**CHAPTER 1**

**INTRODUCTION**

The domestic dog (*Canis lupus familiaris)* which is one of the most widely kept working and companion animals in history is the first domesticated animal in the world ([Larson et al., 2012](#_ENREF_11); [Ovodov et al., 2011](#_ENREF_12)) and a famous quote always goes with this animal that  ["dog is the man’s best friend"](http://en.wikipedia.org/wiki/Man%27s_best_friend_(phrase))([Udell et al., 2014](#_ENREF_17)). That’s why in the developed country the owners always try to fed good food to their dog. But now in developing countries people are keeping pet dogs and try to feed them according to their income limits.

On the other hand, the clinical well-being of an animal depends greatly on the provision of balanced diet. Concepts in nutrition are expanding to include an emphasis on the use of foods to promote a state of wellbeing with better health and to reduce the risk of diseases ([Bontempo, 2005](#_ENREF_4)). The feeding and nutrition of pet dogs are quite different compared to developed countries, attributable to divergent social, economical and cultural factors ([Vijayakumar et al., 2004](#_ENREF_18)). However, there is virtually no information available on the nutritional aspects of pet dogs in Bangladesh.

Nutritional requirements of dog is very unique, varies with age, physiological condition, breed, gender, activity, temperament, environment and state of metabolism ([Schenck, 2011](#_ENREF_15)).From a nutritional point of view, growth is the most important time in a dog's life. By two months of age, pups can be fed with puppy food. This is very critical phase of life-growth; skeletal development is at its peak for the first six months of life. Puppies in their active growth period should be providing a high-quality diet that fulfills their definite nutritional needs. Growing dogs show omnivorous feeding behavior and so, their diet should be comprised of all nutrients in correct proportions. A puppy food which fulfills all requirements is called a “Balanced” or “Complete” diet. The amount of food a puppy requires changes during growth and depends on the puppy's nutritional deficiencies and/or imbalances during this period are more devastating than at any other time ([Hawthorne et al., 2004](#_ENREF_8); [Schenck, 2011](#_ENREF_15)).At this point of age, dog develops a functioning immune system, radically adds bone and muscle mass, and rising proper socialization behaviors. This is the critical time to make sure proper nutrition. Growth diets have been formulated to meet the increased requirements of puppies.

Many pet food trials were conducted to refine pet food or to evaluate the quality of pet food. Evaluation of food was done considering basic criterion like digestibility of food, growth of dog and overall physical condition of dog. Average digestibility coefficients in dogs reported in literature Dry matter 82.3 ± 5.17 %, Crude protein 82.2 ± 4.50 %, Ether extract 92.8 ± 2.60 (Vhile *et al*., 2007). [Krogdahl et al.(2004](#_ENREF_21)) also conducted pet food trial on other Carnivora members to evaluate its digestibility in other carnivore species and average digestibility coefficients in dogs Dry matter 81.69±1.56%, Crude protein 76.64 ± 4.50 %, Ether extract 99.36±0.18 .Crude fiber 19.86±11.

In another experiment, [Dilrukshi et al., (2009](#_ENREF_5)) fed imported food and newly formulated food to pets and found the mean growth rate of 0.0586 kg/day ± 0.022 in formulated feed groups whereas 0.0628 kg/day ± 0.019 was the weight gain in imported feed group. There was no any significance in terms of electrolyte concentration in blood serum in formulated and imported feed trials.

In connection with the well-being with better health status in dog, we went for a trial with prepared biscuit fulfilling all nutritional requirements of dog. After feeding with biscuit, assessment was done by haematological analysis along with body weight gain.

Hematology and serum clinical chemistry analyses are used for health assessment of domestic animals and a wide range of captive wildlife (Smith, 2000; Fowler and Miller, 2003; Kaneko *et al*., 2008).

Blood is an important medium in assessing the health status of animals. Both the physiological and pathological conditions of animals can be assessed by the evaluation of hematological and biochemical analyses of the blood.

