**Chapter 1**

**Introduction**

Bangladesh is basically an agricultural country and about 64.96% of its people live in villages (World Bank collection of development indicators, 2016). Their livelihood is dependent mainly on agriculture and animal husbandry.Bangladesh is a small and developing country overloaded with almost unbearable pressure of human population. In the past, people of Bangladesh were mostly dependent upon land-based proteins. But, the continuous process of industrialization and urbanization consumes the limited land area. Now there is no other way than to depend on animal protein, which can meet the country’s demand. Dairy and poultry farming are the important sectors which provide a large share to the increasing demand for animal protein, cash income and employment opportunities. However, the high price and non-availability of feed ingredients are two major constraints to the growth of commercial livestock enterprises. In Bangladesh, feed cost alone accounts 60-70% of the total production cost (Bulbul and Hossain, 1989). The high and increasing prices for animal feeds have compelled researchers in developing countries to direct their attention to non-conventional feeds, with particular emphasis on protein substitutes ([Gaia](http://www.blogger.com/profile/05732059725931518932), 2005). Now, it is necessary to search and increase the nutrient utilization of crop residues as well as nonconventional feeds.

The non-conventional feed resources (NCFR) refer to all those feeds that have not been traditionally used in animal feeding and or are not normally used in commercially produced rations for livestock. NCFR include commonly, a variety of feeds from perennial crops and feeds of animal and industrial origin. There are serious shortages in animal feeds of the conventional type. The grains are required almost exclusively for human consumption. With increasing demand for livestock products as a result of rapid growth in the world economies and shrinking land area, future hopes of feeding the animals and safeguarding their food security will depend on the better utilization of unconventional feed resources which do not compete with human food. The availability of feed resources and their rational utilization for livestock represents possibly the most compelling task facing planners and animal scientists in the world. The situation is acute in numerous developing countries where chronic annual feed deficits and increasing animal populations are common, thus making the problem a continuing saga. Thus non-conventional feeds could partly fill the gap in the feed supply, decrease competition for food between humans and animals, reduce feed cost, and contribute to self-sufficiency in nutrients from locally available feed sources. It is therefore imperative to examine for cheaper non-conventional feed resources that can improve intake and digestibility of low quality forages. There are certain unconventional feed resources which can be effectively used as feed for poultry and livestock.

The current study was conducted to find out the nutrient content of olive and carambola leaves for poultry and livestock diet. The specific objectives of the present study were-

1. To ascertain the nutrient status of olive and carambola leaves in order to use in poultry and livestock diet
2. To examine for cheaper non-conventional feed resources that can be used as plant protein instead of high cost animal protein

**Chapter 2**

**Materials and method**

* 1. **Study area**

The study was carried out in khulshi, Chittagong. The study area has a latitude of 22°21'N, longitude 91°49'E and elevation of 29 meter. The area is fairly hot with annual average temperature of 25.1°C. The variation of daily average temperature is 8.8°C. Mean monthly temperature has a variation of 9°C the hottest month is May having a mean temperature of 28°C. The coolest month is January which has a mean temperature of 19°C. The average annual relative humidity of the area is 73.7% and average monthly relative humidity ranges from 58% in January to 86% in August. The area has an average of 2735 mm rainfall per year. There are 135 days per year with more than 0.1 mm of rainfall. The driest weather is in January when an average of 6 mm of rainfall. The wettest weather is in July when there occurs an average of 598 mm of rainfall. The longest day of the year is 13:22 hour long and the shortest day is 10:37 hour long. The current study was carried out during October to November 2017.

**2.2. Collection of plant material**

The leaves of Averrhoa carambola, and *Olea europaea* were collected during October-November 2017 from Chittagong district, Bangladesh. Approximately 500 grams of each type fresh green leaves were collected from the tree.Samples were wrapped up by polythene bag and preserved in the laboratory for chemical analysis.

**2.3. Preparation of sample**

The collected leaves were thoroughly washed and dried in the sun. Dried samples were chopped and subjected to grinding to make it homogenous powder.

**2.4. Analysis of sample**

Chemical analyses of the samples were carried out in triplicate for moisture, dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extracts (EE) and total ash in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh as per AOAC (2000).

**Determination of Moisture**

Moisture percentage was determined after determination of DM (dry matter). The enamel disc or crucible was dried in an oven regulated at 105°C which was cooled in a desiccators and weighted. 5gm of sample was weighted into the enamel disc and kept into the oven (105°C) for 24 hours. The enamel disc was removed from the oven with metal tong. After that it was cooled in desiccator and the final weight was taken after getting constant weight AOAC, (2000).

