EFFECT OF ROUGHAGE BASED DIET ON GROWTH PERFORMANCES OF RABBIT

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DR. ND. Mashiur Rahman
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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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DEDICATED TO MY BELOVED PARENTS AND TEACHERS

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The Author

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LIST OF ABBREVIATIONS

Abbreviation	Elaboration
%	Percentage
*	Significant at 1% level of probability
CF	Crude fibre
CP	Crude protein
DE	Digestible energy
DM	Dry matter
EE	Ether extract
FCR	Feed conversion ratio
Gm	Gram
Hb	Hemoglobin
NFE	Nitrogen free extract
PCV	Packed cell volume
SGPT	Serum glutamic pyruvic transaminase
TEC	Total erythrocyte count
TLC	Total leukocyte count

ABSTRACT

The present study was conducted to evaluate the effect of roughage based diet on growth performances of rabbit like growth performances, hematological and sero-biochemical profile and nutrient digestibility of growing rabbit. There were three dietary treatment groups such as traditional diet (Ad-libitum roughage and concentrate) , 50% concentrate+50% roughage (DM basis), 100% roughage treated as T_0, T_1 and T_2 respectively, in which diets T_1 and T_2 are considered as treatment groups and T_0 is control group. A total of forty five (45) weaned crossbred New Zealand White (NZW) growing rabbits (aged about 50 to 60 days) was distributed into three treatment groups each having three replications following Completely Randomized Design (CRD). Green grass and concentrate mixture was offered ad-libitum for control group. From this study it was found that weekly concentrate and DM intake was significantly influenced by 50% concentrate + 50% roughage and 100% roughage diet. Further, the feed conversion ratio (FCR) was significantly differed ($P<0.05$) among the treatment groups. Final body weight, weekly body weight and weight gain was not differed significantly ($P>0.05$) with roughage based diet. The nutrient digestibility of DM, CP, CF, Ash and NFE was also varied significantly ($P<0.05$) among the treatment group compared to control group except EE. There was significant difference existed in serological parameters ($P<0.05$) among the treatment groups. So we can conclude that rabbit can be reared feeding roughage although body weight gain was little decreased than the traditional diet and other concentrate group. A little decreased body weight is less economic loss than feeding concentrate. So it can be concluded that supplementation of roughage based diet can be used in the diet of growing rabbit.

Key words: Roughage based diet, Growing Rabbit, Serology and Serum Biochemistry.

CHAPTER I: INTRODUCTION

Bangladesh is a densely populated country in South Asia with acute crisis of human food. Agriculture is the main driving force of her national economy where the components of agriculture are crop, livestock, fisheries and horticulture. The role of livestock subsector is vital for the economic development of agro-based Bangladesh. The contribution of livestock to National Gross Domestic Product (GDP) is 2.73 percent which is 16.03 percent in Agricultural share (BER, 2010). Poverty reduction, gender equity and empowerment of women are amongst eight set targets of Millennium Development Goals (MDG) of Bangladesh. To achieve these goals, Bangladesh government has identified livestock as one of the key player for poverty reduction strategy. The government has set strategic targets for meeting protein demand, employment generation, up-scaling export earning and women's empowerment through the livestock sub-sector. In many situations, livestock constitute the main, if not only, capital reserve of farming households, serving as a strategic reserve that reduces the risk of the farmer and add stability to the overall farming system (Islam, 2011).

Amongst the components, most of the development efforts in the past were concentrated on crop production and therefore, the livestock sector remained under developed, which can be realized from the unavailability of the livestock products such as milk, meat and egg. Our availability is about 44 ml of milk, 20.6 gm meat every day and 33 eggs in a year (DLS, 2010). Livestock products such as milk, meat and eggs that contain all the essential nutrients required for the normal functioning of the body system, have very high nutritive value. People of advanced countries consume about 70% of their total protein requirement from animal sources but this figure is about 10 to 12% for our country (Islam, 2011). Hence, it is needless to say that urgent and effective steps should be taken to increase the availability of animal protein sources. So, rabbit can be considered as another contributing source of meat along with cattle, goat, sheep and poultry. Rabbit as a micro-livestock (Vietmeyer, 1985) may be a promising enterprise in this respect. Domestic rabbit (*Oryctolagus cuniculus*) is emerging as a viable livestock species which is suited to small-scale production by backyard farming (Cheeke, 1986). Rabbit's meat is acknowledged as of high quality meat being high in protein but low in fat and cholesterol and sodium (Jones, 1990). Rabbit meat is

advocated to people because it does not form uric acid during metabolism (Iyeghe-Erakpotobor, 2007). Rabbits are efficient converters of feed to meat and can utilize up to 30% crude fibre as against 10% by most of the poultry species (Egbo et al., 2001). To make rabbit rearing more viable as a small-scale business, it has been advocated the development of alternative feeding materials that will be relatively cheap when compared with commercial feeds or conventional feedstuffs (Alawa et al., 1990). Rabbit occupies a vital midway between ruminants and monogastric animals. Rabbit can utilize cellulose rich feed with ration containing less than 20% grain. Simple biological characteristics, short breeding cycle, high prolificacy and better feed conversion efficiency logically place rabbit just below the poultry. However, to nourish the rabbit, feed is the major important event to be thought out as it cost around 60-70% of its production cost. Herbs may be alteration source of antibiotic as a growth promoter to rear rabbit commercially.

Fibers are one of the main components of rabbits' diets (García et al., 2000) because they play a key role in rabbit feeding by contributing to cecum activity for efficient digestion (Gidenne, 1992). Moreover that particle size is a major factor of fiber digestibility in rabbits. Crude fiber level in growing rabbits' diet varies from 14 to 16%, whereas in reproductive rabbits' diets that level is between 12 and 13% (Houndonougbo et al., 2012)**.**

According to the statement of previous researchers, it is identified that many works have been performed on rabbit feeding in a broad aspect; however there is a few works have been conducted to observe the effect of only fiber based diet on growth performances of rabbit. The main source of fiber is low in peri-urban areas, and farmers have difficulties to provide rabbits with grass. The processing of complete diets with optimal level of fibers is therefore suitable to efficiently feed rabbits; hence the current research work has been undertaken with the objectives mentioned here under.

Objectives of the study:

a) To evaluate the effect of roughage based diet on growth performance of broiler rabbit.
b) To observe the effect of roughage based diet on serological profile of rabbit.

CHAPTER II: REVIEW OF LITERATURE

Commercial rabbit production in Bangladesh is not common like other domestic animals. So research in this country on various production characteristics of this species is lacking. This chapter discuss about the different research on performances of rabbit. This part also focused about the previous works on effect of roughage based diet on growth performances of rabbit.

