# RUMEN EPITHELIAL SCRAPINGS MEAL AND RUMEN DIGESTA AS ALTERNATIVE PROTEIN SOURCE OF SOYBEAN MEAL IN BROILER RATION

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Roll No.: 0115/08

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**Department of Animal Science and Nutrition**

**Faculty of Veterinary Medicine**

**Chittagong Veterinary and Animal Sciences University**

**Chittagong-4225, Bangladesh**

**December 2016**

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**This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made**

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# List of Abbreviations

| Abbreviation | | Elaboration |
|---|---|---|
| % | - | Per cent |
| < | - | Less than |
| > | - | Greater than |
| °F | - | Degree Fahrenheit |
| ANOVA | - | Analysis of Variance |
| BRETS | - | Bovine Rumen Epithelial Tissue Scrapings |
| BW | - | Body Weight |
| BWG | - | Body Weight Gain |
| CP | - | Crude Protein |
| CRD | - | Completely Randomized Design |
| Ctg. | - | Chittagong |
| CVASU | - | Chittagong Veterinary and Animal Sciences University |
| CY | - | Carcass Yield |
| DOC | - | Day Old Chick |
| DRC | - | Dried Rumen Content |
| EE | - | Ether Extract |
| et al. | - | And his associates |
| etc. | - | Et cetera |
| FC | - | Feed Conversion |
| FM | - | Fish Meal |
| gm | - | Gram |
| Kcal/Kg | - | Kilocalorie per kilogram |
| kg | - | Kilogram |
| ME | - | Metabilizable Energy |
| MS | - | Master of Science |
| NFE | - | Nitrogen Free Extract |
| NS | - | Non-Significant |
| RC | - | Rumen Content |
| RD | - | Rumen Digesta |
| RES | - | Rumen Epithelial Scrapings |
| RESM | - | Rumen Epithelial Scrapings Meal |
| SBM | - | Soybean Meal |
| SEM | - | Standard Error of Mean |
| Sq. Ft. | - | Square Feet |
| Tk. | - | Taka |
| US | - | United State |
| VP | - | Vegetable Protein |
| Wt. | - | Weight |

## Abstract

A four week long study was conducted to measure the effects of Rumen epithelial scrapings meal (RESM) with Rumen digesta (RD) partially replacing soybean meal in broiler diet in terms of improving performance, carcass quality and blood parameters of broiler. Ninety day-old Cobb 500 chicks were randomly distributed into three dietary treatment groups having 30 birds in each. $T_0$ was the control group where no RESM and RD were added. Other dietary treatment groups $T_1$ and $T_2$ were fed with RESM & RD at the level of 5% and 10%, respectively. The diets were iso-caloric and iso-nitrogenous and the ratio of RESM and RD was 1:1. Results showed that a significant difference ($P<0.01$) in feed consumption in different groups at $2^{nd}$ and $4^{th}$ week of age and it was highest in $T_2$ among all the groups. Highly significant ($P<0.01$) differences were observed in case of cumulative feed consumption both at $3^{rd}$ and $4^{th}$ week of age. Live weights differed significantly at $1^{st}$ to $4^{th}$ week of age and were higher in $T_1$ and $T_2$ groups in comparison with $T_0$. The values of feed conversion (FC) was significantly ($P<0.01$) lower in $T_1$ and $T_2$ groups. The increase in final and eviscerated weight observed from both $T_1$ and $T_2$ treatment groups were highly significant ($P\leq0.01$). Drumstick, thigh and feet weight were significantly higher ($P<0.01$) in $T_1$ and $T_2$ groups but better in $T_2$ than $T_1$. Liver and heart weight were significantly higher ($P<0.01$) in $T_1$ and $T_2$. The difference in weights of breast, wing, gizzard and spleen were non-significant ($P>0.05$), though increased values were found in $T_1$ and $T_2$ groups. No Significant ($P>0.05$) difference was found in Cholesterol and Aspartate aminotransferase levels of different groups. Highly significant ($P<0.01$) differences were found in total protein, triglycerides and Alanine aminotransferase level though decreased values were found in all parameters at in $T_1$ and $T_2$ groups in comparison with control. Cost benefit analysis also showed that Net profit (Tk/broiler, Tk/kg live weight) were higher in $T_1$ and $T_2$ groups. Addition of 5% to 10% RESM and RD may be supplied in broiler ration to increase performance, quality of carcass, blood parameters. 10% RESM and RD is recommended for better performance, reducing production cost.



**Keywords:** Rumen epithelial scrapings meal, Rumen digesta, Feed conversion, Carcass characteristics, Blood parameters.

# Chapter I: Introduction

Poultry is a substantial contributor to food supply of Bangladesh. The poultry industry has been successfully becoming a leading industry of Bangladesh (Ali and Hossain, 2012). Feed represents the largest single item of cost in intensive poultry production all over the world. The rising cost of animal feeds has continued to be a major problem in developing countries as feed cost is about 70-75% of the total cost of production, compared consist 50-60% in developed countries (Nworgu et al., 2003). High cost of poultry feed has also cause serious animal protein deficiency among low income earners of Bangladesh, leading to malnutrition (Raha, 2013). Several animal nutritionists involved in feed formulation for monogastric animals have utilized agro industrial by-products regarded as non-conventional feed sources (Alikwe et al., 2013). Apart from the major conventional animal protein sources (fishmeal, meat meal, blood meal) for instance, others like maggot meal, shrimp waste meal, silkworm meal, poultry offal meal, feather meal, crab meal, grasshopper meal have been tried as alternative protein sources for broilers (Ojewola and Annah, 2006).

Rumen epithelial scraping (RES) is an abattoir waste and environmental pollutant, being scraped-off from the rumen (the first compartment of ruminant's stomach) linings, suggesting a high protein source (Bawala et al., 2006). Recently, scraped cattle rumen epithelium are processed and compounded into livestock feed which have been referred to as rumen epithelial scrapings meal (RESM). Reports have shown that RESM has almost similar nutrient constituents to that of fish meal (Bawala et al., 2006). On the other hand, Rumen digesta (RD) is a kind of waste material generated daily at abattoirs which is also found from rumen of cattle (Odunsi et al., 2004). It accounts for about 80% of the capacity of the adult ruminant stomach (Adeniji and Oyeleke, 2008). Rumen digesta contains various plant materials at various stages of digestion rich in protein and other micro-flora such as fungi, protozoa and bacteria (Dairo et al., 2005). It is an important source of energy and vitamins especially vitamin B complex. Its utilization as animal feed will also alleviate and increase the economic value and environmentally being disposal of slaughter house by-products (Esonu et al., 2006).

It was reported that some nutrient rich abattoir wastes (i.e. meat meal, blood meal, rumen digesta, and RESM) can replace the conventional protein sources (Bawala and

Akinsoyinu, 2006). Rumen epithelial scraping meal (RESM) had been used in the feeding of ruminant animals by several researches (Fajemisin et al., 2003; Bawala et al., 2003). However, there is no sufficient information of availability, processing and nutritive value of both cattle rumen epithelial scrapings and rumen digesta and using them as sole or supplementary dietary protein source in monogastric nutrition such as pigs, poultry (Alikwe et al., 2013). Rumen epithelium scraping (RES) inclusion as replacement for fish meal protein could perform well in broiler diet at the finisher phase without adverse effects (Alikwe et al., 2010). Inclusion of rumen digesta with bovine blood had no adverse effect on the health of the broiler birds as shown in the serum biochemistry constituent result of broilers (Okpanachi et al., 2012).

With this background two nonconventional feed ingredients like rumen epithelial scrapings meal (RESM) and rumen digesta (RD) were incorporated in broiler ration for the following objectives:

- To observe the growth performance, carcass quality and organ characteristics of broiler fed with and without RESM and RD
- To measure the blood parameters of broiler
- Economic evaluation of RESM and RD based broiler ration

# Chapter II: Review of literature

There is need to improve the scientific knowledge for utilizing low cost locally available agro-industrial by-products in poultry feed in order to reduce the feed cost. As feed constitutes 60-70% of the total cost of production, any attempt to reduce the feed cost may lead to a significant reduction in the total cost of production (Swain et al., 2014).

The recent innovation in poultry industry is to identify and utilize alternative cheap animal origin protein sources in poultry feed (Laudadio et al., 2012a). In any kind of poultry production, feed is the major cost because some feed materials are shared by both human and birds (Dhama et al., 2015).

## 2.1. Inevitability of protein in broiler nutrition

The usefulness of a protein feedstuff for poultry depends upon its ability to supply a sufficient amount of the essential amino acids (EAA) that the bird requires, as well as the protein digestibility and the level of toxic substances associated with it (Scanes et al., 2004).

Proteins are large molecules made up of amino acids bonded together by peptide linkage. They provide the essential amino acids, which are the initial materials for tissue synthesis and constituent of tissue protein. Thus, it was often referred to as the "currency" of protein nutrition and metabolism. The connective tissues, ligaments, enzymes, hormones, haemoglobin in blood and even the hereditary material (DNA) are all made up of proteins (Aduku, 2004).

Ideal protein should be understood as being that has all amino acids in the exact amount and proportion for attending the maintenance and the highest protein deposition requirements. According to the ideal protein concept, all amino acids are equally limiting to animal growth; therefore, reduction of any amino acid, independent of the level, will cause its own deficiency and protein will become a non-ideal. The first ideal protein profile for broiler chickens was published by Baker & Han (1994).

In various countries, during poultry feed manufacturing, care is taken that animal protein ingredients should be incorporated in the feeds, particularly for young birds,

which require a high level of amino acids. The essential amino acid requirements are gradually decreased as the bird's age, and it is possible to supply diets that contain lower animal protein content and relatively higher levels of plant protein to meet the demands of older birds (Ravindran, 2013).

Broiler chicks have been shown to benefit from immediate access to feed. Although the focus of nutrition has been on provision of energy, chicks would benefit from a more balanced nutrient profile, particularly protein and amino acids. To cope with market demand for protein (meat), modern broilers are reaching market age sooner each year (Kleyn and Chrystal, 2008).

For broiler production there need crude protein 22% in starter, 21% in grower and 20% in finisher diet. The provision of quality protein devoid of any essential amino acids is critical in the early nutrition in the young poultry (Dibner, 2006).

The provision of proper nutrition to chicks in early life is essential, to ensure the rapid growth of the gastrointestinal tract and the rest of the body. Protein appears to be the most essential component of such nutrition, as it drives the initial intestinal development and muscle attachments in later phases. The quality and therefore the source of such protein may be important (Hossain et al., 2012a; 2013).

### 2.2. Sources of protein for broiler nutrition

There are two types of protein sources: animal protein and plant protein. It is easily assumed that no two protein sources are same in characteristics. The pattern of digestibility, biological value, quality, physical or chemical structure or properties of protein sources vary widely between sources. These characteristics of individual protein ingredients may affect neonatal intestinal development and function, and thus subsequent performance of the broiler chickens (Hossain et al., 2014).

The majority of an animal's dietary protein requirement is supplied by plant protein sources. Worldwide, traditionally, the most used energy and protein sources are respectively, maize and soybean. Cereals, like wheat and sorghum, and some plant protein meals are used all over the world as well. Soybean meal (SBM) is the preferred protein source used in poultry feed manufacturing. Its CP content is about 40-48%, and this depends on the quantity of hulls removed and the oil extraction

process. Compared to the protein meal of other oilseed grains, soybean protein is favored due to its well-balanced amino acid profile, especially the essential ones, enabling it to balance most cereal-based diets (Ravindran, 2013). The interaction between dietary nutrients, intestinal growth, and digestive function is crucial during the post-hatch period (Ullah et al., 2012).

### 2.3. Unconventional protein source for replacing conventional protein

Feed ingredients especially protein sources are very expensive and scarce due to high competition among poultry (Laudadio et al., 2012b), human and other animals resulting in the escalating cost of these ingredients. The price of conventional protein feeds resources such as groundnut cake, fish meal and soya bean meal is on the high side and cannot permit profit maximization in poultry ventures. It has also been reported that fish meal is the conventional animal protein source for poultry and that fish meal is scarce and expensive and most importantly in recent times its quality is questionable (Akpodiete and Inoni, 2000). In view of this, current research interest in the poultry industry is aimed at finding alternatives to this elusive feed ingredient.

Soybean meal inclusion level ranges up to 25% in broilers feed (Laudadio et al., 2012b). Increased feed prices in the last decade are making poultry farming out of the reach of small holder farmers. Thus, it is necessary to look for locally available unconventional protein ingredients to substitute soybean meal in poultry ration (Chand et al., 2014a,b). Identification of such cheap protein alternatives such as maggot meal, earthworm meal, silkworm pupae meal, meal worm and other insects as well as Rumen Epithelial Scrapings meal (RESM), Rumen Digesta (RD), Blood meal would help resource poor farmers not only to cut down their production costs, but also to improve the efficiency of their production.