% Moisture = 100 - % DM

**Determination of Ash**

The crucible was cleaned & dried in hot air oven. Than it was cooled in desiccator and weighted. 5 grams of sample was placed there and the sample was burned upto no smoke in heater. The crucible with sample was cooled and transferred to the muffle furnace. Then the sample was ignited at 550-600°C for 6-8 hours until white ash. The furnace was cooled at 150°C & the sample was transferred to desiccators and weighted AOAC, (2000).

**Determination of Crude fiber (CF)**

Two gram sample was weighted and taken into a beaker. 125ml of 1.25% H2SO4 was added into the beaker. Than it wasfitted in condenser and placed on heater**.** The beaker was boiled for 30 minutes and removed from heater. After that it wascooled and filtered through filtering cloth. The sample was washed until it was free from acid. Residue of sample was transferred into same beaker. 125ml of 1.25% NaOH was added there and again fitted in condenser and placed on heater. It was boiled for 30 minutes and removed from heater which was cooled and filtered through filtering cloth. The sample was washed until it was free from alkali. Then residue of sample was transferred in a previously weighted crucible. The crucible was put into the muffle furnace & ignited at 600°C temp. for 5 hours. Then it was weighted after cooling.

**Determination of Crude protein (CP)**

0.5 gram sample was weighted and one spoonful catalyzer mixture (KOH, NaOH, Se) was added there.10ml Conc. H2SO4 was added and the digestion flask was placed in Kjeldhal Digestion Set. After that heat was increased gradually & continued upto clear residue (45 min-1hr). The Flask was removed & cooled.10ml 2%Boric Acid solution and 2 drops mixed indicator was taken in a conical flask. The conical flask was fitted in the collection arm of distillation set. 50ml distilled H2O was added in the digestion tube and fitted in the distillation flask. 40ml of 40%NaOH was added there & the distillation was continued upto 100ml of distillate. The Distillate was titrated against 0.1N HCl. Titration was continued until the color changed into pink. Then the Titration volume was calculated AOAC, (2000).

**Determination of Ether extracts (EE)**

One gram dry sample was taken in an extraction thimble having porosity then placed in the soxhlet flask. The cork of thimble was above the siphon tube. A receiving flask was weighted and fitted with soxhlet apparatus and was placed in water bath(50-60°C). Ether Extract was poured down into the soxhlet flask. The flask under soxhlet was full upto 3/4th portion with ether and was sured that water was running through the condenser. When extraction was over, the thimble with sample was removed and heated in the water bath toremove allthe ether from receiving flask. The receiving flask was placed into the oven (105°C) to eliminate left of the ether and water. After drying, the flask was taken out and weighted AOAC, (2000).

**Calculation of Nitrogen free extracts (NFE)**

The NFE content was calculated by deducting the sum of the values for moisture, crude protein, crude fat, crude fibre and total mineral matter in 100 (Raghuramulu *et al*., 1983).

**2.5. Calculation of ME**

All samples were subjected to proximate analysis in triplicate. Later on, metabolizable energy (ME) available in the leaf samples was calculated by using a standard mathematical formula as ME (kcal/kg) = 32·95 (% crude protein + % ether extract × 2·25 + % available carbohydrate)-29·20 as per (Lodhi *et al*., 1976).

**2.6. Statistical analyses**

All the collected data were subjected to statistical analyses by using one way ANOVA (Minitab version16, 2000). The significance of difference between means was determined by Fisher’s least significant difference at *P≤ 0.05*.

The flow chart of proximate analysis is shown below-

**FEED SAMPLE**

HEAT AT 105˚C FOR 24 HOURS OR UNTIL CONSTANT WEIGHT

LOSS OF WEIGHT

MOISTURE

KJELDAHL METHOD

ACID BOILING FOR 30 MINUTES (1.25% H2SO4, SOLUTION)

**DM**

ETHER EXTRACTION

FILTER

%N2

%CP=%N2×6.25

**EE**

**FILTRATE**

**RESIDUE**

ALKALI BOILING FOR 30 MINUTES (1.25% NaOH SOLUTION)

**FILTRATE**

**RESIDUE**

(CF+ASH+H2O)

HEAT AT 105˚C FOR 24 HOURS

**CF+ASH**

IGNITE AT 600˚C FOR 5 HOURS

LOSS OF WEIGHT

**ASH**

**CF**

**Chapter 3**

**Results**

**Chemical composition of the carambola andolive leaves collected from different areas**

 The results of the chemical composition of carambola and olive leave samples *i.e*dry matter (DM%), crude protein (CP%), crude fiber (CF%), nitrogen free extracts (NFE%), ether extracts (EE%) and total ash contents (TA%) are shown below in Table 1.

