association of official analytical chemists "Official Methods of Analysis" 15th Ed. Published by the AOAC, Washington, D.C.

Abiola, S.S. and Adekunle, A.O. 2002. Nutritive value of melon husk in the diet of chickens. Biores Tech., 81(3):265-267.

Abiola, S.S., Amalime, A.C. and Adekunle, A.O. 2002. The utilization of alkali-treated melon husk by broilers. Biores. Tech., 84(3):247-450.

Adedayo, O., Anderson, W.A., Young, M., Snieckus, V., Patil, P.A. and Kolawole, D.O. 2001. Phytochemistry and antibacterial activity of Senna alata flower. J. Pharm. Biol., 39: 408-412

Aibinu, I., Adenipekun T., Adelowotan T., Ogunsanya T. and Odugbemi T. 2007. Evaluation of the antimicrobial properties of different parts of Citrus aurantifolia (lime fruit) as used locally. Afr. J. Trad. CAM., 4: 185-190.

Ali, A.M. and Shamsuzzaman, M. 1996. Isolation and characterization of antibacterial constituents from the bark of *Psidium guajava*. Bangladesh J. Sci. Ind. Res., 31: 133-140.

Ali, A.M., Shamsuzzaman, M., Rahman, H.M., and Hoque, M.M. 1996. Screening of different solvent extracts of the bark of Psidium guajava for antibacterial activity. Bangladesh J. Sci. Ind. Res., 31: 159-165.

Ali, A.M., Shamsuzzaman, M. 1996. Isolation and characterization of antibacterial constituents from the bark of *Psidium guajava*. Bangladesh J. Sci. Ind. Res. 31(2): 133-139.

Ali, M.A., Shamsuzzaman, M; Rahman, H.M.; Hoque, M.M. 1996. Screening of different solvent extracts of the bark of *Psidium guajava* for antibacterial activity. Bangladesh J. Sci. Ind. Res. 31(1): 159-165.

Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Aly, A. M., 1981. Studies on the unsoponifiable matter of some vegetable oils. M. Sc. Thesis, Faculty of Agri., Minufiae University, Egypt.

Arima, H. and Danno, G. 2002. Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. Bioscience, Biotech. and Biochem., pp. 1727-1730.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Berdy, J., A. Aszalos, M. Bostian, and Mcntt, K.L. 1981. Handbook of Antibacterial Compounds. CRC Press, Bocaratom.

Burkill, H.M. 1997. The useful plants of West Tropical Africa. Edition 2. Vol. 4. Families M-R. Royal Botanic Gardens Kew. ISBN No.1-900347-13-X.

Chen, Kuan-Chou; Hsieh, Chiu-Lan; Peng, Chiung-Chi; Hsieh-Li, Hsiu-Mei; Chiang, Han-Sun; Huang, Kuan-Dar and Peng, Robert, Y. 2007: Brain derived metastatic prostate cancer DU-145 cells are effectively inhibited in vitro by guava (*Psidium guajava* ) leaf extracts. 58(1): 93–106.

Dutta, B. K., Imtiaz Rahman and Das, T. K. 2000. In vitro study on antifungal property of common fruit plants. Biomedicine, pp. 187-189.

El-Deek A. A., Asar, M. A., S. M. Hamdy, and Abdalla. A. A. 2009. Utilization of guava by- products in broiler finisher diets. Poultry Prod. Dept., Fac. of Agric. Alexandria University, Alex. Egypt. Poult. Sci. Vol. 29 (I): 53-75.

Ewers, C., Janssen T. and Wieler L.H. 2003. Avian Pathogenic E. coli (APEC): A review. Berl Muuch Tierarztl Wochenschr, 116: 381-395.

Gadalla, A. 1983. Improved nutrient utilization from a price kernel subjected to some dietary treatments. M.Sc. These Alex. University.

García, M., Lázaro, R, Latorre, M.A., Gracia, M.I., and Mateos, G.G. 2008. Influence of enzyme supplementation and heat processing of barley on digestive traits and productive performance of broilers. Poult. Sci. 87(5):940-948.

Geidam, Y.A., Ambali, A.G. and Onyeyili, P.A. 2007. Phytochemical screening and antibacterial properties of organic solvent fractions of *Psidium guajava* aqueous leaf extracts. Int. J. Pharm., 3: 68-73.

Gnan, S.O. and Demello, M.T. 1999. Inhibition of *Staphylococcus aureus* by aqueous Goiaba extracts. J. Ethnopharmacol., 68: 103-108

González-Alvarado J.M., Jiménez-Moreno E., Lázaro R., and Mateos, G.G. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci. 86(8):1705-1715.

Grabley, S. and Thiericke, R.1999. Drug Discovery from Nature. 1st Ed., Springer, London, ISBN: 978-3-540-64844-4, pp: 5-7.

Greenwood, D. 1989. Antimicrobial Sensitivity Testing. In: Antimicrobial Chemotherapy, Greenwood, D. (Ed.). Oxford University Press, New York, USA, pp: 91-100.

Gutiérrez, R.M.; Mitchell, S. and Solis, R.V. 2008. *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. J. Ethnophar., 117(1): 1–27.

Hassimotto, N.M.; Genovese, M.I. and Lajolo, F.M. 2005. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. J. Ag. and Food Chem. 53(8): 2928–2935.

Hisae Yoshitomi, Xiangyu Guo, Tonghua Liu and Ming Gao 2012. Guava leaf extracts alleviate fatty liver via expression of adiponectin receptors in SHRSP.Z-Leprfa/Izm rats, Nutrition & Metabolism 2012(9):13.