Feed ingredients especially protein sources are very expensive and scarce due to high competition among poultry (Laudadio et al., 2012b), human and other animals resulting in the escalating cost of these ingredients. The price of conventional protein feeds resources such as groundnut cake, fish meal and soya bean meal is on the high side and cannot permit profit maximization in poultry ventures. It has also been reported that fish meal is the conventional animal protein source for poultry and that fish meal is scarce and expensive and most importantly in recent times its quality is

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The expensive soybean meal may possibly be replaceable by the maggot meal (MM) which has a similarity in the amino acid profile (Atteh and Oyedeji, 1994). It has been reported that dried house fly larvae and pupa have high amount of protein (63.1%) and fat (15.5%) contents (Adeniji, 2007). According to Inaoka et al. (1999), dried maggots and pupa contain protein and amino acid composition similar to fish meal and can replace 7% of the fish meal in broiler chicken feed. Recently, Hwangbo et al. (2008) reported higher protein, fats, metabolizable energy and minerals contents, fatty acid and amino acid profile in maggots than soya bean meal. In the same study, it was also reported that apparent digestibility of protein and amino acid was greater for maggots than soya bean. In addition, the short life of maggots and their production in large biomass from materials regarded as waste make them a viable option to explore (Odesanya et al., 2011).

Swain et al. (2014) reported that the chemical composition of agro-industrial by-products and other unconventional feed ingredients, poultry hatchery waste, protein source (sunflower meal) and legume green fodder (cowpea leaf meal) along with their feeding value in broilers, backyard poultry and Japanese quails could be used to economize the cost of production.

Thus, it is necessary to include one or more of these animal protein sources in chicken diets. Hatchery by-products, feather and blood meals, and spent hens, have also been used for feeding non-ruminant animals (Moritz and Latshaw, 2001). To improve performance, there has also been some interest in substituting part of the SBM in poultry diets with animal products. Supplementation of animal protein sources may considerably improve performance parameters over standard diets. However, this may be because of the high concentration of EAA or it may be due to the lower percentage of indigestible carbohydrates present in SBM (Firman and Robbins, 2004).

Shortage and volatility in price of feed ingredients motivated to search for alternative feed source to solve this problem. In recent years, researchers tended to look for traditional an inexpensive sources of feedstuff for inclusion in animal and poultry diets, and from these sources abattoir waste. One of such is from abattoir wastes

comprising rumen content (RC), a potential alternative protein source (Olukayode et al., 2008).

Rumen content is plant material at various stages of digestion rich in microbial protein (McDonald et al., 1990). Blood is a source of high quality protein as blood meal (80-90% crude protein), high in the essential amino acids, especially lysine; (NRC, 1994), and nutritional value of blood meal increases when fed in combination with other protein sources (Ilori et al., 1984; Dafwang et al., 1986). Identification, development, and utilization of potential alternatives to conventional ingredients (such as soybean and fishmeal) are imperative for the sustainability of livestock production. One of such is from abattoir wastes comprising rumen content and blood, a potential alternative protein source (Makinde et al., 2010).

Cattle rumen epithelial scraping is the by-product of processing of cattle rumen into edible meat which is highly relished in most African countries. It is the thin layer of the rumen that is scraped off during the cleaning of this organ for food by man. Rumen epithelial scrap is an abattoir by-product which is rich in protein (about 53% CP) and has amino acids profile which in most cases is similar with those of fish meal (Faremi et al., 2010). The potential of cattle rumen epithelial scraping for goat has been explored (Ogunwole, 2011). Recently, scrapings from Cattle rumen epithelium are processed and compounded into livestock feed which have been referred to as rumen epithelial scrapings meal (RESM) (Bawalaet al., 2006). The protein level and amino acids composition of RESM clearly give it a rating in the category of other conventional protein sources especially of animal origins (Olomu, 2011). Comparing RESM with other protein sources of animal origin, which are recognized for their nutritive values as rations in feeds, RESM is rich in the sulphur hydroxyl amine, aromatic, acidic and basic amino acid. It contains lower level of lysine when compared to hen's egg, cow's milk, fishmeal and larvae meal (Oluokun, 2000) though can easily be supplemented with blood meal.

## 2.4. Sources of animal protein

Protein supplements of animal origin are derived from meat packing and rendering operations, poultry and poultry processing, milk and dairy processing, fish and fish processing etc. (Denton et al., 2005). There is a long history of worldwide animal protein use in the poultry industry (Firman and Robbins, 2004). They are meat and bone meal, protein concentrate, poultry meal, hatchery by product, blood meal, hydrolyzed poultry feather meal, fish meal etc. (Moritz and Latshaw, 2001). Before the discovery of vitamin B-12, it was generally considered necessary to include one or more of these protein supplements in the rations of chickens. Animal proteins are useful constituent of poultry rations. They provide a high level of protein/amino acids, highly available phosphorus, a number of other minerals, and moderate amounts of energy. The animal protein is considered as excellent and high class protein containing all essential amino acid particularly lysine, first limiting amino acids in cereals for broiler (Gianget al., 2001; Robinson and Singh, 2001). Broiler chickens fed on animal protein, found better productivity and performance than those birds fed only on plant-based diets (Hossain et al., 2012; 2013). However, benefits of animal protein, as poultry feed, depend on method of processing and cost effectiveness (Ra'fat, 2008).

Animal proteins are well balanced in terms of EAA that are necessary for body growth and development, but they are expensive for commercial broiler production. Therefore, they are usually used to complement the amino acid balance in the diets rather than as the main protein source. Also the concern associated with disease transmission from products of animal origin is also taken into consideration. In general, the quality of animal protein sources is dependent on the composition of the raw material used. Animal protein supplements are derived from poultry and poultry processing; meat packing and rendering operations; fish and fish processing, and milk and dairy processing (Denton et al., 2005). Bone meal, meat meal, poultry meal, hydrolyzed feather meal and to a lesser extent blood meal have all been used as important feedstuffs for poultry feeding (Pearl, 2002). Animal proteins are a beneficial component of poultry diets because they offer a high level of protein/amino acids, a high level of available phosphorus, reasonable amounts of other minerals, and moderate levels of energy.

## 2.5. Constrains of animal protein

Though animal protein is of excellent quality, it has some drawbacks also. In some countries like India, Pakistan, USA etc.; these feedstuffs are excluded from poultry diets in order to prevent cross-contamination of diets for ruminant animals and to prevent zoonosis (Mendes, 2003; Hossain et al., 2013). Animal protein is the risk factor for spreading infections of zoonotic importance like TB, Salmonellosis, BSE etc. (CEC, 2000; Hofacre et al., 2001). The exclusion of these feed ingredients from formulations not only reduces the nutritive value of the diets, but also limits the ability of the formulations to meet the essential nutrient needs for poultry (Hossain et al., 2011a).

Many protein supplements of animal origin are troublesome to process and store without some spoilage and nutrient loss. If they cannot be dried, they must be usually refrigerated. If not heated to destroy pathogenic bacteria, they may be a source of infection. On the other hand, protein availability will be reduced and some nutrients are lost if the feed is heated excessively (Ra'fat, 2008). Performance of broiler fed with animal byproducts may be highly changeable as a function of raw material type and quality, processing temperature, use of antioxidants to maintain their quality, contamination by pathogenic microorganisms and unwanted substances like sands, fibers, dusts, hair follicles etc., high polyamine content, amino acid unbalance, nutrient content and digestibility, and storage conditions (Bellaver, 2001; Shirley and Parsons, 2001). The use of chemical preservatives in production of animal by-products often causes toxicity to poultry birds (Khatun et al., 2003; Karimi, 2006).

Furthermore, a critical cost appraisal of poultry feed formulations shows that protein of animal origin is more expensive than vegetable protein sources (Oluyemi and Roberts, 2000; Blair, 2008; Chadd, 2008).

Due to these consequences like public health risk, chronic scarcity and high cost of animal protein supplements, particularly fish meal, meat and bone meal have increased interest to seek alternative protein sources for feeding poultry (FAO, 2004).

## 2.6. Sources of plant protein

The sources of plant protein for poultry are soybean meal, cottonseed meal, linseed meal, alfalfa meal, corn gluten meal and legumes. Broiler productions dependent entirely on vegetable ingredients may be an emerging trend for producers, and be in great demand from consumers as well. Broilers grown solely on plant protein diets are preferred in the European Union and Middle East. Meat chickens produced from plant-based diets, except any growth promoter or animal by-products, may be considered as organic meat, which has huge demand in the world food market (Mendes, 2003). This tendency is creating the pressure on feed formulators and nutritionists to supply organic, safe and hygienic poultry products to consumers, by providing quality diets to poultry without using animal by-products or growth promoters. Adding vegetable ingredients, mainly soybean, canola, sunflower and mustard, in diets, instead of using animal meals as a protein source, can lead to optimum broiler performance as long as the diets are properly balanced with necessary nutrients such as digestible amino acids. These ingredients are a good source of nutrients, are comparatively inexpensive, easily available, and easy to process, and pose less risk of disease contamination. Producing poultry products, i.e. meat and eggs, at economical rate may be feasible using plant protein sources in practical diets, as they are considered cheaper and safer than animal protein sources (Hossain and Iji, 2015). Despite cutting feed cost considerably, these ingredients can serve as excellent sources of nutrient for poultry when processed properly and supplemented with other pro-nutrients (Cruz et al., 2009).

The extra fat deposition in the carcass and egg is generally considered as the unfavorable trait in the poultry industry (Remignon and Le Bihan-Duval, 2003). Many researchers have been claimed that broiler chickens deposit less abdominal fat when the birds fed on all vegetable ingredients than those fed on animal protein diets (Pawlak et al., 2005; Hossain et al., 2015).

When lean meat is desired, animals may be fed plant based diets, which will result in low fat and higher protein accumulation in their carcasses (Singh and Panda, 1992). Moreover, broiler meat fed on all vegetable diets may also contribute a better profile of fatty acids in their carcasses, which may enhance the shelf life of meat (Hossain and Iji, 2015).

## 2.7. Constrains of plant protein

However, vegetable sources contain numerous anti-nutritive factors (ANF) and amino acids are imbalanced in the vegetable proteins, even though a single plant protein may contain all essential amino acids but not available in an ideal ration or as per as the requirement of the animals (Hossain et al., 2015a). Two or more plant protein feed ingredients together can make their proper availability in the diet. According to the NRC (1994) excluding sulphur amino acids, all essential amino acids are supplied by the soybean meal. On the other hand, all the other plant proteins have multiple deficiencies. Hossain and Iji (2015) also reported that cotton seed meal was deficiencies of lysine, methionine and leucine, groundnut cake in sulphur amino acids, lysine and threonine, corn gluten meal in arginine, lysine, threonine and tryptophan. Corn gluten meal is notably adequate in sulphur amino acids in contrast to the other proteins. Besides, another plant feed such as lupine seed meal, sunflower meal contains lower protein efficiency ratios because of the relatively low concentration of sulphur amino acids and low available lysine content (Attia et al., 2003).

Because of their deficiency in some amino acids, plant proteins frequently require a supplementary source of amino acids or other protein sources such as animal protein. Plant proteins are usually cheaper than animal proteins; however, there is a limitation to their use because of their content of anti-nutritional factors (ANFs). Most of these ANFs can be destroyed by thermal processing that causes an increase in the nutritional value sometimes and protein level of plant proteins (Adeyemo and Longe, 2007) due to the elimination of ANFs and freeing the protein in the plant protein products.

In general, vegetable (plant) protein sources are nutritionally unbalanced and poor in certain EAA and this decreases their biological value as they may not provide the required limiting amino acids needed by birds for egg and meat production. Poultry nutritionists have paid more concentration to the use of animal protein sources to create balanced diets (Akhter et al., 2008).

The constraints of these vegetable proteins can be overcome by adding different supplemental feeds such as exogenous enzymes, fat or oils, synthetic amino acids, vitamin mineral premix and also growth promoters like feed additives in order to

enrich nutritional quality of feeds for getting optimum performance of poultry (Hossain et al., 2011).

### 2.8. Rumen Epithelial Scrapings Meal (RESM)

Salami et al. (2013) reported that, Bovine rumen epithelial tissue scrapings (BRETS) have crude protein (CP) content of 73%. However, the result obtained for the ether extract (EE), crude fibre (CF), ash, nitrogen free extract (NFE), methionine and lysine in BRETS were 4.05%, 4.35%, 2.03%, 11.28%, 0.92%, 4.00% respectively, and the ash content has a value of 2.03%. The crude protein obtained for BRETS or RESM was higher than the CP obtained from the proximate composition of fishmeal (FM) observed by Awoniyi et al. (2003) and Odunsi (2003), who reported a CP content of 64.5% and 68.5% respectively for FM. However, Faremi et al. (2010) reported a contradictory lower CP and lysine contents of 53% and 0.87% respectively, for BRETS or RESM which if compared with the CP and lysine contents reported for FM, were significantly lower than that of FM. The significant difference between the CP and lysine contents obtained in this result might have been due to the difference in the nutritional status of the rumen of the slaughtered cattle from which the BRETS were obtained. Despite the higher content of CP in BRETS or RESM compared to FM, the values of the two major essential amino acids (methionine 0.92% and lysine 4.0%) analyzed in BRETS or RESM were lower than those observed in FM by Ijaiya and Eko (2009), who reported that FM contained 2.20% of methionine and 4.56% of lysine. However, BRETS showed its superiority in terms of higher CP over other non-conventional protein sources such as maggot meal which contain 55.1% CP (Awoniyi et al., 2003) and shrimp waste meal which contain 46.3% CP (Fanimo and Oduguwa, 1999).