Rabbits are herbivores and can be successfully raised on diets that are low in grains and high in roughage (Cheeke et al., 1986). Rabbits have a number of specific characteristics such as small body size, short generation interval, rapid growth rate and high litter size. Rabbit meat is acknowledged as of high quality being high in protein and low in fat and cholesterol (Jones, 1990). It has been reported that growing rabbits can be maintained satisfactorily on diets consisting of 100-200 gm green roughage and 40-60 gm concentrate mixtures preferably in the form of pellet (Ranjan, 1980). In optimum production about 4 months are required to produce a 2 kg market rabbit under subsistence condition (NRC, 1991).The climatic condition, commercial factors, legal environment, religious, social practices and technological aspects support the rabbit raising potential in Bangladesh (Hasanat et al., 2006).

Nutrition is a major constraint for rabbit rearing in Bangladesh. Supplementation of soybean meal as a source of protein has been suggested on growth and reproductive performance of rabbit (Rahim et al., 1997). Rabbits can utilize low-grain and high-roughage diets (Cheeke et al., 1987). Rabbits are able to breed year-round, and have a quick generation interval; they are uniquely poised to provide animal protein for developing countries, where grain can only be justified for human use. It must be clarified, however, that rabbits are not able to survive solely on poor-quality, low-energy forages. Due to their small size and high metabolic rate, high-quality forage is needed. So, what is the correct way to feed rabbits? An in-depth evaluation of the digestive tract of adult rabbits may help to understand potential complexities when feeding this unique animal. In Benin, 64% of the population has consumed rabbit meat at least once, and 95% of them appreciated it positively. To promote optimal growth performance of rabbits, appropriate feeding strategy is necessary.

2.1. Nutrition of Rabbit

Rabbit can be successfully raised on diets containing forage and cereal by-products and it consumes many kinds of feeds satisfactorily. Rabbits are herbivores animal but they are non-ruminant. They have a distended hindgut (Cheeke et al., 1987). The rabbits are also adapted to the use of a high-roughage diet, but it has a different digestive strategy than the ruminant and the colon fermenter.

In practice, diets can be based largely on herbage, grasses, legumes, leaves, crop residues and kitchen scraps. Fresh green feeds can be used in moderate amounts for feeding rabbits with no drop in production (NRC, 1977).

Rabbits are hind gut fermenters. Jones (1990) concluded that "hind gut fermentation is a superior adaptation for dealing with high fiber herbage, provided that intake is not limited by the actual quantity of herbage available.

Lebas (1983) reported that the daily dry matter requirements can be estimated as 100-120 gm for young animals between 1 to 2.5 months of age. At the age of 3 to 4 months 150-180 gm for the young animal. For pregnant animal, the DM requirement is 150-180gm and for nursing does is 300-400gm depending on the number of young's. Lukefahr and Goldman (1985) concluded that the rabbits can be fed a diet based on legume and grass forages supplemented with table scraps, kitchen waste and crop residues such as surplus or damaged bananas, mangoes and other fruits.

However, Cheeke et al., (1987) stated that full feeding on green grasses may not support a satisfactory growth rate of fryers or maintain a producing doe in adequate condition. A growth rates of 25gm per day over a 4 weeks period when weaning rabbits were fed fresh green clover vegetable leaves with no other supplements. Supplementation is particularly important for new born and lactating females, whose diet must contain about 16% protein and at least 18% fiber (NRC, 1977). Rabbits are rapidly growing animal and required diets that contain high energy, protein and mineral for their growth (Table1).

Table 1: Nutrient requirements of rabbits; second revised edition, NRC (1977).

Nutrient (%)	Requirement
ME (Kcal/kg DM)	2700
CP	16
CF	15-18
Fat	2-3
Calcium	0.4-1.10
Phosphorus	0.20-0.50
Magnesium	0.16-0.35
Potassium	0.5-1.0
Sodium	0.2-0.3
Choline	0.3-0.35

2.1.1. Energy Requirement of Rabbit

Many workers conducted experiments with different levels of energy in the diet to study their effects on growth and reproductive performance of rabbits.

There are differences in energy requirement for growing rabbits with different experiments and detected methods was stated by Xiangmei (2008). Lebas (1975b) concluded that reproductive performance of does was good when fed diets containing 2500- 2900 kcal digestible energy (DE) per kg.

Hasanuzzaman et al. (2001) revealed that growth performances were not affected by energy levels and they concluded that 2500 kcal/kg diet may be appropriate for growth and reproductive performance of rabbit does.

According to NRC (1977) digestible energy of 2500 kcal per kg diet is needed to meet the requirements for rapid growth but at energy levels lower than this the rabbit may not be able to consume sufficient feed to meet its requirements for maximum growth. The daily ME requirement for an adult rabbit is 200 kcal and can be provided with diets containing 2100-2200 kcal of ME per kg (NRC, 1977). About 9.5 kcal of DE is required for each gm of body weight gain and no further increase in growth performance occurs with ME levels exceed 2500 kcal/kg (Lebas, 1975a).

2.1.2. Protein Requirement of Rabbit

Many workers conducted experiments with different levels of protein in the diet to study their effects on growth and reproduction of rabbits.

Protein is the most essential nutrient for better growth and reproductive performance such as growth rate, gestation period, litter interval, litter size, litter weight, litter born alive, age at first kindling etc.

Abdel-Malak (2000) showed that live weight gain, feed conversion, crude protein utilization, performance index and blood urea were increased by increasing dietary protein levels with no significant difference between groups fed 16 or 18% crude protein (CP) in most growth performance traits. Sanchez et al., (1985) reported that live weight of does fed 17.5% CP was lower compared to other treatments (19.0 or 20.5% CP) after 21 and 28 days of lactation. However, Ayyat (1994) reported that daily live weight gain increased with increasing protein concentration in the diet of rabbits.

Protein requirements for rabbits depend on protein quality. For rapid growth, rabbits are dependent upon adequate quantities of dietary essential amino acids. Dietary CP levels of 12, 16, 15 and 17% are recommended for maintenance, growth, pregnancy and lactation respectively for rabbits (NRC, 1977). However, Arrington and Kelley (1976) suggested that 15% dietary crude protein content was adequate for all except young stock and lactating does which needed 16 to 17% CP. Hemid et al. (1988) conducted an experiment to investigate the effect of diets containing two levels of protein (15 or 17.5%) on the reproductive performance of medium New Zealand White (NZW) and light France rabbits. The pregnant and lactating does were fed on diets containing 15 or 17.5% crude protein and digestible energy 2500 kcal/kg. The experiment showed that 17.5% protein diet gave larger litter size, lower mortality and increased number of parities by reducing gestation length and litter intervals compared with 15% protein diet.