Considering the above circumstances in mind, the presented work was undertaken to evaluate the prepared dog biscuits with locally available ingredients by observing the health status of the puppies available at CVASU premises.

**CHAPTER 2**

**MATERIALS AND METHOD**

## 2.1 Study area and period

The study was undertaken for a period of 3 months from February, 2015 to April, 2015 at Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh.

## 2.2 Experimental Plan

A feeding trial was also conducted with 18 puppies for 21 days where body weight gain of every week was recorded as well as blood sample of each puppy was collected. In that experiment, total 18 puppies were allocated to three treatment groups (T0, T1 and T2) with three replications, each having 2 puppies per replication.

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Table 1: Layout of the experiment

|  |  |  |  |
| --- | --- | --- | --- |
| **Dietary treatment groups** | **No. of puppies** | | **Total no. of puppies per treatments** |
| T0 (Commercial food) | T0R1 | 2 | 6 |
| T0R2 | 2 |
| T0R3 | 2 |
| T1 (Prepared biscuits) | T1R1 | 2 | 6 |
| T1R2 | 2 |
| T1R3 | 2 |
| T2 (Homemade food) | T2R1 | 2 | 6 |
| T2R2 | 2 |
| T2R3 | 2 |
| Grand total = | | | 18 |

## 2.3 Formulation of dog biscuits

Biscuit was formulated on the basis of requirement of different nutrients and energy in puppy according to the standard of Association of American Feed Control Officials (AAFCO, 2006). The parameters those were considered in ration formulation are [Metabolizableenergy](https://www.google.com.bd/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&sqi=2&ved=0CDMQFjAB&url=http%3A%2F%2Fmedical-dictionary.thefreedictionary.com%2Fmetabolizable%2Benergy%2B(ME)&ei=ptRIUqPCJMOTrge6t4GQBQ&usg=AFQjCNHaA455Trt1AcUFwcOitAL_bSvvIw&bvm=bv.53217764,d.bmk), Crude protein, Crude fiber, Vitamins and Minerals. Supplementation with different nutrient will also be performed to balance the nutritional composition of prepared dog food.

Table 2: Physical composition of prepared biscuits

|  |  |
| --- | --- |
| **Ingredients (kg/100kg)** | **Amount (kg)** |
| Grounded maize | 22 |
| Grounded wheat | 19.5 |
| Grounded soybean meal | 25 |
| Grounded dry chicken meat | 19 |
| Animal Fat | 6 |
| Vegetable oil | 6 |
| Common salt | .25 |
| Vitamin mineral premix | .25 |
| Egg | 2 pieces |
| Total | 100 |

Table 3: Nutritional composition of prepared biscuit

|  |  |
| --- | --- |
| **Estimated Chemical composition (DM basis)** | |
| Metabolizable Energy (Kcal/kg) | 3384 |
| Crude Protein (%) | 28.5 |
| Crude Fiber (%) | 2.7 |
| Ether Extract (%) | 13 |
| Ash (%) | 4.6 |

## 2.4 Target animals and age groups

A total of eighteen (18) stray puppies from CVASU were selected for this study as target sample were selected between age groups 3-4 months and they were from 6 different parents. The approximate age of the stray puppies was estimated by examining the teeth. According to the statement [Cynthia et al. (2011](#_ENREF_1)) dogs having all white and shiny permanent teeth without worn off cups on the incisors were considered as young (below one year on age).

## 2.5 Dog catching and handling

The process of dog handling and catching was done by humane method (also as ‘ethological’ handling). The process is defined as causing the minimum stress possible during the procedure to both the animal and the people involved (FAO, 2014). In order to achieve humane handling, the individual dog’s behavior and the immediate environment was taken into account.

## 2.6 Feed requirement of dogs

Daily feeding requirement was estimated by determining daily need of metabolizable energy.