### 2.9. Rumen Digesta

Rumen content is plant material at various stages of digestion rich in microbial protein (McDonald et al., 1990). It is a material from the rumen of cattle which is the first stomach compartment of the ruminants. It is account for about 80% of the capacity of the adult ruminant stomach (Adeniji and Oyeleke, 2008). It is plant material at various stage of digestion rich in protein and other micro-flora such as fungi, protozoa and bacteria (Esonu et al., 2006 and Dairo et al., 2005). It is important

source of energy and vitamins especially vitamin B complex. Its utilization as animal feed will also alleviate and max the economic environmentally benign disposal of slaughter house by- products (Esonu et al., 2006). Rumen content is substantial wastes generated daily at abattoirs (Odunsiet al., 2004). Agbabiaka et al. (2011) reported that the proximate compositions of dried rumen digesta (%DM) are Moisture 5.47%, CP 18.58%, CF 34.44%, EE 3.77%, NFE 24.81% and Ash 18.40%. Another study reported that the proximate composition of bovine blood with rumen content mixture showed that it contains 45.35% CP, 4.10% ether extract (EE), 8.81% crude fiber (CF) and 15.42% ash and can replace soybean meal up to 60% level without any deleterious effect on the carcass yield and organ weight of the finishing broilers (Mishra et al. 2015).

Elfaki et al. (2015) reported that dried rumen digesta (RD) contains DM 96.32%, EE 2.99%, ASH 14.23%, CF 28.28%, CP 18.53%, NFE 35.97%, Ca 0.70 %, P 0.69%, ME 2190 kcal/ kg. They concluded that the inclusion of dried rumen content in broiler diets had no adverse effect on performance and biochemical value of plasma in broiler chicks. Therefore, DRC can be used up to 10% as a cheap source of energy and protein with reduced feed cost and environmental pollution.

**2.10. Soybean meal**

A cheap, highly palatable and available feedstuff, soybean meal is the simplest form of soybean protein and a by-product of the oil milling after soybean oil extraction. It contains 44-48% CP, 0.6-07% methionine and 2.7-3% lysine (NRC, 1994). Soybean meal is a qualified source of feed protein, and according to the origin of soybean meal, the quality varies. It is well known that dehulled soybean meal has an excellent nutrient profile and higher energy values and contains more digestible nutrients compared to non-dehulled soybean meals (Swick, 2002). Tacon (1990) reported that, Soybean meal contains Moisture 11.0%, CP 45.0%, EE 1.2%, CF 6.1%, and Ash 6.1%.

It contains higher energy (2,460 metabolizable energy (ME) kcal/kg) and protein than other plant protein sources and has an excellent balance of highly digestible amino acids with the exception of methionine, cystine (both are sulpher containing amino acids) which tends to be low. Soybean meal is however rich in the essential amino

acids like lysine, tryptophan, threonine, isoleucine, and valine which are deficient in cereal grains (corn and sorghum) mostly be utilized by poultry. The bioavailability of the amino acids lysine, threonine, and methionine from soybean meal are 88, 81, and 90%, respectively. Amino acid digestibility is also very high (more than 90 for lysine in poultry) (Sauvant et al., 2004). However, both methionine and lysine supplementation are necessary for increasing feed efficiency in poultry diet based on soybean meal (Douglas and Persons, 2000). Several other studies (Opapeju et al., 2006; Coca-Sinova et al., 2008) have evaluated various methods of enhancing the digestibility of individual amino acids and protein of soybean meal.

Samuel et al. (2011) reported that Soybean meal is deficient in methionine, cystine and to some extent lysine. However, soybean meal possesses anti-nutritional properties which must be overcome to increase its nutritional value. These include trypsin inhibitors, oligosaccharides (rafinose and stachyose) which are poorly utilized by poultry. Phytic acid and antigenic factors found in certain soybean proteins cause inflammatory response in the gastrointestinal tract of monogastric animals. Soybeans also contain lectins, compounds that bind with intestinal cells and interfere with nutrient absorption and other compounds such as saponins, lipoxidase, phytoestrogens and goitrogens whose anti-nutritional effects are not known.

### 2.11. Conventional protein VS Unconventional protein sources

Specially modified rape expellers can serve as a high quality protein feed that can completely replace meat and bone meals in diets used for the fattening of broiler chickens (Suchý et al., 2002). Comparing the inclusion of 4% meat and bone meal, 3% poultry offal meal, and vegetable diets, did not reveal any influence of diets on 21-day-old broiler performance (Bellaver et al., 2005). Ra'fat (2008) found any significant differences among feed intake, weight gain, feed conversion ratio, and carcass characteristics in different dietary treatments using plant and animal protein in broiler ration. The feed intake up to 21 days was highest on the animal protein (AP) diets, and the lowest in the vegetable protein (VP) based diet (Hossain et al., 2013). Broiler chicks can tolerate up to 10% dried rumen content (DRC) replacing other conventional feed in the diets without adverse effect (Elfaki et al., 2015). Combination of rumen content and blood assures a potential alternative protein source (Odunsi, 2003; Odunsi et al., 2004).

The most suitable solution to the problem of high cost of conventional animal protein sources may be the exploitation of vast, cheap and available and underutilized slaughterhouse wastes and animal by-products which often constitute environmental pollutants (Ogunwole, 2011). Oladunjoye et al. (2013) designed a study to measure the effect of substituting cattle rumen epithelial tissue scrapings instead fishmeal a conventional protein source on the production performance of the growing rabbits. It is also reported that, RESM has similar nutrients constituents to that of fish meal (Isah, 2001; Bawala et al. 2003; Fajemisin et al., 2003).The ever-increasing cost of livestock feeds with the attendant increase in the cost of animal products such as meat, eggs and milk shows the use of nonconventional feed ingredients in the feeding of domestic animals (Ani and Omeje, 2007; Owen et al., 2009). It was reported that some nutrient rich abattoir wastes (i.e. meat meal, blood meal, rumen digesta and RESM) can replace the conventional protein sources existing in market (Bawala and Akinsoyinu, 2006).

In developing countries, feed cost accounts for 70-75% of the total production cost, compared to about 50-60% in developed countries (Nworgu et al., 2003). Major part of feed cost comes from protein cost. If we could use the alternative protein rich feed resources that might reduce the feed cost. Unconventional protein sources would be the good sources of protein for broiler feeding.

# Chapter III: Materials and Methods

## 3.1. Location of the experimental shed

This research aimed to study the growth performance, carcass quality as well as blood parameters of broiler fed with rumen epithelial scrapings meal along with rumen digesta partially replacing soybean meal at 5% and 10% level. The study was conducted from 03 March to 27 May 2016, at the Experimental Poultry Farm and Laboratory of Department of Animal Science and Nutrition & Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong-4225, Bangladesh.

## 3.2. Preparation of poultry shed for the experiment

Poultry shed was selected and prepared for broiler rearing. At first, the selected broiler shed was carefully dry cleaning 3 times for 2 days then washed and cleaned up by using tap water with disinfectant. Phenyl solution was also spread on the floor and ceiling, then brushing was done by using steel brush along with clean water. Brooding boxes and broiler cages were also cleaned by using tap water with disinfectant for 2 times. Then copper sulphate (1gm/2 liter water) solution was used as sprayer for 2 days. Formalin solution was also used as disinfectant for two days. After that potassium permanganate (1gm/3 liter water) solution was used for two days. After cleaning and disinfecting, the house was left for one week. All windows were opened for proper ventilation. After one-week lime was spread around the shed for bio-security. In our experimental shed, floor space for each bird was 0.17 sq. ft. in brooding box and 0.57 sq. ft. in the cage.

## 3.3. Experimental design

The experiment was carried out for 28 days where starter period was 0 to 14 days and finisher period was 15 to 28 days. The statistical design used for the experiment was CRD (Completely Randomized Design). In this experiment, total 90 birds were allocated for three treatment groups with three replications in each. Chicks were equally and randomly distributed in three dietary treatment groups ($T_0$, $T_1$ and $T_2$) having 30 birds per treatment group and 10 birds per replication. Diet $T_0$ was the control diet formulated without the inclusion of RESM and RD. Diets $T_1$ and $T_2$ were

formulated with RESM & RD was incorporated at 5% and 10% respectively that partially replace the Soybean meal. All diets were also supplemented with feed grade methionine and lysine. All rations during starter (0-14 days) and finisher periods (15-28 days) supplied in both cases were iso-caloric and iso-nitrogenous. Layout of the experiment is shown in Table 3.3.a.

**Table 3.3:** Layout of the experiment

| **Dietary treatment groups** | **No. of broilers per replication** | | **Total no. of broilers per treatment** |
|---|---|---|---|
| $T_0$ (Without RESM and RD) | $R_1$ | 10 | 30 |
| | $R_2$ | 10 | |
| | $R_3$ | 10 | |
| $T_1$ (With 5% RESM and RD) | $R_1$ | 10 | 30 |
| | $R_2$ | 10 | |
| | $R_3$ | 10 | |
| $T_2$ (With 10% RESM and RD) | $R_1$ | 10 | 30 |
| | $R_2$ | 10 | |
| | $R_3$ | 10 | |
| **Grand total** | | | **90** |

RESM= Rumen Epithelial scrapings meal, RD= Rumen Digesta

### 3.4. Collection of day-old chicks

A total of 90 unsexed Day-Old Chicks (Cobb 500 strain) were purchased from an agent of M. M. Agha Ltd., Khatunganj, Chittagong, Bangladesh on 22 March, 2016. During purchasing all chicks were examined for uniform size and free from any kind of abnormalities.

**Figure 3.4:** Day old chick in Brooding box

### 3.5. Collection of Feed ingredients

#### 3.5.1. Collection of Rumen epithelial scraping (RES) and Rumen digesta (RD)

Rumen epithelial scraping and rumen digesta were collected from abattoir at Jhautala Bazar, Khulshi, Chittagong Metropolitan at morning. At every morning, rumen epitheliums were collected from slaughter house and conventional beef selling shop in Jhautala Bazar and stored into plastic bags. Rumen digesta was also collected from slaughter house during offal processing, slaughter house garbage storing area and finally stored into plastic bags. The bags with rumen epithelium and rumen digesta were carried to the processing plant.

#### 3.5.2. Collection of other Feed ingredients

Feed ingredients and feed additives were collected from abattoir at Pahartoli Bazar, Khulshi, Chittagong Metropolitan.

### 3.6. Processing of RESM and RD

At first RESM and RD were boiled followed by sun-drying for about 8 hours daily for 4 days until they become crispy. The sun-dried scrapings and rumen digesta were grinded in a hammer mill and sieved and then proximate analysis of samples was done following the method described by AOAC (2006) before incorporating them into the experimental dietat 5% and 10% levels as the replacer of soybean meal. RESM and RD were equal amount in weight (1:1 ratio).

**Figure 3.6.a:** Boiling of Rumen digesta

**Figure 3.6.b:** Boiling of Rumen Epithelial Scraping

**Figure 3.6.c:** Sun drying of Rumen digesta

**Figure 3.6.d:** Sun drying of Rumen Epithelial Scraping

**Figure 3.6.e:** Grinding of Rumen Digesta

**Figure 3.6.f:** Grinding of Rumen Epithelial Scraping

### 3.7. Feeding standard

Feeding standard followed in the experiment was that of Bangladesh standard specification for poultry feed (2nd Revision, BDS 233: 2003). The birds were provided with dry mash feed throughout the experimental period. All the rations were iso-caloric and iso-nitrogenous. Feeds were supplied ad-libitum along with fresh clean drinking water for all the time.

### 3.8. Feed formulation and feeding the birds

The birds were supplied mash feed. Mash feed was prepared manually from raw feed ingredients, which were collected from retail and wholesale market. Three types of ration were used for two phases such as broiler starter for $T_0$, $T_1$ (5% RESM & RD), $T_2$ (10% RESM & RD) and broiler finisher for $T_0$, $T_1$ (5% RESM & RD), $T_2$ (10% RESM & RD). Rations were formulated according to the requirement of birds. Feed was supplied ad-libitum along with fresh clean drinking water. The composition of different feed ingredients and nutritive value of starter and finisher rations are given in Table 3.8.a and 3.8.b.