Khan et al. (2000) conducted an experiment suggested that adlibitum green grass plus supplementary concentrate mixture of 15.71% CP content was the best for optimum growth and carcass characteristics of rabbits under Bangladesh condition. Similarly, another study with New Zealand White (NZW) rabbits indicated that soybean meal diet

containing 17.5 or 20% CP gave highest live weight gain followed by gluten and soybean meal plus maize gluten diets with 17.5% CP (Sonbol et al., 1992). An experiment was performed by Salma (2000) to study the effect of feeding different levels of supplemental protein on reproductive performance of rabbit does and effect of mother nutrition on the subsequent performance of weaned litter. Three supplemental diets (concentrate mixture) were formulated with wheat, wheat bran, maize, til oil cake, soybean meal to contain three levels of protein i.e. 13.71, 16.64 and 21.00% CP along with ad-libitum green grass. Higher CP% improved reproductive performance of rabbit does and the post weaning performance of litters.

2.2. Digestibility of Rabbit

The study was conducted with the influence of supplemental *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* on nutrient digestibility in Soviet Chinchilla male rabbits randomly divided into four equal groups (Saijpaul et al., 2005). Their study concluded that a combination of Lactobacillus and Saccharomyces was beneficial in improving the CP and CF digestibility of the diet in rabbits in comparison to either of the two alone.

In another study to determine the effect of feed restriction on performance and feed digestibility in rabbits (Meo et al., 2007). Two hundred and fifty-six rabbits were equally divided into two groups fed the same commercial concentrates supplied adlibitum or restricted to 90% of adlibitum from weaning (35 d) to slaughter (85 d). Mortality was recorded daily. The feed restriction group rabbits showed significantly higher apparent digestibility for almost all the nutrients (except crude protein and ether extract) in particular for the crude fiber, NDF and ADF, confirming a higher residence time of the feeds in the digestive system.

2.3. Feed Intake and Growth Performance of Rabbit

The effect of feeding different levels of concentrate on the production performance of broiler rabbits was shown by Bhatt et al. (2005). Rabbits (n=83) were divided into 3 groups and were offered concentrate pellets at 50 gm, 80 gm and adlibitum. The effect of the level of concentrate feeding was significant on growth, plane of nutrition and digestibility of nutrients. The gain/day was highest (27.6+or-0.5 g) in adlibitum fed groups and the differences were significant when compared with the group fed 50 gm

and non-significant with the group fed 80 gm concentrate. Feed gain was best (3.15) in 50 gm fed group and deteriorated with increasing level of concentrate supplementation. No significant effect of concentrate feeding was observed on carcass and gastrointestinal attributes. Digestibility of crude fibre and cellulose was affected by concentrate feeding with the highest value (40.4+or-2.4 and 49.3+or-2.9%) in the group 50 gm and the lowest value (29.9+or-4.1 and 42.7+or-1.0%) in the group fed adlibitum. It is concluded that for economical broiler rabbit production, the rabbits must not be fed more than 50 gm of concentrate per day incorporated with quality green fodder.

2.4. Biochemical Changes

Adefunke (2005)conducted an experiment with growing rabbit to evaluate the effect of utilizing newsprint waste (NPW) as replacement for maize on performance and hematological parameters of grower rabbits revealed that, there was significant ($P<0.05$) dietary effect on average daily weight gain which ranges between 9.17 and 14.52 gm feed intake, feed conversion ratio, the feed cost, total serum protein, glucose ranges from 52.7 to 114.90 and hematological parameters. The study concluded that 7.5% maize could be replaced by newsprint waste successfully.

Toghyani et al. (2010) revealed that inclusion of *M. puberula* leaf meal up to 30% dietary level would be of benefit in raising grower rabbit in the humid tropics in which hematological parameters were not significantly differed among the treatment groups ($P>0.05$). Again serum biochemical constituents like urea, creatinine, cholesterol, sodium, chloride, bicarbonate and serum protein values were similar for all groups whereas potassium level was significantly ($P<0.05$) differed among all treatments.

CHAPTER III: MATERIALS AND METHODS

The whole study includes collection of feed stuffs, chemical analysis, diet formulation, management and feedings of rabbits and growth and digestibility trial. The methodologies followed in this experiment are discussed below:

3.1. Location and Duration of Experiment

The present study was conducted in laboratory of animal research unit, Chittagong Veterinary and Animal Sciences University located at Chittagong region (lat 22°21'N, lon 91°49'E and elevation 95 ft) of Bangladesh. The average temperature was 25.1° C with a variation of ±8°C. The average annual relative humidity of the area was 73.7% and average monthly relative humidity ranges from 58% in January to 86% in August. Chittagong has an average of 2735 millimeters (mm) of rainfall per year. This study was conducted between the periods of July, 2015 to December, 2015.

3.2. Collection and Processing of Roughage

The roughage (green grass) was collected from the fodder plot of Animal science and Nutrition department, Chittagong Veterinary and Animal Sciences University. Then washed and chopped. Then roughage (green grass) was offered to the rabbits.

3.3. Experimental Design and Dietary Treatment Groups

A total of 45 weaned rabbits of 50-60 day were procured from a market of Chittagong district in Bangladesh. Forty five rabbit were distributed into three treatment groups consisting of three replication having five rabbits in each in a completely randomized design (CRD). All the rabbits were housed in iron cages (55cm x 40cm x 40 cm) during the experimental period. The rabbits were supplied water and feed twice daily at 8am and 4pm according to 3 dietary treatment groups. Dietary treatment diets were 1 as the control (traditional) diet, diet 2 (50% concentrate + 50% roughage on DM basis) and diet 3 (100% roughage). The diet was formulated to meet the nutrient requirements of rabbits recommended by NRC (1977) and contained no antibiotics. Weekly body weight changes and daily feed intake of individual rabbit was monitored during the experimental period. Feed conversion ratio was estimated from dry matter intake and weight gain.

The initial body weight of experimental rabbit was done using digital weighing balance and the group weight as well as individual weight was calculated after formatting 3 treatments.

Table 2: Initial live weight of rabbits in different treatment groups

Treatment groups	**T_0**	**T_1**	**T_2**
Body weight (Mean)	349.31	348.74	351.23

T_0=Control group (Traditional diet); T_1=50% concentrate + 50% roughage on dry matter basis; T_2=100% roughage with no concentrate.