Resting Energy requirement (RER) of puppies was estimated according to [Schenck (2011](#_ENREF_2))

(RER) = 70× (Body weight in kilograms) 0.75

Daily energy requirement for dog is depend on the age, weight, physiological status.

For a growing puppy the Daily Energy Requirement (DER) is

DER= 3×RER ([Schenck, 2011](#_ENREF_2))

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Plate 5: Feeding trial of the puppies

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## 2.7 Feeding trial

A total of 21days (April 09th to April 29th 2015) growth trial was conducted on preselected street puppies. Trial was conducted with three different groups of dogs: (i) with newly formulated dog biscuit, (ii) with the market available dog biscuit (iii) with traditional foods. All three groups were intensively maintained in the field laboratory under the Department of Animal Science and Nutrition. Feed was provided to puppies 2 times in a day i.e. 9.00 a.m. in the morning and 7.00 p.m. in the evening. Regular clean drinking water was provided as ad-lib basis.

## 2.8 Weekly measuring of weight gain

Weekly body weight was taken to estimate the weight gain and also for the feed requirement. Weighing was done by electronic scale (± 10gm) in the morning in empty gut.

|  |
| --- |
| IMG_2677_2.JPG |

Plate 6: Weighing of dog

## 2.9 Weekly collection of Blood samples

Blood was collected on weekly basis to do hematological and biochemical analysis. Blood were collected through cephalic vain puncture in two sterile vacutainer (3 ml for each). One containing EDTA (anticoagulant) for hematology and another do not contain anticoagulant which was used for serum separation for biochemical analysis. During blood collection the collection site was disinfected with 70% alcohol solution.

** **

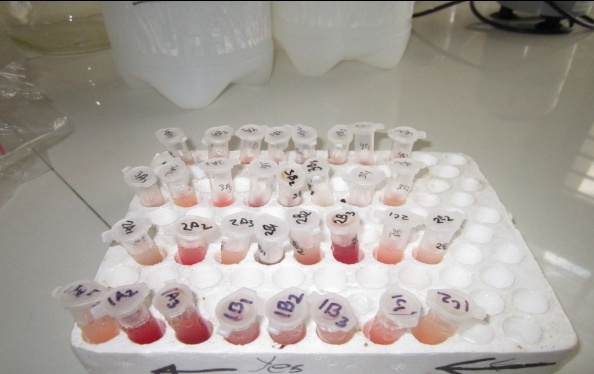
** **

Plate 7: Collection of blood and performing heamatological test.

### 2.9.1 Hematological Analysis

The samples collected with anticoagulant were analyzed for routine examination of blood as per Weiss and Wardrop (2011). The samples were analyzed within 24 hours of collection. Hemoglobin (Hb), Packed cell volume (PCV), Erythrocyte sedimentation rate (ESR), Total leukocyte count (TLC), Total Erythrocyte count (TEC) and Differential leukocyte count (DLC) were performed in Physiology laboratory of Chittagong Veterinary and Animal Sciences University (CVASU).

#### 2.9.1.1 Haemoglobin (Hb)

Haemoglobin (Hb) was determined by acid hematin method. Hb is converted to acid hematin by dilute HCl which in solution brown in colour. The intensity of this colour depends on the amount of acid hematin in solution which in turn depends on Hb concentration. The colour of the solution is matched against brown tinted glass filter by direct vision and the results were expressed as gm/100ml blood (gm %).

#### 2.9.1.2 Packed Cell Volume (PCV)

#### Blood samples were centrifuged in a haematocrit tube. The RBC (Sp. gr. =1.09) being heavier than plasma (Sp. gr. = 1.03) get pack towards the bottom of the tube by centrifugal force. The reading of the percentage of blood that is red cells was then noted.

#### 2.9.1.3 Erythrocyte Sedimentation Rate (ESR)

ESR was estimated by Wintrobe’s method. Blood samples were added to hematocrit tube up to the mark 10. The RBC (Sp. gr. = 1.09) being heavier than plasma (Sp. gr. = 1.03) settle down gradually towards the bottom of the tube. The rate in mm at which the RBC settles was noted at the end of certain period.