**Figure 3.8.a:** RESM

**Figure 3.8.b:** RESM & RD mixing with vegetable oil

**Figure 3.8.c:** Mixing of feed ingredients


**Figure 3.8.d:** Mixed feed stored in plastic bag

**Table 3.8.a:** Feed ingredients used in experimental broiler starter diets

| **Ingredients (Kg/100kg)** | **Starter ration (0-14 days)** | | |
|---|---|---|---|
| | **$T_0$** | **$T_1$** | **$T_2$** |
| Maize | 61.153 | 59.153 | 57.403 |
| Rice Polish | 0 | 2.25 | 3.85 |
| **Soybean meal** | **29** | **24** | **19** |
| **RESM & RD** | **0** | **5** | **10** |
| Molasses | 0.5 | 0.5 | 0.55 |
| Vegetable oil | 1.5 | 1 | 0.6 |
| Fish meal | 5.25 | 5.5 | 6 |
| Limestone | 0.75 | 0.75 | 0.75 |
| DCP | 0.9 | 0.9 | 0.9 |
| Methionine | 0.2 | 0.2 | 0.2 |
| Lysine | 0.1 | 0.1 | 0.1 |
| Salt | 0.3 | 0.3 | 0.3 |
| Vitamin mineral premix | 0.25 | 0.25 | 0.25 |
| Maduramycin | 0.06 | 0.06 | 0.06 |
| Enzyme | 0.025 | 0.025 | 0.025 |
| Antioxidant | 0.012 | 0.012 | 0.012 |
| **Total** | **100** | **100** | **100** |

**N.B:** $T_0$ = Control diet without RESM & RD, $T_1$ = Experimental diet with 5% RESM & RD, $T_2$ = Experimental diet with 10% RESM & RD

Vitamin Mineral Premix in Rations that mentioned in table 3.8.a contains following ingredients per kg diet: Vitamin A = 5000 IU, Vitamin $D_3$ = 1000 IU, Vitamin K = 1.6 mg, Vitamin $B_1$ = 1 mg, Vitamin $B_2$ = 2mg, Vitamin $B_3$ = 16 mg, Vitamin $B_6$ = 1.6 mg, Vitamin $B_9$ = 320 µg, Vitamin $B_{12}$ = 4.8 µg, H = 40 mg, Cu = 4 mg, Mn = 40 mg, Zn = 20 mg, Fe = 2.4 mg, I = 160 µg.

**Table 3.8.b:** Feed ingredients used in experimental broiler finisher diets

| **Ingredients (Kg/100kg)** | **Finisher ration (15-28 days)** | | |
|---|---|---|---|
| | **$T_0$** | **$T_1$** | **$T_2$** |
| Maize | 61.5 | 61 | 58.75 |
| Rice Polish | 1 | 1.2 | 3.75 |
| **Soybean meal** | **27.153** | **22.153** | **17.153** |
| **RESM & RD** | **0** | **5** | **10** |
| Molasses | 0.5 | 0.5 | 0.4 |
| Vegetable oil | 3 | 2.35 | 1.85 |
| Fish meal | 3.5 | 4.45 | 4.75 |
| Limestone | 1.5 | 1.5 | 1.5 |
| DCP | 0.9 | 0.9 | 0.9 |
| Methionine | 0.2 | 0.2 | 0.2 |
| Lysine | 0.1 | 0.1 | 0.1 |
| Salt | 0.3 | 0.3 | 0.3 |
| Vitamin mineral premix | 0.25 | 0.25 | 0.25 |
| Maduramycin | 0.06 | 0.06 | 0.06 |
| Enzyme | 0.025 | 0.025 | 0.025 |
| Antioxidant | 0.012 | 0.012 | 0.012 |
| **Total** | **100** | **100** | **100** |

**N.B:** $T_0$ = Control diet without RESM & RD, $T_1$ = Experimental diet with 5% RESM & RD, $T_2$ = Experimental diet with 10% RESM & RD

Vitamin Mineral Premix in Rations that mentioned in table 3.8.b contains following ingredients per kg diet: Vitamin A = 5000 IU, Vitamin $D_3$ = 1000 IU, Vitamin K = 1.6 mg, Vitamin $B_1$ = 1 mg, Vitamin $B_2$ = 2mg, Vitamin $B_3$ = 16 mg, Vitamin $B_6$ = 1.6 mg, Vitamin $B_9$ = 320 µg, Vitamin $B_{12}$ = 4.8 µg, H = 40 mg, Cu = 4 mg, Mn = 40 mg, Zn = 20 mg, Fe = 2.4 mg, I = 160 µg.

**Table 3.8.c:** Estimated nutritional composition (DM basis) of the experimental broiler starter diets

| Parameters | Starter ration (0-14 days) | | |
|---|---|---|---|
| | **$T_0$** | **$T_1$** | **$T_2$** |
| ME (Kcal/kg) | **2964.83** | **2971.91** | **2985.24** |
| CP (gm/100gm) | **22.03** | **21.96** | **21.97** |
| CF (gm/100gm) | 3.41 | 4.13 | 4.77 |
| EE (gm/100gm) | 4.40 | 4.23 | 4.11 |
| Ca (gm/100gm) | 1.03 | 1.22 | 1.43 |
| P (gm/100gm) | 0.81 | 0.89 | 0.96 |
| Lysine (gm/100gm) | 1.44 | 1.31 | 1.2 |
| Methionine (gm/100gm) | 0.56 | 0.54 | 0.52 |

**N.B:** In table 3.8.c, $T_0$ = Control diet without RESM & RD, $T_1$ = Experimental diet with 5% RESM & RD, $T_2$ = Experimental diet with 10% RESM & RD, ME = Metabolizable energy, CP = Crude protein, CF = Crude fibre, EE = Ether extract, Ca = Calcium, P = Phosphorus.

**Table 3.8.d:** Estimated nutritional composition (DM basis) of the experimental broiler finisher diets

| Parameters | Finisher ration (15-28 days) | | |
|---|---|---|---|
| | **$T_0$** | **$T_1$** | **$T_2$** |
| ME (Kcal/kg) | **3050.08** | **3052.55** | **3059.15** |
| CP (gm/100gm) | **20.34** | **20.56** | **20.52** |
| CF (gm/100gm) | 3.37 | 3.89 | 4.64 |
| EE (gm/100gm) | 5.83 | 5.35 | 5.21 |
| Ca (gm/100gm) | 1.16 | 1.39 | 1.59 |
| P (gm/100gm) | 0.74 | 0.83 | 0.91 |
| Lysine (gm/100gm) | 1.27 | 1.19 | 1.07 |
| Methionine (gm/100gm) | 0.52 | 0.51 | 0.49 |

**N.B:** In table 3.8.d, $T_0$ = Control diet without RESM & RD, $T_1$ = Experimental diet with 5% RESM & RD, $T_2$ = Experimental diet with 10% RESM & RD, ME = Metabolizable energy, CP = Crude protein, CF = Crude fibre, EE = Ether extract, Ca = Calcium, P = Phosphorus.

### 3.9. Managemental procedure

The following management procedures were followed during the whole experimental period and the uniformity in the management practices were maintained as much as possible.

#### 3.9.1. Brooding of the chicks

After proper cleaning and drying, the brooding boxes were ready for broiler chicks rearing under strict hygienic conditions. The experiment was conducted in summer season. Dry and clean newspaper was also placed in the brooding box. Newspaper was changed four times in a day from the floor of the brooding box. This was sustained for 7 days. During the brooding period chicks were brooded at a temperature of 90-95°F during 1st week and 90-85°F during 2nd week respectively with the help of electric bulbs.

**Figure 3.9.1:** Brooding of the chicks

#### 3.9.2. Maintaining room temperature

Basis on requirement, temperature was increased and decreased in the brooding box as well as in the whole house. The key concern was the comfort of broiler birds. Electric bulbs and fans were used to maintaining the temperature. Temperature was maintained according to age of birds as 1st week 95°F, 2nd week 90°F, 3rd week 85°F, 4th week 80°F.

### 3.9.3. Brooder and cage spaces

Each box brooder having 2.38 ft. × 2.08 ft. was owed for 30 birds. After 12 days later broiler birds were transferred to cage having 3.5 ft. × 1.63 ft. for 10 birds. Therefore, floor space for each bird in the brooding box was 0.17 sq. ft. and cage was 0.57 sq. ft. respectively.

### 3.9.4. Feeder and drinker spaces

In the early stage of brooding feed and water were given to birds on paper and small drinker. Feeding and watering were performed by using one small round plastic feeder and one round drinker with a capacity of 1.5 liter in each brooding box. The feeders and drinker were fixed in such a way so that the birds could eat and drink conveniently. After $5^{th}$ day small round feeder was replaced by small liner feeder (2.21 ft. × 0.25 ft.) in each brooding box. During the period of cage rearing large liner feeder (3.5 ft. × 0.38 ft.) and large round drinker with a capacity of three liters was used for feeding and drinking.

### 3.9.5. Method of feeding, watering and lighting

Formulated mash feed and fresh clean drinking water was supplied ad-libitum to the birds throughout the experimental period. Feed and drinking water were given three times a day. Starter ration was supplied for 0 to 14 days and finisher ration for 15 to 28 days. During the early stage of growth feed and water were given to birds on paper and small drinkers. The birds were exposed to a continuous lighting of 24 hours of photo period.

**Figure 3.9.5:** Feeding of broiler

### 3.9.6. Litter management

Dry newspapers were used as litter materials at a considerable depth during the brooding period. After the ends of brooding period birds were replaced in the cage for rearing until the end of experiment. Litter materials were cleaned by dandy brush form the tray and disinfected hygienically with detergent for two times in a day.

**Figure 3.9.6:** Cleaning of litter tray using dandy brush

### 3.9.7. Vaccination and chemo prophylaxis/medication

All birds were vaccinated properly against Newcastle disease on the 4$^{th}$ day and booster dose again on 14$^{th}$ day according to the following schedule:

**Table 3.9.7.a:** Schedule of vaccination used during experiment period

| Age of birds | Name of diseases | Name of the vaccines | Route of administration |
|---|---|---|---|
| 4$^{th}$ day | New Castle Disease | BCRDV (Live) | One drop in each eye |
| 14$^{th}$ day | Infectious Bursal Disease | IBD (Live) | One drop in each eye |

After each vaccination, Rena -WS multivitamin was supplied at 1g/5 liter of drinking water along with vitamin-C to overcome the stressed effect of vaccination and hot weather.


**Figure 3.9.7.a:** Vaccination of broiler

Chemo prophylactic measures/medication with water soluble vitamins, minerals and electrolyte were used at different ages of birds, details of which are given below:

**Table 3.9.7.b:** Schedule of chemo prophylaxis/medication

| Age of the birds | Drugs used through water |
|---|---|
| 1-7 days | Rena-WS + Electrolyte + Gluco-C |
| 10-17 days | Rena-WS + Electrolyte + Gluco-C |
| 18-28 days | Rena-WS + Electrolyte + Lemon + Gluco-C |

### 3.9.8. Bio-security/Sanitation

Drinkers were washed with caustic soda and dried up daily in the morning, and feeders were also cleaned and washed with caustic soda every 3 day after. Potassium permanganate was used for washing the floor & nearer places of the shed. Lime powder and bleaching powder was also used for strict bio-security measures those were followed during the whole experimental period.

## 3.10. Record keeping

Following parameters were recorded throughout the experimental period.

### Body weight

Body weight of the chicks was recorded at first day and then regular basis at the weekly intervals by a digital weighing balance for whole experimental period.

**Figure 3.10:** Weight measurement of broiler

**Feed intake**

Weekly feed intake was calculated by deducting the left over feeds from the total amount of supplied feed to the broilers.

**Mortality**

Mortality was recorded throughout the experimental period when death occurred in any replication.

### 3.11. Calculation of data

**Body weight gain**

The body weight gain was calculated by deducting initial body weight from the final body weight of the birds.

Body weight gain = Final body weight - Initial body weight

**Feed intake**

Quantity of offered feed was weighed weekly. Refusal feed was recorded to determine the feed intake per week. Average feed intake was calculated weekly as gm/bird.

**Feed conversion (FC)**

The amount of feed intake per unit of weight gain is the feed conversion (FC). This was calculated by using following formula.

$$FC = \frac{\text{Feed intake (kg)}}{\text{Weight gain (kg)}}$$

**Mortality**

It was calculated on the basis of total number of birds housed and number of birds died during the experimental period. The mortality was expressed in percent.

### 3.12. Collection of blood and serum separation

On the day 28, two birds were selected from each replication randomly for collection of blood. About 2.5 ml of blood was collected from every bird by sterile syringe and put those syringe in refrigerator vertically. After 6 hours serum was collected in sterile plastic vial to estimate serum parameters.