3.4. Collection of Feedstuffs

Green grasses were collected from the fodder plot of Animal Science and Nutrition Department, Chittagong Veterinary and Animal Sciences University and then chopped prior to offer to rabbits. Maize, wheat bran, rice polish, pea bran, soybean meal, mustard oil cake, common salt and DCP plus was purchased from local market.

3.5. Preparation of Concentrate Mixture

The concentrate mixture was formulated by mixing different feed ingredients in such a way to fulfill the nutrient requirement of growing rabbits (Table 3).

Table 3: Ingredients composition of experimental diets (concentrate mixture)

Ingredients (%)	**T_0**
Maize broken (%)	52.50
Wheat bran (%)	6.00
Rice polish (%)	11.00
Pea bran (%)	4.00
Mustard oil cake (%)	3.00
Soybean meal (%)	21.00
DCP (%)	2.25
Salt (%)	0.25
Total	100.00

T_0= Concentrate + Green grass (Traditional diet); T_1= 50% concentrate + 50% roughage on dry matter basis; T_2=100% roughage with no concentrate

Table 4: Proximate composition of experimental diets for growing rabbits

Parameters	T_0	T_1	T_2
Dry matter (%)	59.10	52.65	32.00
Crude Protein (%)	16.86	16.27	15.13
Crude fibre (%)	20.17	12.96	38.00
Ether extract (%)	3.31	1.92	1.43
Ash (%)	8.72	7.17	11.00
Nitrogen free extract (%)	50.93	61.68	34.44

T_0= Concentrate + Green grass (Traditional diet); T_1=50% concentrate + 50% roughage on dry matter basis and T_2= 100% roughage with no concentrate.

Table 5: Proximate composition of green grass offered to growing rabbits

Parameters	DM	CP	CF	Ash	EE	NFE
Percentages (%)	32.00	15.13	38.00	11.00	1.43	34.44

3.6. Experimental Feeding Practices

Fresh, clean and safe drinking water was supplied to the rabbits at all time. Concentrate and green grass ad-libitum were offered only to the control group. Green grass was collected every morning and afternoon followed by cleaning, chopped, weighted and supplied to the rabbits. Concentrate mixture and green grass was offered twice daily, once in the morning at 8.00 A.M. and another in the afternoon at 4.00 P.M.

3.7. Management Practices

The cages, feeders and water pots were cleaned with water and then washed with washing powder and sun dried. The feeder and waterer were then placed in the individual steel cage pens. All cages and floor of the room were cleaned with disinfectant every week, though floor was cleaned every day. Feeder and waterer were also cleaned everyday in the morning with water but these were cleaned with washing powder in every week. The feces were taken a way to a safe place in order to provide proper hygienic condition. The rabbits of different treatment groups were provided with identical care and management throughout the experimental period.

3.8. Recording of Feed Intake

The rabbits were supplied experimental diets; twice daily and leftover was collected. The refusal of concentrate feed of the subsequent days were collected, weighed and recorded in the following morning before offering feed. Feed intake was calculated after subtracting left over from the feed supplied.

Feed consumption (g/rabbit) = Feed supply (g) – [left over (g) + waste]

3.9. Measurement of Live Weight, Live Weight Gain and FCR

The rabbits were weighed individually at the beginning of the experiment and the average weight was taken as the initial body weight. There after the rabbits were weighed individually in every week by using an electric digital weighing balance before morning feeding. The weekly live weight gain was calculated by subtracting the weight at starting of the week from end of the week.

FCR= Total Feed intake (g)/ Live weight gain (g)

Weight gain for a particular week = Final body weight of a particular week – Initial body weight of a particular week.

3.10. Digestibility Trial

A digestibility trial was conducted in the last 7 days of the experimental period to evaluate the effect of roughage in digestibility of Nutrients. Feed supply and feces collection was performed two times daily. During digestibility trial, the quantity of feed supplied and feces collected were recorded carefully. After collection of feces it was immediately stored in a freezer. Both the feed and feces were subjected to proximate analysis following the standard procedure (AOAC, 2004) to determine nutrient contents of feed and feces. The digestibility of each nutrient was estimated by the following formula.

$$\%\ \text{digestibility of Nutrient} = \frac{\text{Nutrient intake through feed (gm)} - \text{Nutrient in feces (gm)}}{\text{Nutrient intake through feed (gm)}}$$

3.11. Chemical Analysis

Samples of feed, feces and green grass were analyzed for moisture, crude protein(CP), crude fiber(CF), ether extract(EE), ash and nitrogen free extract (NFE) following the methods of AOAC(2004). All the samples were analyzed in duplicates and mean value were recorded.

3.12. Collection, Preservation and Analysis of Blood Sample

At the end of the feeding trial, 2 rabbits were selected from each replicate, blood samples were collected through heart puncture about 4ml from each rabbit. Blood sample was taken into two separate vials. One containing EDTA (anticoagulant) for hematology and another do not contain anticoagulant which was used for serum preparation for biochemical analysis. The blood sample with anticoagulant was analyzed for HB, PCV, TEC and TLC within 24 hours. The separated serum samples were preserved into deep freeze at -18 °C and biochemical analysis were done within 7 days.

3.13. Biochemical Analysis

The biochemical analysis was performed from the preserved serum sample. The samples were allowed to be in room temperature before starting the analysis. The serum total protein (TP), Albumin, Cholesterol, Urea and creatinine level were estimated by using biochemical analyzer (Humalyzer-3000 chemistry analyzer, semi automated Benchtop chemistry photometer) in biochemistry laboratory of CVASU. For each parameters the commercial kit of RANDOX company (http://www.randox.com/reagent) were used and followed the manufacturer's procedure.

3.14. Statistical Analysis

All collected data and sample evaluated values were imported in Microsoft office excel-2007 and transferred to SPSS-16 (Statistical Package for the Social Sciences) software for analysis. Descriptive statistics of some parameters were done. Quantitative performance parameters from different groups of dietary treatment, values of digestibility trial and hematological parameter were compared by one way ANOVA by using SPSS-16. The differences of different parameters were considered significant when the p- value was < 0.05 and highly significant when p – value was < 0.01.