#### 2.9.1.4 Total Erythrocyte Count (TEC)

The number of RBC was estimated by using Neubaur Haemocytometer. The blood was diluted 200 times with Hayem’s solution. Red blood cells were then counted into Neubaur Haemocytometer under microscope in diluted blood. The TEC in undiluted blood was calculated by multiplying volume correction factor and dilution factor. The results were expressed as number of RBC per ml of blood.

#### 2.9.1.5 Total Leukocyte Count (TLC)

The blood was diluted with 0.1N HCl which destroys the red cells and stains the nuclei of WBC. White blood cells (WBC) were then counted into a Haemocytometer under microscope in diluted blood. The TLC in undiluted blood was calculated by multiplying volume correction factor and dilution factor. The results were expressed as number of WBC per ml of blood.

#### 2.9.1.6 Differential Leukocyte Count (DLC)

A small drop of blood used to make a thin film of blood on a glass slide. Blood film was then stained with Wright’s stain. The different white blood cells on stained film were then counted under microscope based on their morphology. The results were expressed as percentages of different white blood cells.

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## 2.10 Statistical analysis

The collected data were imported in Microsoft Excel 2007. Descriptive analysis of different parameters was done. Comparison of different variable of three different treatment groups was performed by one way ANOVA by using SPSS 16. Comparisons of digestibility trial of two groups were completed by T-test using STATA 11 software.

**CHAPTER 3**

**RESULTS AND DISCUSSION**

## 3.1 Growth trial

## A comparative growth trial was conducted with prepared dog biscuit and available dog feed.

### 3.1.1 Food offered

Food was offered to the puppies according to the standard requirements suggested by AAFCO (2006) to maintain the nutritional level as 3.5-4 Kcal/gm of ME and 28% CP. However, food was offered by following the feeding rule of [Schenck (2011](#_ENREF_2)), every week after weighing the body weight, feeding was adjusted with the weight gain after satisfying the maintenance requirements of the experimental dogs.

Table 4: Daily offered food (gm/puppies) in different treatment group.

|  |  |  |  |
| --- | --- | --- | --- |
| **Average feeding amount (Mean±SE)** | | | |
| Treatment group | Day (1-7) | Day (8-14) | Day (15-21) |
| T0 | 233.68±13.38 | 261.33±17.91 | 276.33±18.22 |
| T1 | 235.66±14.72 | 262.33±12.20 | 277.33±11.55 |
| T2 | 231±1 | 252.67±1.20 | 267.33±1.45 |

T0=Diet containing commercial food; T1=Diet containing prepared biscuits; T2=Diet containing homemade food. SE= Standard Error.

## 3.1.2 Body weight gain of dogs

Table 5: Trends of Body weight (kg) of different treatment groups

|  |  |  |  |
| --- | --- | --- | --- |
| **Week** | **Dietary treatment groups** | | |
| **T0 (Mean±SE)** | **T1 (Mean±SE)** | **T2 (Mean±SE)** |
| **Initial day** | 5.96±0.46 | 6.18±0.5 | 5.42±0.03 |
| **1st** | 6.89±0.63 | 7.12±0.43 | 6.09±0.04 |
| **2nd** | 7.44±0.67 | 7.71±0.41 | 6.56±0.05 |
| **3rd** | 7.98±0.71 | 8.29±0.39 | 7.03±0.03 |

T0=Diet containing commercial food; T1=Diet containing prepared biscuits; T2=Diet containing homemade food. SE= Standard Error.