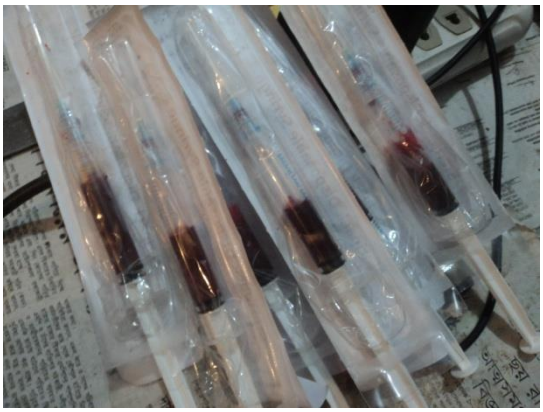

**Figure 3.12:** Collection of blood from broiler

### 3.13. Evaluation of carcass traits

On day 28, five birds per experimental unit representative of average body weight were selected for the evaluation of carcass traits. Replicate groups were randomly selected for carcass and organ weight evaluation after fasting them over night with supplementation of only drinking water. The birds were weighed, slaughtered by severing the jugular vein and allowed to bleed thoroughly. Birds were scalded at 75°C in a water bath for about 30 seconds before defeathering and then the birds were

reweighed to calculate feathers weight by difference. The dressed chicks were later eviscerated. The wings were removed by cutting anteriorly severing at the humeoscapular joint, the cuts were made through the rib head to the shoulder girdle, the back were removed intact by pulling anteriorly. Thighs and drum stick were dissected from each carcass and weighed separately. The measurement of the carcass traits (dressed weight %, eviscerated weight %, thigh, shank, chest, back, neck, wing, abdominal fat and head) were taken before dissecting out the organs. All the carcass traits except the dressed and eviscerated weight were expressed as percentages of the live weight while the organs weight were expressed in g/kg body weight. The following traits were evaluated: carcass yield (CY), weight of primal parts (drumstick, thigh, breast, back, neck, wing and feet) and weight of internal edible offal (gizzard and proventriculus, heart, liver, abdominal fat and neck fat). Carcass yield (CY) was calculated relative to live weight before slaughter.

$$\text{Carcass yield (CY)}\% = \frac{\text{Carcass weight} \times 100}{\text{Live weight}}$$

**Figure 3.13:** Evaluation of carcass traits

### 3.14. Chemical analysis of RESM, RD, formulated feed and Meat

After processing of RESM and RD about 200 gm sample was collected for chemical analysis. There were two different sample was collected for each in case of RESM and RD. After chemical analysis the rations were formulated as needed as experiment. After formulation of diets about 200 gm of sample (two samples) from each diet was taken for chemical analysis. These laboratory works were done before the arrival of DOC in poultry shed.

After determination of carcass traits, 150-200 gm meat sample (breast meat) was collected from each of 15 slaughtered broilers of 28 day old (five birds per experimental unit) for chemical analysis of meat samples. The samples were preserved in plastic bag, minced, dried in oven and grinded. Dry matter (DM), crude protein (CP), crude fiber (CF), ether extracts (EE) along with other proximate components were estimated according to the methodology of AOAC method (AOAC, 2006).

The experimental samples were also subjected for proximate analysis for moisture, dry matter (DM), crude protein (CP), ether extracts (EE), crude fiber (CF), total ash and insoluble ash in the Animal Nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh in accordance with standard methods described by the AOAC (2006).

**Table 3.14.a:** Proximate composition of RESM

| **Traits** | **Proximate value (%)** |
|---|---|
| Dry Matter (DM) | 89.12 |
| Crude Protein (CP) | 52.09 |
| Crude Fibre (CF) | 2.79 |
| Ether Extract (EE) | 6.32 |
| Ash | 15.25 |
| Nitrogen Free Extract (NFE) | 23.56 |

**N.B.:** ME value of RESM = **4090** kcal/kg (Alikwe et al., 2013)

**Table 3.14.c:** Proximate composition of RD

| **Traits** | **Proximate value (%)** |
|---|---|
| Dry Matter (DM) | 89.61 |
| Crude Protein (CP) | 18.97 |
| Crude Fibre (CF) | 27.43 |
| Ether Extract (EE) | 3.15 |
| Ash | 15.31 |
| Nitrogen Free Extract (NFE) | 35.15 |

**N.B.:** ME value of RD = **2190** kcal/kg (Elfaki et al., 2015)

**Table 3.14.c:** Proximate composition of the experimental broiler starter diets

| **Traits** | **Proximate value (%)** | | |
|---|---|---|---|
| | **$T_0$** | **$T_1$** | **$T_2$** |
| Dry Matter (DM) | 84.31 | 85.27 | 86.69 |
| Crude Protein (CP) | **21.87** | **21.89** | **21.98** |
| Crude Fibre (CF) | 3.55 | 4.27 | 4.58 |
| Ether Extract (EE) | 4.48 | 4.55 | 4.09 |
| Ash | 7.40 | 6.77 | 6.09 |
| Nitrogen Free Extract (NFE) | 62.7 | 62.52 | 63.26 |

In table, $T_0$ = Control diet without RESM & RD, $T_1$ = Experimental diet with 5% RESM & RD, $T_2$ = Experimental diet with 10% RESM & RD

**Table 3.14.d:** Proximate composition of the experimental broiler finisher diets

| **Traits** | **Proximate value (%)** | | |
|---|---|---|---|
| | **$T_0$** | **$T_1$** | **$T_2$** |
| Dry Matter (DM) | 87.03 | 86.39 | 86.80 |
| Crude Protein (CP) | **20.48** | **20.39** | **20.53** |
| Crude Fibre (CF) | 3.69 | 3.92 | 4.58 |
| Ether Extract (EE) | 5.12 | 5.47 | 5.38 |
| Ash | 5.69 | 4.95 | 4.32 |
| Nitrogen Free Extract (NFE) | 65.02 | 65.27 | 65.19 |

In table, $T_0$ = Control diet without RESM & RD, $T_1$ = Experimental diet with 5% RESM & RD, $T_2$ = Experimental diet with 10% RESM & RD

**Table 3.14.e:** Proximate composition of the experimental broiler meat of different treatment groups

| **Traits** | **Proximate value (%)** | | |
|---|---|---|---|
| | **$T_0$** | **$T_1$** | **$T_2$** |
| Dry Matter (DM) | 26.69 | 29.93 | 27.54 |
| Crude Protein (CP) | 63.24 | 65.09 | 65.30 |
| Crude Fibre (CF) | 0 | 0 | 0 |
| Ether Extract (EE) | 19.80 | 24.32 | 25.11 |
| Ash | 2.47 | 2.13 | 3.46 |
| Nitrogen Free Extract (NFE) | 14.50 | 8.46 | 6.13 |

In table, $T_0$ = Control diet without RESM & RD, $T_1$ = Experimental diet with 5% RESM & RD, $T_2$ = Experimental diet with 10% RESM & RD

**Figure 3.14.a:** Dry matter determination

**Figure 3.14.b:** Crude protein determination

**Figure 3.14.c:** Crude fiber determination

**Figure 3.14.d:** Ether extract determination

### 3.15. Cost-benefit analysis

In case of cost analysis, chick cost, total feed cost, management cost and finally total cost were calculated in Taka per bird. Total feed cost included to feed raw materials cost. Management cost included vaccination cost, labour cost, electricity cost, disinfectant cost and litter materials cost. In case of return, market sale price, total sale price and net profit were calculated in Taka per bird.

### 3.16. Blood parameter estimation

Blood was collected without anticoagulant from a total 6 birds from each group (2 birds from each replicate) at $30^{th}$ days of age of broilers. Serum was separated after centrifugation at 3,000 rpm for 15 minutes. Different blood parameters were measured in the post graduate laboratory under the department of Physiology, Biochemistry and Pharmacology, CVASU using standard kits (BioMereux, France) and automatic analyzer (Humalyzer 300, Merck®, Germany) according to the manufacturer's instruction (FVMAAU; Addis Ababa, Ethiopia).

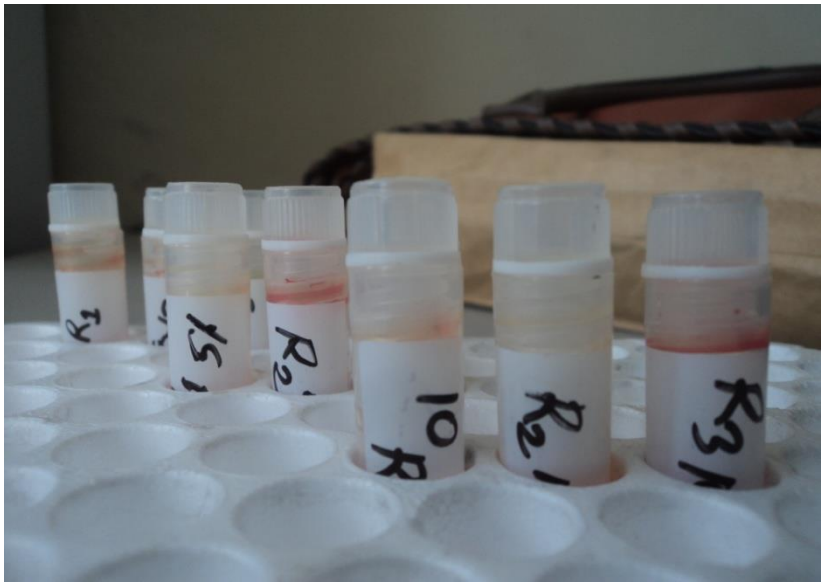

**Figure 3.16:** Serum samples for chemical analysis

### 3.17. Statistical analysis

All the data of live weight, weight gain, feed consumption and feed conversion etc., related to carcass parameters, blood parameters and chemical analysis of meat were entered into MS excel (Microsoft office excel-2007, USA). Data were compared among the groups by one way ANOVA in STATA version-12.1 (STATA Corporation, College Station, Texas) and subsequent Duncan's Multiple Range Tests (DMRT). Results were expressed as means and SEM. All P values of ≤0.05 and ≤0.01 were considered significant and highly significant, respectively.

# Chapter IV: Results

## 4.1. Feed consumption

**Table 4.1.1:** Weekly feed intake of broilers among different dietary treatment groups (gm/broiler)

| Age of birds | Feed intake (gm/bird) | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| 1st week | 137.0 | 142.50 | 149.41 | 0.18 | 0.08 | NS |
| 2nd week | 264.04[a] | 276.82[b] | 255.40[c] | 0.65 | 0.01 | ** |
| 3rd week | 717.76 | 708.39 | 726.84 | 0.58 | 0.07 | NS |
| 4th week | 1080.18[a] | 1420.09[b] | 1521.22[b] | 2.13 | 0.00 | ** |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, NS = Non significant, SEM = Standard error of mean, ** = significant at 1% level

It was represented that the amount of feed consumption of birds at from 1st to 4th weeks of age of broilers (Table 4.1.1). No significant difference ($P<0.05$) in feed consumption of birds in different groups was observed at 1st week of age. Feed consumption by birds was found significantly ($P\leq0.01$) increased in birds treated with 5% RESM and RD ($T_1$) in comparison with other groups at 2nd week of age. At 3rd week of age, there was no significant ($P<0.05$) difference in feed consumption of birds in different treatment groups. At the end of the experiment (4th week of age) feed consumption by birds was found significantly ($P<0.01$) increased in $T_1$ and $T_2$ groups. Highest feed consumption was found in birds of 10% RESM and RD treatment group ($T_2$) among all the groups. However, lowest feed intake was observed in $T_0$ or control group.

**Table 4.1.2:** Cumulative feed intake of broilers among different dietary treatment groups (gm/broiler)

| Age of birds | Cumulative feed intake (gm/bird) | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| 1-2 weeks | 401.04 | 419.32 | 404.81 | 0.03 | 0.09 | NS |
| 1-3 weeks | $1118.8^a$ | $1127.71^b$ | $1131.65^c$ | 0.11 | 0.00 | ** |
| 1-4 weeks | $2198.98^a$ | $2547.80^b$ | $2652.87^c$ | 0.57 | 0.00 | ** |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS = Non significant, ** = significant at 1% level

Table 4.1.2 indicated that, the difference in cumulative feed intake of birds among different groups was not significant (P>0.05), statistically up to 2nd week of age of birds. However, highly significant (P<0.01) differences were observed both at 3rd and 4th weeks of age. An increased cumulative feed consumption was observed both in $T_1$ and $T_2$ groups in comparison with control group ($T_0$).

### 4.2. Live weight

**Table 4.2.1:** Weekly live weight of broilers among different dietary treatment groups (gm/broiler)

| Age of birds | Live weight (gm/bird) | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| Day 1 | 38.8 | 38.33 | 38.70 | 0.13 | 0.29 | NS |
| 1st week | $157.93^a$ | $169.06^a$ | $182.36^b$ | 1.67 | 0.04 | * |
| 2nd week | $369.16^a$ | $407.7^b$ | $410.4^b$ | 3.46 | 0.00 | ** |
| 3rd week | $796.4^a$ | $853.23^b$ | $867.53^b$ | 6.32 | 0.00 | ** |
| 4th week | $1431.8^a$ | $1698.52^b$ | $1773.02^c$ | 14.96 | 0.00 | ** |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS = Non significant, * = significant at 5% level, ** = significant at 1% level

From table 4.2.1 it was found that, initially there was no significant difference (P>0.05) in live weight of the birds in different dietary treatment groups ($T_0$, $T_1$, $T_2$). Difference in live weight of broilers among the groups was observed significant (P<0.05) with increasing age (1st week). Live weight among broilers differed

significantly (P<0.01) at 2nd week of age also and was higher in $T_1$ and $T_2$ groups in comparison with control group ($T_0$). Concordant results were found both at 3rd and 4th weeks of age of broilers and highly significant (P<0.01) differences in live weight of broilers were observed among the dietary treatment groups. 10% RESM & RD treatment group ($T_2$) gained highest weight among the treatment groups.