**Table 1:** Chemical composition (DM, CP, CF, EE, NFE and TA)% of carambola (T1) and olive leaves (T2)

|  |  |
| --- | --- |
| **Treatment**  | **Chemical composition (%)** |
| **DM** | **CP** | **CF** | **EE** | **NFE** | **TA** |
| T1 | 89.02b | 12.54a | 14.76b | 2.02a | 52.24a | 7.27a |
| T2 | 92.78a | 10.44b | 35.94a | 1.60b | 41.40b | 3.45b |
| SEM | 0.063 | 0.165 | 0.124 | 0.041 | 0.160 | 0.039 |
| P- value | 0.001 | 0.003 | 0.001 | 0.001 | 0.001 | 0.001 |

[Data refer to mean values of two treatment consisting of three replicates; T1= Carambola leaves; T2= Olive leaves; a,bMeans bearing uncommon superscripts within a column is significantly different at the level cited in the Table; DM=Dry matter; CP=Crude protein; CF=Crude fiber; NFE=Nitrogen free extract; TA=Total ash; SEM=Standard error of the mean]

**3.1. Dry matter**

The result of dry matter content of two treatments (T1 and T2) was 89.02 % and 92.78%, respectively (Table 1). The data showed that the DM% of T2 was significantly better (P<0.001) than that of T1. From the result it was observed that the moisture content of the two treatmentswas 11% and 7.2%, respectively.

**3.2. Crude protein**

The result of crude protein content of two treatments (T1 and T2) was 12.54% and 10.44%, respectively (Table 1). The data showed that the CP% of T1 was significantly better (P<0.01) than that of T2.

**3.3. Crude fiber**

The result of crude fiber content of two treatments (T1 and T2) was 14.76% and 35.94%, respectively (Table 1). The data showed that the CF% of T2 was significantly better (P<0.001) than that of T1. Dairy ration includes 60% roughage and 40% concentrate. So, these samples are a good source of roughage for dairy cattle. Poultry can digest least amount of fiberin their caeca (Grower: 2-5% and layer: 5-8%). So, these dried leaf powder can be used in certain amount in poultry diet.

**3.4. Ether extract**

The result ofether extract content of two treatments (T1 and T2) was 2.02% and 1.60%, respectively (Table 1). The data showed that the EE% of T1 was significantly better (P<0.001) than that of T2.

**3.5 Nitrogen free extract**

The nitrogen free extractcontent of two treatments (T1 and T2) was 52.24% and 41.40%, respectively (Table 1).The data showed that the NEE% of T1 was significantly better (P<0.001) than that of T2.

**3.6. Total ash**

The total ash content of two treatments (T1 and T2) was 7.27% and 3.45%, respectively (Table 1). The data showed that the TA% of T1 was significantly better (P<0.001) than that of T2. Further study should be done in order to know the specific minerals present in the ash content.

**3.7. Metabolizable energy content (ME%) of the carambola and olive leaves**

The result of ME values of two treatment are shown below in graph (Figure 1).

 Figure 1 : ME value of carambola (T1) and olive (T2) leaves; Bar with different letter is significantly different between treatment (P<0.001).

**Chapter 4**

**Discussion**

The main reason for the poor animal production is the inadequate supply and low level of feeding due to serious shortage of feedstuffs. A major gap exists between the requirements and supplies of nutrients for feeding of animal, the non-conventional feeds could partly fill this gap. We need to know more information on chemical composition, nutritive value and their utilization for their better utilization in livestock ration.  Farmers are not aware of the nutritive value of some feed sources and the way for their efficient   integration in livestock feeding. The involvement of local extension agencies in technology development for efficient use of NCFR, assessment and transfer is equally important. Several factors may account for their limited use, among which is their low nutritive value, seasonal availability, high cost of handling and transportation from the production site to the farm, presence of anti-nutritional factors. It is essential to increase feeds by growing more fodders, propagating agro and social forestry, improving the nutritive value of crop residues and utilizing other NCFRs. Crop residues, AIBPs and browse foliage are certain an increasingly important role as feeds in the future, as human and livestock populations expand.