A. Making balanced ration

B. Proper mixing of feed ingredient

C. Feeding of rabbit

D. Feeding of rabbit

E. Different treatment groups

F. Weekly weighing of rabbit

Figure 1: Growth trial of rabbit

A. Preparation of sample (dry grass)

B. Weighting of sample

C. Sampling feed for proximate analysis

D. Washing samples during CF estimation

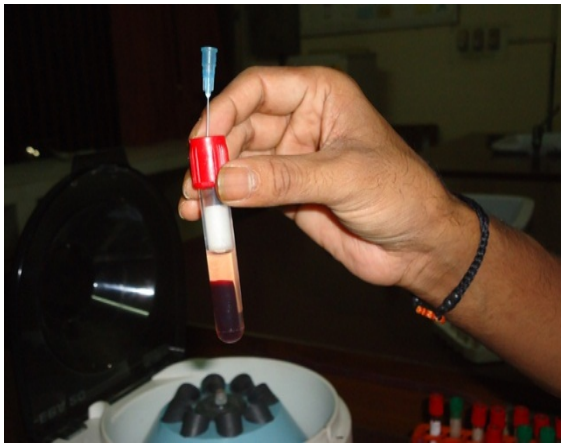
E. Crude fibre estimation

F. Ether extract estimation

Figure 2: Proximate analysis

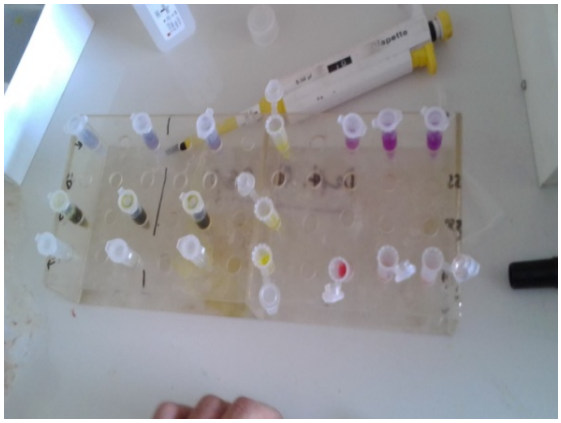
A. Blood collection

B. Blood collection from ear vein

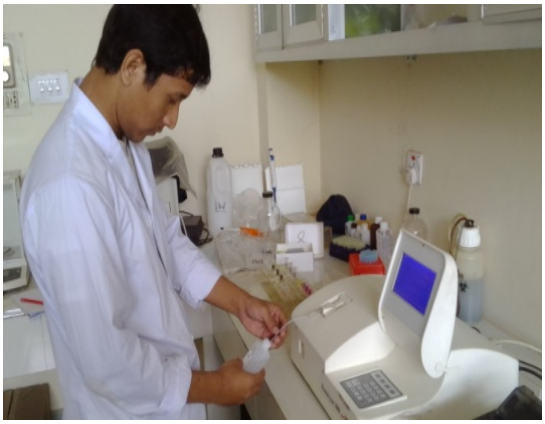


C. Blood serum separation

D. Transfering serum sample



E. Placement of sample



F. Biochemical analysis

Figure 3: Serum biochemical parameters

CHAPTER IV: RESULTS

Growth and digestibility trail was conducted according to the design schedule and the findings are discussed in this chapter chronologically.

4.1. Effect of Roughage Based Diet on Rabbit Growth Performances

The findings on growth performances are discussed below following some different sub headings.

4.1.1. Effect on Live Weight of Rabbit

From the data in table 6, it was found that initial, and 1st week body weight did not differ significantly (P>0.05). Whereas, the data on body weight at 2nd week and 3rd week differed significantly (P<0.05) among all the treatment groups. The highest body weight was observed in To group and lowest body weight was observed in T_3 group where 100% roughage diet was offered to the rabbit.

Table 6: Effect of roughage based diet on body weight (gm) of rabbit.

Age	Body weight (Mean± SE)			Level of significance
	T_0	T_1	T_2	
Initial	349.3±0.56	348.74 ±0.49	351.23±0.52	NS
1st week	421.5 ±1.14	416.8 ±0.65	411.9 ±0.53	NS
2nd week	560.8^{b}±1.10	551.80^{a} ±1.05	539.3^{a} ±1.01	*
3rd week	727.6^{b} ±1.24	712.04^{a} ±1.04	690.5^{a} ±1.01	**

N= 15; S.E=Standard Error; NS=Non-Significant (P>0.05); **=Significant (P<0.01) (a,b,c,..) Means with different superscripts in the same row differ significantly (P>0.05). T_0=control group (Traditional diet); T_1= 50% Concentrate + 50%roughage on DM basis; T_2= 100% Roughage.

4.1.2. Live Weight Gain

From the data in table 7 it was showed that there was insignificant difference among live weight at 1st week though at 2nd week and 3rd week data of body weight gain differed significantly (P<0.05). Highest body weight gain was observed in T_0 group and lowest in T_2 group.

Table 7: Live weight gain (gm) in different week among various treatment groups.

Age	Live weight gain (Mean± SE)			Level of significance
	T_0	T_1	T_2	
1st week	72.22±1.10	68.06±1.03	60.67±0.50	NS
2nd week	139.35 [b] ±1.24	135.0 [a]±1.43	127.4[ab]±1.14	*
3rd week	166.75 [a] ±1.58	160.24 [a] ±1.04	151.2 [a] ±1.46	**

N= 15; S.E=Standard Error; NS=Non-Significant (P>0.05); **=Significant (P<0.01) (a,b,c,..) Means with different superscripts in the same row differ significantly (P<0.05). T_0=control group (Traditional diet); T_1= 50% Concentrate + 50% roughage on DM basis; T_2= 100% Roughage.

4.1.3. Feed Conversion Ratio

The effect of roughage based diet on FCR of growing rabbit is shown in table 8. There was insignificant difference among the weekly FCR of rabbit among the treatment groups. However, the numerically higher FCR was inT_2 group and lower FCR was in T_0 group.

Table 8 : Weekly feed conversion ratio (FCR).

Parameters	Weekly FCR (Mean± SE)			Level of significance
	T_0	T_1	T_2	
1st week	4.90±.07	4.91±.07	4.99±.03	NS
2nd week	3.07±.028	3.129±.03	3.175±.025	NS
3rd week	2.70 ±.02	2.786±.01	2.857 ±.02	NS

N= 15; S.E=Standard Error; NS=Non-Significant (P>0.05); T_0=control group (Traditional diet); T_1= 50% Concentrate + 50% roughage on DM basis; T_2= 100% Roughage.

4.1.4. Nutrient Digestibility

A digestibility trial was conducted at the last week of the experimental period. The co-efficient of digestibility of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen free extract (NFE) and organic matter (OM) was analyzed and the data found from that had been presented in table 9.

4.1.4.1. Digestibility Co-efficient of Dry Matter (DM)

The digestibility co-efficient of DM was found as 53.236, 62.114 and 64.522 percent respectively for T_0, T_1 and T_2 group. The highest DM value was found in T_2 and lowest

in T_0 group whereas the T_1 stands moderate in position. The result of statistical analysis of the value of digestibility of DM showed highly significant (P<0.01) effect of treatment among the groups.