**Table 4.2.2:** Cumulative live weight of broilers among different dietary treatment groups (gm/broiler)

| Age of birds | Cumulative live weight (gm/bird) | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| 1-2 weeks | 527.09 | 576.76 | 592.76 | 0.13 | 0.07 | NS |
| 1-3 weeks | 1323.49[a] | 1429.99[b] | 1460.29[b] | 1.17 | 0.00 | ** |
| 1-4 weeks | 2755.29[a] | 3128.51[b] | 3233.31[b] | 5.12 | 0.04 | * |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS = Non significant, * = significant at 5% level, ** = significant at 1% level

The differences in cumulative live weight of birds were not significant (P<0.05) up to 2nd weeks of age of birds from different treatment groups. Highly significant difference (P<0.01) was observed at 3rd week of age of broilers and control group showed lower weight than other two groups ($T_1$ & $T_2$). Significant (P<0.05) difference was observed at the end of the experiment (4th week of age of broilers). Cumulative live weights in both $T_1$ & $T_2$ groups were significantly (P<0.05) higher compared to $T_0$ group (Control).

### 4.3. Live weight gain

**Table 4.3.1:** Weekly live weight gain of broilers among different dietary treatment groups (gm/broiler)

| Age of birds | Live weight gain (gm/bird) | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| 1st week | 119.13 | 130.73 | 143.66 | 1.68 | 0.09 | NS |
| 2nd week | 211.23 | 238.64 | 228.04 | 3.12 | 0.07 | NS |
| 3rd week | 427.24 | 445.53 | 457.13 | 5.59 | 0.06 | NS |
| 4th week | $635.4^a$ | $845.29^b$ | $905.49^c$ | 15.14 | 0.04 | * |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS = Non significant, * = significant at 5% level

Table 4.3.1 represented that, from 1st to 3rd weeks of age, the difference in live weight gain of broilers among dietary treatment groups were not significant ($P>0.05$), statistically. However, $T_1$ and $T_2$ groups gained slightly higher body weight than control group. Significant difference ($P<0.05$) in weight gain was observed with the advancement of age of birds. At the end of the experiment (4th weeks of age), birds from $T_1$ and $T_2$ groups gained significantly ($P<0.05$) higher live weight than $T_0$ group.

**Table 4.3.2:** Cumulative live weight gain of broilers among different dietary treatment groups (gm/broiler)

| Age of birds | Cumulative live weight gain (gm/bird) | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| 1-2 weeks | $330.36^a$ | $369.37^b$ | $371.70^b$ | 1.16 | 0.00 | ** |
| 1-3 weeks | $757.60^a$ | $814.90^b$ | $828.83^b$ | 4,21 | 0.04 | * |
| 1-4 weeks | $1393.0^a$ | $1660.19^b$ | $1734.32^c$ | 8.55 | 0.02 | * |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, ** = significant at 1% level, * = significant at 5% level

Cumulative live weight gain in birds among different dietary treatment groups differed significantly throughout the whole experimental period which was highly significant ($P<0.01$) up to 2nd weeks of age of birds (table 4.3.2). At 3rd and 4th weeks of age there were highly significant ($P<0.05$) differences in cumulative live weight

gain among the treatment groups. $T_1$ and $T_2$ groups represented higher cumulative live weight gain compared to $T_0$ group.

### 4.4. Feed conversion (FC)

**Table 4.4.1:** Weekly feed conversion of broilers among different dietary treatment groups

| Age of birds | Feed conversion (FC) | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| 1st week | 1.15[a] | 1.09[b] | 1.04[b] | 0.02 | 0.00 | ** |
| 2nd week | 1.25[a] | 1.16[b] | 1.12[b] | 0.03 | 0.00 | ** |
| 3rd week | 1.68[a] | 1.59[b] | 1.58[b] | 0.02 | 0.00 | ** |
| 4th week | 1.70[a] | 1.68[b] | 1.64[b] | 0.05 | 0.00 | ** |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, SEM = Standard error of mean, ** = significant at 1% level

Feed conversion was significantly higher (P<0.01) in control group than RESM & RD supplemented groups at 1st and 2nd weeks of age of broilers (table 4.4.1). Likewise it was found significantly (P<0.01) lower in RESM & RD treatment groups of broiler both at 3rd and 4th weeks of age of birds. However, 10% RESM & RD supplemented group ($T_2$) showed better feed conversion than 5% RESM & RD supplemented groups ($T_1$). Highest feed conversion was observed in control group.

**Table 4.4.2:** Cumulative feed conversion of broilers among different dietary treatment groups

| Age of birds | Cumulative feed conversion | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| 1-2 weeks | 1.21[a] | 1.14[b] | 1.09[b] | 0.05 | 0.01 | ** |
| 1-3 weeks | 1.48[a] | 1.38[b] | 1.37[b] | 0.06 | 0.00 | ** |
| 1-4 weeks | 1.58[a] | 1.54[b] | 1.53[b] | 0.04 | 0.00 | ** |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, ** = significant at 1% level

Table 4.4.2 depicted that, cumulative feed conversion of broilers differed significantly (P≤0.01) throughout the whole experimental period. Significantly (P≤0.01) better feed conversion was found in RESM & RD treatment groups ($T_1$ and $T_2$) than control

group from 1st to 4th weeks of age of broilers. Cumulative feed conversion was lowest in $T_2$ group among all the groups.

### 4.5. Effect of different diets on Carcass quality of broilers

**Table 4.5.1:** Final body weight, carcass weight and carcass yield of broiler among different treatment groups at 28th days of broilers (gm/broiler)

| Traits | Mean | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| Final body weight (gm) | $1422.8^a$ | $1522.4^b$ | $1621.2^c$ | 6.53 | 0.01 | ** |
| Carcass weight (gm) | $887.8^a$ | $929^b$ | $975.4^c$ | 5.31 | 0.02 | ** |
| Carcass yield (CY) % | 60.71 | 61.02 | 60.15 | 0.30 | 0.40 | NS |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS = Non significant, ** = significant at 1% level

Table 4.5.1 illustrated that, the increase in final and eviscerated weight of birds observed from both 5% and 10% RESM & RD treatment groups were highly significant ($P<0.01$), statistically. However, there was an increase in carcass yield in 5% RESM & RD treatment group ($T_1$). The difference was insignificant ($P>0.05$), statistically.

**Table 4.5.2:** Weight of primal parts and internal edible organs of broilers at 28th days of age (gm/broiler)

| Traits (gm) | Mean | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| **Primal Parts** | | | | | | |
| Drumstick | 62.4[a] | 68.0[b] | 73.2[c] | 0.81 | 0.01 | ** |
| Thigh | 132.8[a] | 140.4[b] | 153.0[c] | 2.28 | 0.00 | ** |
| Breast | 284.4 | 305.2 | 310.2 | 5.32 | 0.07 | NS |
| Back | 197.8[a] | 211.8[b] | 214.6[c] | 3.22 | 0.05 | * |
| Neck | 42.6[a] | 42.8[a] | 46.4[b] | 0.62 | 0.02 | * |
| Wing | 36.4 | 36.6 | 40.6 | 1.66 | 0.06 | NS |
| Feet | 32.9[a] | 33.4[b] | 35.4[c] | 0.30 | 0.01 | ** |
| Head | 40.6[a] | 42.4[b] | 42.8[b] | 0.44 | 0.02 | * |
| **Internal Edible Organs** | | | | | | |
| Liver | 33.2[a] | 35.5[b] | 37.6[c] | 0.64 | 0.01 | ** |
| Heart | 8.72[a] | 11.09[c] | 10.58[b] | 0.29 | 0.00 | ** |
| Gizzard | 31.4 | 32.8 | 33.2 | 0.37 | 0.07 | NS |
| Spleen | 1.61 | 1.73 | 1.82 | 0.04 | 0.09 | NS |
| Abdominal fat | 10.57 | 11.40 | 11.59 | 0.24 | 0.14 | NS |
| Neck region fat | 4.94[a] | 5.13[b] | 5.58[b] | 0.09 | 0.02 | * |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, SEM = Standard error of mean, NS = Non significant, * = significant at 5% level, ** = significant at 1% level

Highly significant differences ($P<0.01$) were observed in weight of drumstick, thigh, feet of birds (table 4.5.2). Control group showed lower weight than other two groups. Significant differences ($P<0.05$) were observed in weight of back, neck and head in different dietary treatment groups. Highest weight was observed in all parameters of 10% RESM & RD supplemented group ($T_2$). Next to this group of birds from $T_1$ group (5% RESM & RD supplemented group) gained higher weight. However, there was no significant difference ($P>0.05$) in weight of breast, wing among different dietary treatment groups.

Weights of internal organs weight were also measured in this experiment. At the end of the experiment weight of liver, heart became significantly (P<0.01) lower in control group than other two groups. The differences in weight of gizzard, spleen and abdominal fat were not significant (P>0.05), statistically. Those parameters were apparently higher in RESM & RD treatment groups than control group. Significant (P<0.05) increase in neck region fat weight was observed in 5% and 10% RESM & RD treatment groups than control group (table 4.5.2).

### 4.6. Effect of RESM & RD on cost benefit analysis of broiler

**Table 4.6.1:** Cost of production and returns in different treatment groups

| **Cost items** | | | | |
|---|---|---|---|---|
| **Parameters** | **$T_0$** Mean±SEM | **$T_1$** Mean±SEM | **$T_2$** Mean±SEM | **Level of sig.** |
| Chick cost (Tk./Chick) | 50.00 | 50.00 | 50.00 | NS |
| Total feed cost (Tk./Kg) | 35.06 | 33.04 | 32.23 | NS |
| Management cost (Tk./broiler) | 17.00 | 17.00 | 17.00 | NS |
| Total feed cost (Tk./broiler) | $77.13^{a}$±0.03 | $84.25^{b}$±0.14 | $85.41^{b}$±0.10 | * |
| Total cost (Tk./broiler) | $144.13^{a}$±0.03 | $151.25^{b}$±0.09 | $152.41^{b}$±0.15 | * |
| Total cost (Tk./Kg live broiler) | $100.79^{b}$±0.46 | $88.97^{a}$±0.25 | $86.11^{a}$±0.78 | * |
| **Income** | | | | |
| Market sale price (Tk./Kg broiler) | 120.00 | 120.00 | 120.00 | NS |
| Total sale price (Tk./broiler) | $171.60^{a}$±0.72 | $204.00^{b}$±0.81 | $212.40^{c}$±0.71 | * |
| Net Profit (Tk./broiler) | $27.47^{a}$±0.71 | $52.75^{b}$±0.69 | $59.99^{c}$±0.58 | * |
| Net Profit (Tk./Kg live broiler) | $19.21^{a}$±0.24 | $31.03^{b}$±0.16 | $33.89^{b}$±0.78 | * |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS = Non significant, * = significant at 5% level, ** = significant at 1% level

**N.B.** Total feed cost included feed raw materials cost; Management cost included vaccination cost, electricity cost, disinfectant cost and litter material's cost.

Significant (P<0.05) differences were found in total feed cost, total cost, total sale price and net profit among the dietary treatment groups (Table 4.6.1). Total feed cost (Tk./broiler) was less in RESM & RD based diet than control. No significant (P>0.05) differences were observed in chick cost (Tk./chick), management cost (Tk./broiler). In terms of profit, net profit (Tk./broiler) and net profit (Tk./Kg live broiler) differed significantly (P<0.05) among the treatment groups. RESM & RD treated groups showed higher net profit than control group. Significant increase (P<0.05) in profit was observed with increasing level of RESM & RD. Highest profit was observed in 10% RESM & RD treated group. Next to this group 5% RESM & RD treated group gained higher net profit

### 4.7. Effect of different diets on blood parameters of broilers

**Table 4.7.1:** Different serum constituents level of broilers at 28$^{th}$ days of age

| Parameter | Serum constituents level | | | SEM | P value | Level of Sig. |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | | | |
| Cholesterol (mg/dl) | 141.7 | 125.46 | 122.26 | 4.18 | 0.06 | NS |
| Total Protein (gm/dl) | 6.07$^a$ | 5.00$^b$ | 5.87$^c$ | 0.16 | 0.00 | ** |
| Albumin (gm/dl) | 1.47$^a$ | 1.20$^b$ | 0.81$^c$ | 0.11 | 0.03 | * |
| Triglyceride (mg/dl) | 218.91$^a$ | 190.78$^b$ | 151.56$^c$ | 8.62 | 0.00 | ** |
| ALT (U/L) | 10.16$^a$ | 12.25$^a$ | 6.05$^b$ | 3.43 | 0.01 | ** |
| AST (U/L) | 326.61 | 319.86 | 347.25 | 15.79 | 0.67 | NS |

$T_0$ = Control, $T_1$ = 5% RESM & RD, $T_2$ = 10% RESM & RD, Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS = Non significant, * = significant at 5% level, ** = significant at 1% level

Table 4.6.1 indicated that, cholesterol (mg/dl) level was decreased in $T_1$ and $T_2$ groups in comparison with control though the difference was non-significant (P>0.05), statistically. The decrease in total protein level of both $T_1$ and $T_2$ groups than $T_0$, was highly significant (P<0.01), statistically. Increased value was observed in $T_2$ than $T_1$ group. Significant difference (P<0.05) was observed in albumin (gm/dl) level of blood of broilers among the treatment groups at 28$^{th}$ days of age. However, decreased values of albumin were observed in $T_1$ and $T_2$ groups. Control group showed highest value than other two groups. Triglyceride levels in blood of birds of $T_1$ and $T_2$ groups were lower than control group ($T_0$) and this difference was highly significant (P<0.01),

statistically. The increase in ALT level of 5% RESM & RD treatment group was highly significant ($P \leq 0.01$) which was lowest in 10% RESM & RD dietary treatment group ($T_2$). The difference in AST level of different treatment groups was significant ($P > 0.05$). However, decreased AST level was found in $T_1$ group than other two groups.