Iwu, M.M. 1993. Handbook of African Medicinal Plants. 1st Ed., CRC Press, Boca Raton, FL., ISBN-10: 084934266X, pp: 464.

Jaiarj, P., P. Khoohaswan, Y. Wongkrajang, P. Peungvicha, P. Suriyawong, M.L. Saraya and Ruangsomboom, O. 1999. Anticough and antimicrobial activity of *Psidium guajava* leaf extract. J. Ethnophar., 67: 203-212.

Jiménez-Escrig, A., Rincón, M., Pulido, R. and Saura-Calixto, F. (2001): Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. J. Ag. and Food Chem. 49(11): 5489–5493.

Jordan, F.T.W. and Pattison, M. 1999. Yolk Sac Infection: Poultry Diseases. 4th Ed., WB Saunders Co. Ltd., London, pp: 42-44.

Jordan, M.J., Margaria, C.A., Shaw, P.E. and Goodner, K.I. 2003. Volatile components and aroma active compounds in aqueous essence and fresh pink guava fruit puree (*Psidium guajava* ) by GC-MS and multidimensional GC/GC-O. J. Agri. Food Chem., 51: 1421-1426.

Kaljee, Linda M., Thiem, Vu Dinh, von Seidlein, Lorenz; Genberg, Becky L.; Canh, Do Gia; Tho, Le Huu; Minh, Truong Tan; Thoa, Le Thi Kim; Clemens, John D. & Trach, Dang Duc. 2004: Healthcare Use for Diarrhoea and Dysentery in Actual and Hypothetical Cases, Nha Trang, Viet Nam. J. Heal. Pop. and Nut. 22(2): 139-149.

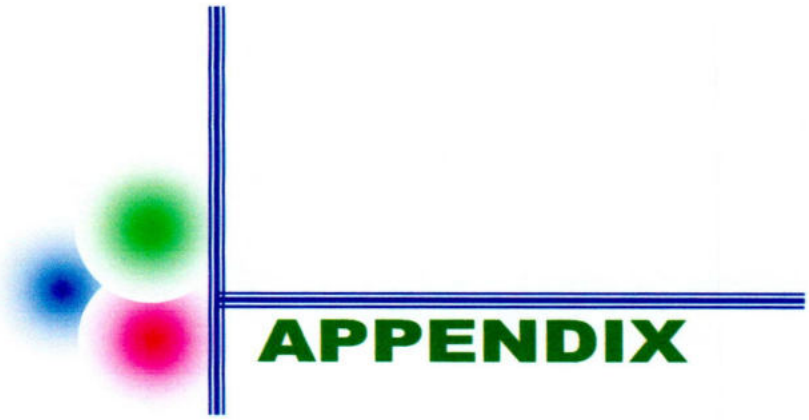
Koteeswaran, A., S. Manoharan, Chandramohan A. and Chandran, N.D.J. 2004. Studies on physical and microbiological qualities of day old chicks. Cheiron, 33: pp. 63-68.

Mahattanatawee, K.; Manthey, J.A.; Luzio, G.; Talcott, S.T.; Goodner, K. and Baldwin, E.A. 2006: Total antioxidant activity and fiber content of select Florida-grown tropical fruits. J. Ag. and Food Chem. 54(19): 7355–7363.

Mahfuzul Hoque, M.D.; Bari, M.L.; Inatsu, Y.; Juneja, V.K. and Kawamoto, S. 2007: Antibacterial activity of guava (*Psidium guajava* ) and Neem (*Azadirachta indica* A. Juss.) extracts against food borne pathogens and spoilage bacteria. Food born Pathogens and Disease 4(4): 481–488.

Manosroi, J., Dhumtanom, P. and Manosroi, A. 2006. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Letters* 235(1): 114–120.

Meijerhof, R., 2005. Counts for chicks quality. Hybro B.V P.O. Box 30, 5830 AA, Boxmeer, Netherlands.


APPENDIX

# APPENDICES

**Appendix I : Composition of different feed ingredient**

| Ingredients | DM | ME | CP | CF | EE | NFE | ASH | Ca | p | Lys | Meth | Cst-M | Tryp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maize | 89.5 | 3309 | 9.2 | 2.4 | 2.6 | 82.8 | 1.9 | 0.07 | 0.4 | 0.25 | 0.13 | 0.4 | 0.09 |
| Soybean meal | 89.9 | 2240 | 45 | 6 | 2.9 | 25 | 7.30.32 | 0.67 | 2.9 | 0.65 | 1.6 | 0.6 | |
| Rice polish | 91.8 | 2937 | 11.9 | 12.4 | 12.7 | 48.6 | 4.6 | 0.35 | 1.2 | 0.4 | 0.39 | 0.4 | 0.19 |
| Meat & bone meal | 91.5 | 2111 | 53.8 | 2.3 | 8 | | 18 | 11.3 | 5.39 | 3.72 | 0.75 | 0.64 | 0.25 |
| DCP | 98 | | | | | | | 24.3 | 18.2 | | | | |
| Soybean oil | 100 | 8950 | | | 100 | | | | | | | | |
| Guava leaves | 94.1 | 2226 | 9.08 | 39.5 | 10.00 | 32.97 | 2.55 | 0.38 | 0.10 | | | | |

Accumulated from-

1. The encyclopedia of Farm Animal Nutrition Editor-in-cheif M. F. Fuller, Rowett Research Institute, Aberdeen, UK.

2. A Text Book of Animal Husbanry by G.C. Banergee.