4.1.4.2. Digestibility Co-efficient of Crude Protein (CP)

The digestibility co-efficient of CP was found as 55.65, 63.266 and 63.05 percent respectively for T_0, T_1 and T_2 group. The highest CP value in T_1 and lowest in T_0 group whereas the T_2 stands moderate in position. The result of statistical analysis of the value of digestibility of CP showed highly significant (P< 0.01) effect of treatment among the groups.

Table 9: Digestibility co-efficient of different nutrients

Parameters	Nutrient digestibility of different treatment groups (Mean± SE)			Level of significance
	T_0	T_1	T_2	
DM%	53.236^{a}±0.12	62.114^{b}±0.14	64.522^{c}±0.19	**
CP%	55.65^{a} ±0.21	63.266^{b}±0.20	63.050^{b}±0.19	**
CF%	51.75±0.21	55.48±0.23	55.60 ±0.29	NS
EE%	54.83±0.21	55.64±0.12	55.51±0.30	NS
ASH%	53.99^{a}±0.19	63.072^{b}±0.21	62.74^{b}±0.22	**
NFE%	52.30^{a}±0.14	63.072^{b} ±0.21	62.74^{b}±0.22	**

N= 6; S.E=Standard Error; NS=Non-Significant (P>0.05);**=Significant (P<0.01) (a,b,c,..) Means with different superscripts in the same row differ significantly P> 0.05). T_0=control group (Traditional diet); T_1= 50% Concentrate + 50% Roughage on DM basis; T_2= 100% Roughage.

4.1.4.3. Digestibility Co-efficient of Crude Fiber (CF)

The digestibility co-efficient of CF was found as 51.75, 55.48 and 55.60 percent respectively for T_0, $T_1$and T_2group. The result of statistical analysis of the value of digestibility of CF showed non-significant (P>0.05) effect of treatment among all groups.

4.1.4.4. Digestibility Co-efficient of Ether Extract (EE)

The digestibility co-efficient of EE was found as 54.83, 55.64 and 55.51 percent respectively for T_0, T_1 and T_2 group. The result of statistical analysis of the value of digestibility of EE showed non-significant ($P>0.05$) effect of treatment among the groups.

4.1.4.5. Digestibility Co-efficient of Ash

The digestibility co-efficient of ASH was found as 52.30, 63.07 and 62.74 percent respectively for T_0, T_1 and T_2 group. The highest CF value was in T_1 and lowest in T_0 group whereas the T_2 stands moderate in position. The result of statistical analysis of the value of digestibility of ASH showed highly significant ($P< 0.01$) effect of treatment among all groups.

4.1.4.6. Digestibility Co-efficient of Nitrogen Free Extract (NFE)

The digestibility co-efficient of NFE was found as 53.99, 63.07 and 62.74 percent respectively for T_0, T_1 and T_2 group. The highest NFE value was found in T_1 and lowest in T_0 group whereas the T_2 stands moderate in position. The result of statistical analysis of the value of digestibility of NFE revealed highly significant ($P<0.01$) effect of treatment among the group.

4.1.5. Biochemical Changes

The blood parameter (biochemical changes) was observed after analyzing it in the Dept. of Physiology, Pharmacology and Biochemistry and the results founds are shown below:

Table 10: The effect of experimental diet on serum biochemical parameter of rabbits

Parameter	Serum biochemical parameter of rabbits (Mean± SE)			Level of significance
	T_0	T_1	T_2	
Protein (g/dl)	4.53^{a}±0.02	5.28^{b}±0.09	5.56^{c} ±0.76	**
Albumin(g/l)	9.10^{a}±0.01	9.78^{b}±0.01	9.7^{b}±0.11	**
Phosphorus (mg/dl)	6.53^{a}±0.03	10.07^{b}±0.08	11.30^{b}±0.12	**
Calcium (mg/dl)	6.11^{a}±0.01	10.83^{b}±0.10	12.10^{b}±0.10	**
Glucose (mg/dl)	131.80^{a}±0.8	195.46^{b}±0.85	198.73^{b}±0.97	**
Creatinine (mg/dl)	0.783^{b}±0.01	0.440^{a}±0.03	0.446^{a}±0.02	**
Urea (mg/dl)	33.50^{a}±0.33	51.42^{b} ±0.46	57.31^{c}±0.34	**
SGPT (u/l)	45.01a±0.35	51.47b±0.67	56.15c ±0.52	**

N=3; S.E=Standard Error; NS=Non-Significant (P>0.05); **=Significant (P<0.01) (a,b,c,..) Means with different superscripts in the same row differ significantly (P> 0.05). T_0=control group (Traditional diet); T_1= 50% Concentrate + 50% roughage on DM basis; T_2= 100% Roughage.

From the above table 10 it was revealed that total protein level in blood was higher ($P < 0.05$) in T_2 and lower in T_0 (control) group. It was found that blood albumin level was significantly ($P>0.01$) differed among different treatment group where the highest value was found in T_1 group and the lowest was in T_2 group. The highest phosphorus level was found in T_2 group and the lowest in T_1 group. The highest calcium was found in T_2 group and the lowest in T_1 group. The highest glucose level was found in group T_2 and the lowest level in T_1 group. The highest creatinine level was found in T_0 group and the lowest level in group T_2 and T_1. The highest SGPT level was found in group T_2 and the lowest level in T_1 group. Blood urea level was also significantly ($P<0.01$) different among treatment group where the highest value was found in T_2 group and the lowest value was found T_1.In conclusion it can be said that the increment of total protein, albumin, calcium, phosphorus, glucose, creatinine, urea and SGPT is highly significant ($P<0.01$).

CHAPTER V: DISCUSSION

The current study investigated the effect of roughage based diet on growth performances of rabbit. So, the current study was conducted with the hypothesis that roughage may be the sole source of nutrients for successful rabbit performances in terms of body weight, body weight gain, feed conversion ratio, nutrient digestibility and serological profile. However, the results found in the study will be discussed in this chapter.