# Chapter V: Discussion

## 5.1. Growth performance of broilers

### 5.1.1. Feed consumption of broiler

Table 4.1.1 indicated that significant differences were found in feed consumption of birds at 1st and 3rd weeks of age of birds (P>0.05). At 2nd week, there were highly significant differences (P≤0.01) in feed consumption of bird groups among the groups supplemented with RESM & RD or without RESM & RD. Though feed intake was lowest in 10% RESM & RD supplemented group ($T_2$) and highest in $T_1$ group. At the end of the experiment highest feed consumption was observed in $T_2$ group.

Table 4.1.2 represented differences in cumulative feed consumption by birds were not significant (P>0.05) up to 2nd weeks of age. Highly significant (P<0.01) differences were observed both up to 3rd and 4th weeks of age. Control group showed lower feed consumption than other two groups at 2nd and 4th weeks of age. At 4th week of age highest feed consumption was observed in 10% RESM & RD supplemented groups among the treatment groups.

The results of increasing feed consumption with increasing level of RESM are in concordance with Alikwe et al. (2013). They observed that feed intake of birds supplemented with 25%, 50% or 75% RESM (Rumen Epithelial Scrapings Meal) was higher than control group and that trend of increasing feed consumption was observed with increasing level. Another study in rabbit also revealed higher feed consumption in Cattle Rumen Epithelial Scrapings Meal (CRESM) treatment group than control. Higher feed consumption was recorded with increasing level of RESM (Oladunjoye et al., 2013a). However, it was reported that no significant difference (P>0.05) was observed in feed consumption by birds treated with either RESM or fish meal (FM) though it was higher compared to control group (Faremi et al., 2010). Another study revealed that dietary treatment with CRESM had no significant (P>0.05) effect on feed intake of birds (Oladunjoye et al., 2013b).

Alikwe et al. (2014) reported that the equality in feed consumption of birds treated either with RESM or Fish Meal (FM) may be due to equal crude fibre (around 9.5%)

content in both meals. Results of the feed intake in the starters depicted an increase with increasing level of RESM up to 75%.

Fish meal was replaced by Bovine Rumen Epithelial Tissue Scrapings (BRETS) with 0%, 50%, and 100% level in feed of broiler. Significant difference ($P<0.05$) in both the total feed intake and daily feed intake was found. Birds in dietary treatment A (0% BRETS) consumed most, followed by birds in dietary treatment C (100% BRETS) while birds fed diet B (50% BRETS) consumed least (Salami et al., 2013).

There was found significant difference ($P<0.05$) among the control and dried rumen digesta (DRD) groups in case of feed intake. The result shown that DRD at 40% dietary inclusion could replace soybean in the diet of *Oreochromis niloticus* fingerlings without compromising growth (Agbabiaka et al., 2011). Another study reported that feed intake of the birds on varying inclusion levels of dried rumen content were significantly ($P<0.05$) higher than the control group (Elfaki et al., 2015).There was significant difference in ($P<0.05$) in both the total feed intake and daily feed intake. Birds in dietary treatment A [0% Bovine rumen epithelial tissue scrapings (BRETS)] consumed most, followed by birds in dietary treatment C (100% BRETS). The probable reason behind higher feed intake of birds fed diet A was that they consumed more to meet their protein requirement due to lower CP content in fish meal compared to Bovine rumen epithelial tissue scrapings (BRETS) (Salami et al., 2013).

### 5.1.2. Live weight of broilers

The responses of broilers in acquiring live weight treated with or without RESM & RD were shown in table 4.2.1. Initial body weight of birds among different groups differed slightly but those values were not significant ($P>0.05$), statistically. It indicates a higher possibility of having similar weighted birds in different groups prior the beginning of the treatment. Live weight of broilers was improved significantly ($P<0.01$) in RESM & RD treatments compared to control group throughout the whole experimental period. 10% RESM & RD supplemented group ($T_2$) gained slightly higher weight gain than 5% RESM & RD supplemented group ($T_1$).

Table 4.2.2 depicted the differences in cumulative live weight of birds in different treatment groups. It was insignificant ($P<0.05$) up to $2^{nd}$ weeks of age of birds. At $3^{rd}$

week of age, the difference was highly significant (P<0.01) among different dietary treatment groups. At 4th week of age of broiler the difference in cumulative live weight was significant (P<0.05), statistically among the groups. In both 3rd and 4th weeks of age cumulative live weights of birds were higher in RESM & RD treatment groups. $T_2$ group showed highest cumulative live weight among all the treatment groups. Next to this group was $T_1$ group representing higher cumulative live weight.

The result of increasing weight with increasing level of RESM is in agreement with Alikwe et al., 2014; Alikwe et al., 2013 and Faremi et al., 2010. In addition, Oladunjoye et al. (2013a) recorded that though live weight increased in rabbit with increasing level of RESM it decreased with 100% RESM. Alikwe et al. (2014) reported that drying ensures reduction or destruction of microbes in feed. Processing method and the nutrient content of RESM both affect in live weight and weight gain of broilers. Live weight value of birds in the control was superior to all others though the result was not significant (P>0.05). Birds with 75% RESM had the least live and eviscerated weight. In another study it was recorded that dietary treatment with CRESM (Cattle Rumen Epithelial Scrapings Meal) had no significant (p>0.05) effect on weight gain and live weight gain (Oladunjoye et al., 2013b).

### 5.1.3. Live weight gain of broilers

The weekly live weight gain in different ages of broilers fed diets supplemented with or without RESM & RD has presented in table 4.3.1. Up to 3rd weeks of age no significant difference (P>0.05) was observed among different dietary treatment groups though control groups gained lower weight than either 5% or 10% RESM & RD treatment groups. However, the differences in live weight gain of birds at 4th week of age of birds were significant (P<0.05).

Table 4.3.2 represented the difference in cumulative live weight gain in birds among different dietary treatment groups which was highly significant (P<0.01) up to 2nd weeks of age. Significant (P<0.05) differences were observed in both 3rd and 4th weeks of age of broilers. $T_1$ and $T_2$ groups represented higher cumulative live weight gain than $T_0$ group (Control).

No significant difference was observed in final weight, total weight gain and daily weight gain of different treatment groups i.e. control group and those with 25% and

50% CRESM (Cattle Rumen Epithelial Scrapings Meal) replacing fish meal (Oladunjoye et al., 2013b). It was observed that 100% RESM supplemented group gained lower weight at finisher stage among different levels of RESM supplemented groups (Alikwe et al., 2013). The result of the performance of starter for average body weight gain was significantly differed ($P<0.05$). The general trend for this parameter in the starter phase was increased with increasing levels of dietary RESM in replacement of fish meal. The replacement of fish meal with RESM in broiler chicken starter, grower and finisher diet resulted higher weight gain compared to control diets (Alikwe et al., 2014).

At the starter phase, birds treated with 100% RESM and 0% FM with the least feed intake had the highest body weight gain though the result was statistically similar in birds treated with 75% RESM and 25% FM. It was different from $T_1$ and $T_3$ groups with 0% RESM and 100% FM, 50%RESM and 50% FM respectively (Alikwe et al., 2014). In another study, there was no significant difference ($P>0.05$) in mean final body weights for the three dietary treatments i.e. A (0% BRETS), B (50% BRETS), C (100% BRETS). Here Fish meal was replaced by Bovine Rumen Epithelial Tissue Scrapings (BRETS) with 0%, 50%, and 100% level in feed of broiler (Salami et al., 2013).

Birds on diets containing dried rumen content recorded higher weight gain than the control group. The body weight gain was significantly ($P<0.01$) affected by different dietary treatments. Treatments dried rumen content supplemented with enzymes resulted in a significant improvement in growth rate (Elfaki et al., 2015). Nile Tilapia fingerlings were assigned to five different diets such that dried rumen digesta (DRD) replaced soybean meal at 0, 10, 20, 30 and 40% levels. Result showed that all fish fed DRD based diets performed better than the control group. There was significant difference ($P<0.05$) in weight gain among the control groups and those fed DRD based diets (Agbabiaka et al., 2011).

### 5.1.4. Feed conversion (FC) of broilers

Table 4.4.1 represented weekly feed conversion (FC) of broilers supplemented with or without RESM. The analysis of data revealed that supplementation of RESM & RD at different percentages resulted a significant impact ($P<0.01$) in feed conversion at

different ages of broilers. Feed conversion was significantly ($P<0.01$) improved in bird groups supplemented with either 5% or 10% RESM & RD compared to control group. At 1st to 4th weeks of age feed conversion was better in 10% RESM & RD supplemented groups than 5% RESM & RD supplemented group and control group, showed significantly ($P<0.01$) better feed conversion among all the treatment groups.

The result of cumulative feed conversion of broilers ate different ages indicated in table 4.4.2. Significantly ($P\leq0.01$) higher feed conversion was observed in control in comparison with other groups ($T_1$ & $T_2$) from 1st to 4th weeks of ages. 10% RESM & RD treatment group ($T_2$) showed lower and better cumulative feed conversion up to 4th weeks of age of birds. Cumulative feed conversion was significantly lowest in 10% RESM & RD treatment group ($T_2$) among all the treatment groups both at 1st to 4th weeks of age of broilers.

Similar findings of better feed conversion with supplementing RESM were recorded previously by several researchers (Alikwe et al., 2014; Faremi et al., 2010). Faremi et al. (2010) reported better feed conversion in RESM and fish meal (FM) treatment groups in comparison with control group. In another experiment, FCR was not significantly ($P>0.05$) affected by inclusion of dried rumen content in the diets (Elfaki et al., 2015). Another study also revealed that, dietary treatment with CRESM (Cattle Rumen Epithelial Scrapings Meal) had no significant ($P>0.05$) effect on feed conversion ratio (Oladunjoye et al., 2013b). At that experiment, fish meal was replaced by Bovine Rumen Epithelial Tissue Scrapings (BRETS) with 0%, 50%, and 100% levels in feed of broiler. However, significant difference ($P<0.05$) was observed in the FCR of the three dietary treatments. Birds fed diet A (0% BRETS) having the highest FCR and birds fed diet B (50% BRETS) having the lowest FCR (Salami et al., 2013).

### 5.2. Carcass quality and organ characteristics of broilers

Table 4.5.1 represented final body weight, eviscerated weight of birds in different dietary treatment groups. Highly significant ($P<0.01$) increase in final weight and eviscerated weight was observed in $T_1$ and $T_2$ treatment groups compared to control group ($T_0$). Difference in carcass yield (CY) percentage in different treatment groups were not significant ($P>0.05$) statistically, though it was apparently higher in 5%

RESM & RD supplemented group in comparison with other two groups. Those results of increasing weight, eviscerated weight, carcass yield or dressing percentage with RESM treatment are in concordant with previous findings.

Alikwe et al., (2014) showed that the dressing% and eviscerated% in case of 100% RESM group were superior to all other treatments though the result were not significant ($P>0.05$), statistically. They also observed that eviscerated weight of 25% RESM was higher in comparison with control group.

Table 4.5.2 illustrated the weight of primal parts and internal edible organs of broilers at $28^{th}$ days of age. There were highly significant differences ($P<0.01$) among the treatment groups in case of weights of drumstick, thigh, feet of birds. Higher weights were found in all the parameters in RESM & RD treatment groups ($T_1$ & $T_2$) compared to control. Weights of back, neck and head also significantly ($P<0.05$) differed and were highest in 10% RESM & RD treatment group ($T_2$). Lowest weight was found in control group ($T_0$). No significant differences ($P>0.05$) were found in weight of breast, wing among the groups. Liver and heart weights were significantly ($P<0.01$) lower in control group than both 5% and 10% RESM & RD treatment groups. However, no significant ($P>0.05$) differences were observed in weight of gizzard, spleen and abdominal fat though the weights were apparently lower in control group. Neck region fat weight was significantly ($P<0.05$) higher in both $T_1$ and $T_2$ groups than control.