Table 6: Body weight gain (kg/week/dog) of experimental dogs of different treatment groups

|  |  |  |  |
| --- | --- | --- | --- |
| **Week** | **Dietary treatment groups** | | |
|  | **T0 (Mean±SE)** | **T1 (Mean±SE** | **T2 (Mean±SE)** |
| **1st** | 0.94a±0.19 | 0.95a±0.08 | 0.67b±0.02 |
| **2nd** | 0.55±0.04 | 0.58±0.03 | 0.47±0.01 |
| **3rd** | 0.54±0.04 | 0.58±0.03 | 0.47±0.02 |
| **Total** | 2.02a±0.27 | 2.11a±0.13 | 1.62b±0.04 |

T0=Diet containing commercial food; T1=Diet containing prepared biscuits; T2=Diet containing homemade food. SE= Standard Error; Means with different superscripts in the same row differ significantly (p> 0.05)

The mean growth rate of puppies in group T0 (Commercial food) and T1 (Prepared biscuit) showed a significantly higher growth rate (2.02±0.27kg/week/puppy and 2.11±0.13kg/week/puppy respectively) than puppies in group T2 (Homemade food) (1.62±0.04kg/week/puppy). This could be due to inadequate nutritional composition of homemade food. During the adaptation period (1st week) there was significantly higher growth rate (0.94kg±0.19 kg/week/puppy) and 0.95kg±0.08 kg/week/puppy, respectively in group T0 and T1 than group number T2 (0.67kg±0.02 kg/week/puppy) was observed. The result is somewhat similar with the research works of Dilrukshi et al. (2009), who mentioned the weight gain as 1.27±0.43 kg/week/puppy in imported feed and 0.25kg±0.17 kg/week/puppy in homemade food. The present study found that, there was no significant difference (p>0.05) in growth rate of puppies during 2nd and 3rd week though rate of weight gain was higher in T0 &T1 group that were fed commercial food and prepared biscuit than the T2 group that was fed homemade food. The mean weight gain (kg/week/puppy) were 0.58±0.03 in prepared biscuits group where 0.54kg/week ±0.04 was the mean weight gain of commercial food group which was slightly higher than Dilrukshi et al. (2009) found mean weight gain (kg/week/puppies) 0.41 ±0.15 in formulated food and 0.43± 0.13 in imported food. Again in this study there was no significance difference in total mean weight gain (kg/week/puppies) between the groups that were fed prepared biscuits (2.11 ±0.13) and commercial food (2.02 ±0.27). This may be the consequence of similar nutritional, mainly energy and protein balance in prepared biscuit and commercial food.

## 3.2 Hematological changes

Table 7: Values (Mean±SE) of different hematological parameters in different treatment groups