5.1. Effect of Roughage Based Diet on Growth Performance of Rabbit

From the current study it was revealed that the live weight of different weeks like-initial, 1st week, 2nd week and 3rd week did not differ significantly among the various treatment groups. Although numerically the highest body weight was observed in traditional diet (control diet) group and lowest body weight was in 100% roughage group. The previous study revealed that roughage supplementation along with rice bran can be used as a potential nutrient for good growth performance of rabbit but only unconventional roughage like Maize offal can be used to increase live body weight of rabbit (Raharjo et al., 1987; Onifade and Tewe, 2010) which support the findings of the current study. Some other study did not support the current study where unconventional roughage feeding was done and found that cassava root and cassava can be fed to rabbit as a strong energy booster for good growth performance of rabbit (Omole and Onwudike, 1983). The reason may be the seasonal effect of the current work. The current findings was also supported by other researcher and found that Lucerne which is known as alfalfa grass rich in fibre helps to improve growth performance (Omole and Onwudike, 1983; Lowe, 2010). Another similar study revealed that star grass improves digestibility of rabbit which promotes growth performance (Nehring et al., 1963). So we can conclude that rabbit can be reared feeding roughage although body weight gain is little lower than the traditional diet and other concentrate group because a little lower body weight is less economic loss than feeding concentrate.

5.2. Serum Biochemical Changes

Significant variations of serum biochemical parameters were observed in different treatment groups. As all animals were in same environment and same age so the variation may be raised from the supplied feed. In this study blood albumin level was significantly ($P>0.01$) differed among different treatment groups where highest value was found in T_1 group and lowest was in T_2 group. Highest phosphorus level was found in T_2 group and lowest found in T_1 group. Highest calcium found in T_2 group and lowest found in T_1 group. Highest glucose level found in group T_2 and lowest level found in T_1 group which is not agreed with Onifade and Tewe (2010) where they found that the hypoglycemic impact of dietary fibre both in fasting and post prandial condition was reflected by lower level of serum glucose in rabbits fed on cassava peel which is highly fibrous. Highest creatinine level found in T_0 group and lowest level found in group T_2 followed by T_1group. Highest SGPT level found in group T_2 and lowest level found in T_1 group. Blood urea level was also significantly ($P<0.01$) differed among treatment group where highest value was found in T_2 group and lowest value was found in T_1 group. These findings agreed with Baron (1982) who revealed that there was a negative correlation between urea nitrogen and protein intake and the highest urea level in blood was found in those animals feeding roughage.

In conclusion it can be said that the increment of total protein, albumin, calcium, phosphorus, glucose, creatinine, urea and SGPT is highly significant ($P<0.01$) where there was no alteration of uric acid based on protein intake which is supported by (Clifford, 1977).Actually, there was no improved growth performances in supplementation with roughage only. However the present study revealed that supplementation of roughage with some concentrate can improve growth performance.

CHAPTER VI: CONCLUSION

The experiment was conducted at the Animal Nutrition Lab (small animal unit) under Chittagong Veterinary and Animal Sciences University for a period of 6 months to study the effect of roughage based diet on feed intake, growth performances, nutrient digestibility and feed conversion efficiency of crossbred New Zealand White (NZW) growing rabbit. Forty five weaned growing rabbits (aged about 50-60 days) were divided into three dietary treatment groups and arranged in a completely randomized design each having three replications. From this experiment, final body weight, weekly body weight and body weight gain of 100% roughage group was not improved significantly compared to control group. There was insignificant difference among the weekly FCR of rabbit among the treatment groups. However, the numerically higher FCR was in 100% roughage group and lower FCR was in control group.

In nutrient digestibility, there found significant difference among the treatment group for the value of DM, CP, CF, Ash and NFE but insignificant difference in EE. DM digestibility was higher in 100% roughage group and CP digestibility was higher in 50% roughage + 50% concentrate group.

In the serum biochemical study, the increment of total protein, albumin, calcium, phosphorus, glucose, creatinine, urea and SGPT is highly significant among the treatment group. From these findings, it can be said that there was no improved growth performances in supplementation with roughage only. Although body weight gain was little decreased than the traditional diet and other concentrate group. A little decreased body weight is less economic loss than feeding concentrate. So it can be concluded that supplementation of roughage based diet can be used in the diet of growing rabbit. However the present study revealed that supplementation of roughage with some concentrate can improve growth performances.

CHAPTER VII: RECOMMENDATION

This study on the investigation of the effect of roughage based diet on growth performance of rabbit suggests the following recommendations:

- The effect of roughage based diet showed less body weight gain, effective outcome in FCR and feed intake. So, farmers can use supplementation of roughage with some concentrate can improve growth performance.
- However, Rabbit is very sensitive animal. So proper care and hygiene should be maintained for better performances of rabbit.

Future perspective

The current study only indicates the effect of roughage based diet on rabbit growth performances. Some extra parameters of hematological and biochemical study could be taken into consideration like the level of TEC, TLC, PCV, Hemoglobin etc. The meat composition was not studied in this study. So further study could include the meat composition effects by roughage based diet in rabbit ration.

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Annex 1: Serum Biochemical Study

Blood urea estimation:

Principal: Urea reacts with diacetylmonoxime in acidic conditions at nearly 100^{o}C to give a red colored product which is measured colorimetrically at 520nm. Thiosemicarbazide and ferric ions are added to catalyse the reaction and increase the intensity of color. This method is linear only up to 300mg% urea. For higher values if expected, the blood sample should be diluted.

Reagents

1) Reagent A: Dissolve 5g of ferric chloride in 20ml of water. Transfer this to a graduated cylinder and add 100ml of orthophosphoric acid (85%) slowly with strring. Make up the volume to 250ml with water. Keep in brown bottle at 4oC.
2) Reagent B: Add 200 ml conc, H_2SO_4 to 800 ml water in 2L flask slowly with stirring and cooling.
3) Acid Reagent: Add 0.5 ml of reagent A to 1 L of reagent B. keep in brown bottle at 4^{o}C.
4) Reagent C: Diacetylmonoxime 20g/L of water. Filter and keep in brown bottle at 4^{o}C.
5) Reagent D: Thiosemicarbazide 5g/L of water.
6) Colour Reagent: Mix 67 ml of C with 67 ml of D and make up the volume to 1000 ml with D.H_2O keep in brown bottle at 4^{o}C.
7) Stock urea standard: 100mg/100 ml water.
8) Working urea standard: Dilute 1 ml stock to 100ml with DH_2O so conc. is 1 mg/100ml.

Procedure: 0.1 ml if serum/plasma is diluted to 10 ml. set up the test tubes as follows:

	B	T	S1	S2	S3	S4	S5
Serum (ml)	-	1.0	-	-	-	-	-
(dil 1:100)							
Std (ml)	-	-	0.2	0.4	0.6	0.8	1.0
D. Water (ml)	2	1.0	1.8	1.6	1.4	1.2	1.0
Color Reagent (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Acid reagent (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0

Mix all the tube thoroughly. Keep in boiling water bath for exactly 30 mins. Then cool and read absorbance at 520nm.