Alikwe et al. (2014) found no significant difference ($P>0.05$) in weight of wing, head, drum stick, thigh, breast among control and 25%, 75%, 100% RESM treatment groups though they were apparently lower in control group. The back weight significantly differed ($P<0.05$) among the treatment groups with highest weight in 25% treatment group. They also reported that weight of liver, heart and gizzard was higher in 25% RESM treatment group than control group.

The live weight and dressed weight of the birds were significantly ($P<0.05$) affected by dietary treatments with cattle rumen epithelial scrapings meal (CRESM). Final live weight and dressed weight of birds that had 25% and 50% fish meal in their diets replaced with CRESM were similar to that of the control. These were however higher than the values observed for those that had 75 and 100% fish meal in their diets

replaced with CRESM. No significant difference was observed in carcass yield and weights of abdominal fat, liver, kidneys, lung, heart, spleen and gizzard of the birds (Oladunjoye et al., 2013b).

Salami et al. (2013) observed that, the various carcass characteristics parameters of broiler chickens fed graded level of bovine rumen epithelial tissue scrapings (BRETS) showed that significant differences ($P<0.05$) were observed for the values obtained for the live weights, defeathered weights, eviscerated weights and carcass weights. Treatment group A with 0% Bovine rumen epithelial tissue scrapings (BRETS) showed the highest live weights, defeathered weights, eviscerated weights and carcass weights, followed by treatment B (50% BRETS) and treatment C (100% BRETS) had the least values. Significant differences ($P<0.05$) were observed for the values obtained for the head, neck, wing, breast, back and thigh. The breast, thigh and back are regarded as the major meaty parts of the broiler chicken. Birds fed diet B (50% BRETS) had the highest weights of breast, thigh and back. There was no significant difference ($P>0.05$) in the lung and heart weights. Birds in dietary treatment A had the highest lung weight value followed by birds fed diet B and birds fed diet C had the least value.

The report of Ojewola et al. (2005) contrasts with the findings of a study, as significant difference was only observed for kidney weights when evaluating comparative utilization of three animal protein sources by broiler chickens on various organ proportions. However, the non-significant difference observed in the lung and heart weights.

Another study in birds fed diets containing dried rumen content were significantly ($P<0.05$) effected with higher carcass weight than the control group. The highest value recorded in birds fed dried rumen content supplemented with enzymes. Dressing percentage was not significantly ($P>0.05$) influenced by levels of dried rumen content. The liver, spleen, heart, small intestine and abdominal fat were not significantly ($P>0.05$) affected by dietary treatments. The gizzard weight was significantly ($P<0.05$) affected by inclusion of dried rumen content in the diets (Elfaki et al., 2015).

### 5.3. Cost benefit analysis

There were significant ($P<0.05$) differences in total feed cost, total cost, total sale price and net profit among the dietary treatment groups (Table 4.6.1). Total feed cost (Tk./broiler) was less in RESM & RD based diet than control. No significant ($P>0.05$) differences were observed in chick cost (Tk./chick), management cost (Tk./broiler). In terms of profit, net profit (Tk./broiler) and net profit (Tk./Kg live broiler) differed significantly ($P<0.05$) among the treatment groups. RESM & RD treated groups showed higher net profit than control group. Significant increase ($P<0.05$) in profit was observed with increasing level of RESM & RD. Highest profit was observed in 10% RESM & RD treated group. Next to this group 5% RESM & RD treated group gained higher net profit

Feed cost also reduced ($P<0.05$) progressively with increased level of CRESM substitution in the diet at the finisher phase. Feed cost per kilogram weight gain was significantly ($P<0.05$) different among the treatments. Highest cost was observed on birds that were fed 100% CRESM (Cattle Rumen Epithelial Scrapings Meal) as the main source of animal protein, followed by 75%, 0%, 25% and 50% in that order (Oladunjoye et al., 2013b).

Dietary inclusion of dried rumen content (DRC) reduced cost of feed and cost of production, this reflected in the cost and price of meat (Elfaki et al., 2015). Birds fed Sun dried rumen content and blood meal (SDRBM) at 0%, 5% and 10% had higher feed intake ($p<0.05$) than birds fed 15% SDRBM and feed cost per unit weight gain lower ($p<0.05$) for all SDRBM diets than the SDRBM-free diet (Makinde et al., 2008).

### 5.4. Blood parameters of broiler

Table 4.6.1 represented that the cholesterol (mg/dl) level was decreased in 5% and 10% RESM & RD treatment groups ($T_1$ & $T_2$) in comparison with control ($T_0$) though the difference was non-significant ($P>0.05$), statistically. The increase in total protein level of both $T_1$ and $T_2$ groups was highly significant ($P<0.01$), statistically. Significant differences ($P<0.05$) were observed in albumin (gm/dl) level in blood of broilers at $28^{th}$ days of age. However, decreased values were observed in $T_1$ and $T_2$ groups and control group showed highest value than other two groups. Triglyceride

levels in blood of birds of 5% and 10% RESM & RD treatment groups ($T_1$ and $T_2$) were lower than control group and this difference was highly significant ($P<0.01$), statistically. The increase in ALT levels of 5% RESM & RD treatment groups was highly significant ($P\leq0.01$) which was lowest in 10% RESM & RD dietary treatment group. The difference in AST level of different treatment groups was significant ($P>0.05$). However, decreased AST level was found in 5% RESM & RD treatment groups ($T_1$).

Total serum protein e.g. albumin and globulin was highly significant in 75% RES inclusion than 0, 25, 50 and 100% inclusion of RES. On the other hand, Alanine transaminase and Aspartate transaminase in the liver and serum showed no significant difference in case of control and experimental diet with RES (Alikwe et al., 2010). The results of hematological variables of broiler fed with RES recommend that the experimental diets did not effect on chick's health. However, the MCH value in broiler fed 0% and 25% RES were significantly lower than 50%, 75%, 100% RES inclusion groups (Alikwe et al., 2010). Those values were generally higher in the study of Agbede and Aletor (2003), where fish meal was replaced by leaf protein concentrate from Glyricidia.

Blood represents the means of assessing clinical and nutritional health status of animals in feeding trial and the hematological parameters most usually used in nutritional studies include PCV, HBC, MCHC, MCV and clotting time (Adeyemi et al., 2000). Plasma calcium, total protein, glucose, total lipids and cholesterol were not significantly ($P>0.05$) influenced by dietary treatments with dried rumen content (DRC) (Elfaki et al., 2015).

Processed mixtures (discarded vegetable-blood-rumen content) had no effect on hematological parameters but significantly affected serum parameters except albumin and cholesterol values at the starting phase of growth. This indicates that the test additives had no any beneficial effect on hematological status of birds and are nearly similar to each other in hematological parameters. There was a significant increase in the erythrocytic parameters (except erythrocyte indices: MCV, MCH and MCHC) with increasing level of inclusion with the least value obtained in birds fed at 0% inclusion (Ekunseitan et al., 2013).

# Chapter VI: Conclusion

By-products coming from abattoir can be used in the diet of poultry as an animal protein source. Rumen epithelial scrapings meal (RESM) and Rumen digesta (RD) might be successfully used in broiler feeding. It has been reduced both the feed cost and the chances of environmental pollution.

RESM & RD based feed partially replaced soybean meal significantly increased feed consumption that reflected on live weight gain with higher feed conversion (FC) efficiency. So, it can be concluded that RESM & RD (1:1) at the level of either 5% or 10% can be incorporated in broiler ration.

# Chapter VII: Recommendations and Future perspectives

In present experimentation it is revealed that supplementation of RESM & RD partially replacing soybean meal increased the performance of broilers. As it is a pilot study, further studies may be conducted on parallel field to make a concrete remark. However, according to this research work, the following recommendations may be done:

- Soybean meal in broiler diet may be replaced partially by RESM & RD and the inclusion level could be up to 10%.
- The future studies may be with increasing changing levels of RESM and RD (i.e. 12%, 15% etc.).

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## Appendices

**Method of estimating different biochemical parameters of serum (According to manufactures instruction):**

- **Total protein assay**

**Assay principle**

The principle outcome of total protein is based on the principle of competitive bindings between cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample.

**Materials and reagents**

1. Serum sample
2. Total protein conjugate reagent
3. Precision pipettes: 20µl and 1.0ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

**Procedure**

This was a photometric colorimetric test for total proteins are called Biuret method. The sterile eppendorf tubes were taken. Then 20µl of total protein standards was taken in an eppendorf tube and 20µl of sample serums were taken in each 24 eppendorf tube. 1000µl of total protein conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Total protein standards with conjugate.

- **Albumin assay**

**Assay principle**

The principles outcome of albumin is based on the principle of competitive bindings between albumin and albumin reagent. Bromocresol green forms with albumin in citrate buffer a colored complex. The absorbance of this complex is proportional to the albumin concentration in the sample.

**Materials and reagents**

1. Serum sample
2. Albumin conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

**Procedure**

This is a photometric colorimetric test for albumin called Bromocresol Green method. The sterile eppendorf tubes were taken. Then 10µl of albumin standards was taken in an eppendorf tube and 10µl of sample serum were taken in each eppendorf tube. 1000µl of albumin conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 5 minutes. Albumin standards with conjugate reagent were examined first for determined of the standard value. Then all 100 eppendorf tubes containing sample serum with albumin conjugate reagent was examined using automated humalyzer and the reading was taken. The standard value was used as a compared tool.

- **Cholesterol assay**

**Assay principle**

The principles outcome of cholesterol is based on the principle of competitive bindings between cholesterol and cholesterol reagent. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The absorbance of this complex is proportional to the cholesterol concentration in the sample.

**Reaction**

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterage}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

**Materials and reagents**

1. Serum sample
2. Cholesterol conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

**Procedure**

This was an enzmatic colorimetric test for cholesterol is called CHOD-PAP method. The sterile eppendorf tube was taken. Then 10µl of cholesterol standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube. 1000µl of cholesterol conjugate reagent was then added to each eppendorf tube. The

eppendorf tube was then incubated at 37ºC for 10 minutes. Cholesterol standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with cholesterol conjugate reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

- **Triglyceride assay**

**Assay Principle**

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4–aminophenezone and 4–Chlorophenol under the catalytic influences of peroxidease.

**Materials and reagent**

1. Serum sample
2. TG conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves

**Procedure**

The sterile eppendorf tubes were taken. Then 1000µl TG standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube. The eppendorf tube was then kept in room temperature for 10 minute. TG standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

❖ **AST (Aspartate Aminotransferase) or SGOT assay**

**Procedure:**

Aspirate fresh dd$H_2O$ and perform a new Gain Calibration in flow cell mode. Select AST in the Run Test screen and carry out water blank as instructed.

| **Pipette into a test tube:** | |
|---|---|
| Sample | 0.05 ml |
| Reagent | 0.5 ml |

Mix and aspirate into the Rx Monza.

| **Pipette into cuvette:** | | |
|---|---|---|
| | Macro | Micro |
| Sample | 0.2 ml | 0.1 ml |
| $R_1$ Enzyme/ Coenzyme/ α-oxoglutarate | 2.0 ml | 1.0 ml |

Mix, read initial absorbance after 1 min. Read again 1, 2 and 3 min. Note if the absorbance change per minute is between

- 0.11 and 0.16 at 340/ Hg 334 nm
- 0.06 and 0.08 at Hg 365 nm

Use only the values for the first 2 minutes for the calculation.

❖ **ALT (Alanine Aminotransferase) or SGPT assay**

**Procedure:**

Aspirate fresh dd$H_2O$ and perform a new Gain Calibration in flow cell mode. Select ALT in the Run Test screen and carry out water blank as instructed.

| **Pipette into a test tube:** | |
|---|---|
| Sample | 0.05 ml |
| Reagent | 0.5 ml |

Mix and aspirate into the Rx Monza.

| **Pipette into cuvette:** | | |
|---|---|---|
| | Macro | Micro |
| Sample | 0.2 ml | 0.1 ml |
| $R_1$ Enzyme/Coenzyme/α-oxoglutarate | 2.0 ml | 1.0 ml |

Mix, read initial absorbance after 1 min. Read again 1, 2 and 3 min. Note: if the absorbance change per minute is between

- 0.11 and 0.16 at 340/ Hg 334 nm
- 0.06 and 0.08 at Hg 365 nm

Use only the values for the first 2 minutes for the calculation.

Reference: Randox Laboratories Limited, 55 Diamond Road, Crumlin, Country Antrim, BT29 4QY, United Kingdom. www.randox.com

## Brief biography of the author

Kalyanashish Bhadury completed his graduation degree on Doctor of Veterinary Medicine (DVM) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. As an intern student he received clinical training from Madras Veterinary College and Veterinary College & Research Institute, Namakkal, Tamilnadu, India. Kalyanashish has a great enthusiasm in research and has done some nutritional and clinical research works. He has measured the possibility of using biogas effluent as feed for livestock during his internship at Chittagong. He has studied on clinical management of uterine torsion followed by complete post-partum uterine prolapse in murrah buffalo during his internship in VC&RI, Namakkal, Tamil Nadu, India. His research interest is to provide quality and less costly livestock and poultry feed by using unconventional feed ingredients.