Olive trees are rich in phenolic substances with having significant biological properties, the most important of which is oleuropein. Whilst it was discovered in 1908 by Bourquelot and Vintilesco, the exact structure of this compound could only be determined in 1960 ([Pannizzi](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b24-ajas-28-4-538) *[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b24-ajas-28-4-538)*[.,1960)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b24-ajas-28-4-538). Oleuropein is the heterosidic ester of elenolic acid and hydroxytyrosol ([Bouaziz](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b8-ajas-28-4-538) *[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b8-ajas-28-4-538)*[., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b8-ajas-28-4-538)). Although oleuropein is found in all tissues and parts of olive trees—and can be present in olive pulp, olive oil, and the wastes (alperujo) generated during olive oil production—the most important natural source of this compound is the olive leaf ([Soler-Rivas](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b28-ajas-28-4-538) *[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b28-ajas-28-4-538)*[., 2000](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b28-ajas-28-4-538); [Gikas](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b17-ajas-28-4-538) *[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b17-ajas-28-4-538)*[., 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b17-ajas-28-4-538)). In previous studies, oleuropein content was determined as 0.005% to 2.0% in olive oil; 0.87% in alperujo; and 1.0 to 14.0% in olive leaves ([Priego-Capote](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b26-ajas-28-4-538) *[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b26-ajas-28-4-538)*[., 2004](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b26-ajas-28-4-538); [Beauchamp *et al*., 2005](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/#b4-ajas-28-4-538)). Studies on olive leaf demonstrated that it includes some medical compounds having antihypertensive, antiatherogenic, cardioprotective, hypocholesterolemic, hypoglycemic, antimicrobial, antiviral, antitumor, anti-inflammatory and antioxidant properties ([Visioli](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b31-ajas-28-4-538) *[et al](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b31-ajas-28-4-538)*[., 2002](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b31-ajas-28-4-538); [Botsoglou](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b6-ajas-28-4-538) *[et al.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b6-ajas-28-4-538)*[, 2013](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4341103/%22%20%5Cl%20%22b6-ajas-28-4-538)).

There is very limited information available about the nutritional value of *A. carambola* (Kamranga leaves) regarding dietary supplementation in livestock or poultry. However, kamrangaplant is used in the folk medicinal system of Bangladesh; the leaves and fruits of A. carambola are used for treatment of diabetes, colic and fever. *Averrhoa carambola* is fully packed with vital nutrients. It is a very good source of natural antioxidants like L-ascorbic acid, (-) epicatechin and gallic acid in gallotannin forms. Consuming 108 g of this fruit can provide, 33 Kcal calories, 1.12 g proteins, 7.27 g carbohydrates, 3 g dietaryfiber, 0.36 g fat, 3 mg calcium, 0.09 mg iron, 13 mg phosphorus, 144 mg potassium. Moreover, various amino acids like 0.009 g of tryptophan, 0.023 g of methionine and 0.083 gm of lysine are also present in 108 g of the fruit.

So, the carambola and olive leaves may be considered as non-conventional feed sources in poultry and livestock diet as they contain moderate levels of fiberand protein and also other nutrients. This study will also help in further study if a feed trial in poultry and livestock with these leaves will be held.

**Conclusion**

Livestock feed costs in developing countries are a continuing challenge. Therefore, it is important to explore rational feedstuff to enhance productivity. Concerning the feeds of crop origin, the majority are bulky poor-quality cellulosic roughages with a high crude fiber and low nitrogen contents, suitable for feeding to ruminants. They have considerable potential as feed materials and their value can be increased if they are converted into some usable products. This study will help in further study.

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**Limitations**

In this proximate analysis, we estimate total N2, not the ultimate protein & NPN (NonProtein Nitrogenous Substance). Again it estimates %CP from N2 multiplying by 6.25 assuming that all protein contains 14-18% N2. So over & under estimation of N2 can be happened. During estimation of %CF, acid & alkali boiling is going on the hemicelluloses is partially destroyed. So there can be a little variation from the real value of %CF. We can’t estimate vitamins, calcium and phosphorus level of feed by using this method.Any deviation in results may be due to environmental or experimental error. The study area was also limited.

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**Biography**

This is Umme Salma Amin from Chittagong, girl of Md. Nurul Amin and Nur Jahan. I have completed my Secondary School Certificate from Kulgaon City Corporation High School and Higher Secondary Certificate from Chittagong Cantonment Public College in 2009 and 2011 respectively with CGPA 5.0 out of 5.0 scale in both exams under Chittagong board. As a successful candidate for DVM degree, I have achieved CGPA 3.91 out of 4.00 in the taught courses/ in-campus study placing myself in the 2nd position. Now I am enrolled in the year long internship program. I have immense interest to do the higher study and research in the field of Veterinary Medicine.