Estimation of Total Protein:

Principle: Cupric ions form chelates with the peptide bonds of proteins in an alkaline medium. Sodium potassium tartrate keeps the cupric ions in solution. The intensity of the violet color that is formed is proportional to the number of peptide bonds which, in turn, depends upon the amount f proteins in the specimen.

Reagents

(i) Biuret Reagent – 3 mg of copper sulphate is dissolved in 500 ml of water. 9 gm of sodium potassium tartrate and 5 gm of potassium iodide are added and dissolved. 24 gm of sodium hydroxide, dissolved separately in 100 ml of water is added. The volume is made up to 1 litre with water. The reagent is stored in a well-stoppered polythene bottle.

(ii) Biuret blank – this is prepared in the same way as the bi uret reagent with the difference that copper sulphate is not added.

(iii) Standard protein solution – the best way is to determine the total protein concentration in pooled human serum by Kjeldahl method, dilute it to bring the protein concentration to the desired level, say 6 gm/100 ml and use it as standard. Alternatively, a 6 gm/100 ml solution of bovine albumin in water may be prepared and used as standard.

Procedure: label 3 test tubes 'Unknown', 'Standard' and 'Blank', Measure 5 ml of biuret reagent into each. Wash 0.1 ml of serum into 'Unknown', 0.1` ml of standard protein solution into ‘Standard' and 0.1 ml of water into 'Blank'. Mix and allow to stand for 30 minutes.

Read 'Unknown' and 'Standard' against 'Blank' at 540 nm or using a green filter.

Calculations:

$$\text{Serum total protein (gm/dl)} = 6 \times \frac{Au}{As}$$

Estimation of serum Albumin:

Bromocresol Green Method:

Principle: The method is based on the protein error of indicators. Biding of a protein to an indicator changes its color. Among serum proteins, only albumin binds to BCG this binding produces a change in the color of BCG which is measured colorimetrically. The pH is maintained during the reaction by a buffer.

Reagents

(i) Succinate buffer - 11.8 gm of soccinic acid is dissolved in about 800 ml of water. The pH is adjusted to 4.0 with 0.1 N sodium hydroxide. The volume is made up to 1 litre with water. This solution should be stored in refrigerator.

(ii) BCG solution - 419 mg of bromocresol green is dissolved in 10 ml of water. The solution is stored in refrigerator.

(iii) Buffered BCG solution – 250 ml of BCG solutions is mixed with 750 ml of succinate buffer. The pH is adjusted to 4.2 with 0.1 N sodium hydroxide solution. 4 ml of Brij – 35 solution (30%) is added.

(iv) Standard albumin solution – an aqueous solution of human albumin with a concentration of 4 gm/100 ml can be prepared and used as a standard. Sodium azide should be included in this solution (50 mg in every 100 ml) as a preservative. Pooled human serum (preserved with sodium azide) or a control serum having an albumin concentration of 4 gm/100 ml can also be used as a standard.

Procedure: Level 3 test tubes 'Unknown', 'Standard' and 'Blank'. Measure 4 ml of buffered BCG solution into each. Wash 0.02 ml of serum into 'Unknown', 0.02 ml of standard albumin solution into 'Standard' and 0.02 ml of water into 'Blank'. Mix and allow the tubes to stand for 5 minutes.

Read 'Unknown' and 'Standard' against 'Blank' at 630 nm or using a red filter.

Calculations:

Serum Albumin (gm/dl) = $4 \times \frac{\text{Au}}{\text{As}}$

Estimation of serum cholesterol:

Test Principle: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Procedure:

Both reagent and sample brought at room temperature and mixed 1.0 ml reagent with 10µl sample in test tube. Let waited for 10 minutes and placed mixture in cuvette. The cuvette was sated in spectrophotometer at 550 nm and recorded the reading. The reading was calculated by comparing with standard value and multiplied by 200mg/dl. So the result was expressed as mg/dl.

Estimation of serum creatinine:

Before performing the test, the “Reflotron” instrument was switched on when “Ready” appears on the display, argalment carrier strip out of the wall was taken and the vial was closed immediately with the desiccant stopper. The foil was removed protecting the test area; taking could not too overhead the strip. By using “Reflotron pipette, the sample material was drawn up (0.3 ml) avoiding the formation of bubbles and applied that as a drop the centre of the red application zone without allowing the pipette tip to touch the zone. Within 15 seconds, the flap was opened; the strip was placed on the guide and inserted the strip horizontally into the instrument until hearing a click. Closing flap the display “creatinine” confirmed that the rest specific

magnetic code has been correctly read into the instrument. The time before the results appeared in displayed in seconds. After particular time, the creatinine concentration displayed in for mg/dl 37ºC, 30ºC depending upon the reference temperature selected. The range of measurement was 5.00-500 mg/dl, (37ºC).

Annex 2: Nutrient composition of feed (concentrate)

Nutrient composition of feeding ingredients used for concentrate mixture (NRC, 1977)

Ingredients	DM (%)	CP (%)	CF (%)	EE (%)	Ca (%)	P (%)	ME (Kcal/KG)
Maize	89.50	8.30	2.90	4.60	0.13	0.21	3350
Rice polish	92.45	14.12	4.10	11.00	0.05	1.31	3100
Wheat bran	88.30	14.50	7.40	4.80	0.18	0.92	1300
Pea bran	88.60	17.67	23.78	1.01	-	-	1812
Soybean	89.70	46.58	6.25	1.10	0.29	0.58	2230
Mustard oil	92.70	33.90	6.20	5.50	2.00	0.30	2200
DCP	0.99	_	_	_	28.00	37.34	_

Annex 3: The nutritive value of green grass

Nutrients	(%)
DM	20.00
CP	14.24
CF	34.80
EE	1.435
NFE	38.01
Ash	11.00
*Ca	0.01
*p	0.02
*ME (Kcal/kg DM)	1000

*According to NRC (1977)

Brief Biography

This is DR.ND. Mashiur Rahman; son of MD. Abdul Quduis Bhuyain and Shahnaz Begum from Belabo Upazila under Narsingdi district of Bangladesh. He has passed the secondary school certificate examination in 2005 followed by higher secondary certificate examination in 2007. He obtained his Doctor of Veterinary Medicine Degree in 2012 (held in 2014) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh with CGPA 3.41 (Out of 4.00). Now, he is a candidate for the degree of MS in Animal and Poultry Nutrition under the Department of Animal Science and Nutrition, Faculty of Veterinary Medicine, CVASU.