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Time period** | **Dietary treatment groups** | | | **P value** | **Reference value\*** |
| **T0 (Mean±SE)** | **T1 (Mean±SE)** | **T2 (Mean±SE)** |
| **HB**  **(g/dl)** | Initial day | 6.1±0.06 | 7.3±0.70 | 7.47±0.42 | 0.16 | 12-19 |
| 1st week | 7.37±0.42 | 7.03±0.12 | 7.37±0.22 |
| 2nd week | 7.80±1.53 | 10.03±0.89 | 8.4±0.7 |
| 3rd week | 7.60±1.36 | 9.70±0.70 | 7.47±0.62 |
| **PCV**  **(%)** | Initial day | 19.33±1.45 | 33±2.52 | 29±1.52 | 0.01 | 25-34 |
| 1st week | 40±6.08 | 52.33±2.84 | 52.33±2.72 |
| 2nd week | 25.67±1.20 | 40.67±2.33 | 42±4.62 |
| 3rd week | 25.33±1.20 | 38.33±1.45 | 39±3.46 |
| **TLC**  **(103/µl)** | Initial day | 7.5±0.42 | 9.07±1.54 | 8.33±1.48 | 0.69 | 6-17 |
| 1st week | 7.47±0.42 | 9.2±1.03 | 8.03±1.59 |
| 2nd week | 7.70±0.46 | 9.13±0.61 | 8.03±1.09 |
| 3rd week | 7.73±0.48 | 9.17±1.02 | 8.3±1.07 |
| **TEC**  **(106/ µl)** | Initial day | 10.6±0.35 | 11.73±0.43 | 11.03±0.80 | 0.41 | 5.6-18.7 |
| 1st week | 10.47±0.59 | 11.17±0.49 | 11.87±0.59 |
| 2nd week | 10.13±0.48 | 10.97±0.37 | 10.70±0.71 |
| 3rd week | 10.37±0.60 | 10.83±0.34 | 11.17±0.67 |
| **Lymphocyte (%)** | Initial day | 28±4.04 | 26±3.79 | 17.67±2.96 | 0.18 | 8-21 |
| 1st week | 35.33±7.88 | 30.67±6.96 | 28.33±1.67 |
| 2nd week | 33±3.51 | 24.33±4.48 | 27.67±0.33 |
| 3rd week | 32.33±3.33 | 25.67±4.33 | 29±1 |
| **Monocyte (%)** | Initial day | 7±2.52 | 3.67±1.20 | 4.67±0.67 | 0.40 | 2-10 |
| 1st week | 10.67±2.33 | 9±2.52 | 7.33±0.67 |
| 2nd week | 3.67±0.33 | 2.67±0.33 | 3.33±0.33 |
| 3rd week | 3.67±0.33 | 2.67±0.33 | 3.33±0.33 |
| **Neutrophil (%)** | Initial day | 51.67±3.84 | 61.67±3.48 | 72±3.05 | 0.02 | 58-85 |
| 1st week | 51.33±8.84 | 52.33±5.36 | 53.67±2.96 |
| 2nd week | 55±3.51 | 62.67±5.36 | 59.11±2.02 |
| 3rd week | 56±3.51 | 63.33±4.33 | 60±1.54 |
| **Eosinophil (%)** | Initial day | 12.67±3.71 | 8.33±2.03 | 5.67±2.33 | 0.28 | 0-9 |
| 1st week | 2.67±0.88 | 11±3.21 | 10.67±3.71 |
| 2nd week | 8.33±0.88 | 10±1.73 | 9.33±1.76 |
| 3rd week | 8±0.58 | 8±0.58 | 9±0.58 |
| **Baso**  **phil**  **(%)** | Initial day | 0.67±0.33 | 0.33±0.33 | 0 | 0.30 | 0-1 |
| 1st week | 0 | 0.67±0.67 | 0.67±0.33 |
| 2nd week | 0 | 0.33±0.33 | 0 |
| 3rd week | 0 | 0.33±0.33 | 0 |

T0=Diet containing commercial food; T1=Diet containing prepared biscuits; T2=Diet containing homemade food. **\*** Ref. value [Kaneko et al. (1997](#_ENREF_18))

Hematological changes in this study showed almost no significant differences (p>0.05) except PCV and neutrophil (p<0.05) count. Complete blood counts are done to monitor overall health, to [screen](https://en.wikipedia.org/wiki/Screening_(medicine)) for some diseases, to confirm a diagnosis of some medical conditions, to monitor a medical condition, and to monitor changes in the body caused by medical treatments ([Bourgès‐Abella et al., 2014](#_ENREF_4)). In the current study complete blood count done mainly to observe the overall health condition of the experimental dogs and to justify whether the newly prepared biscuit have the potentiality to create food allergy symptoms to the dogs. All the hematological parameters were within the range of reference value except Hb. The research work conducted by [Weiss and Wardrop (2011](#_ENREF_40)) and [Rørtveit et al. (2015](#_ENREF_31)) showed lower Hb levels in puppies compared to adults. It can be concluded that the dogs are in good health in terms of hematological parameters. As the eosinophil counts were within the range of reference value in all dietary treatment groups, the comments may be drawn that the newly prepared dog biscuit are safe interms of food allergy ([Lund et al., 1999](#_ENREF_23)).

## 3.3 Metabolic profile test

The values obtained in this research work are well compared with those of the literature reference (Kaneko et al., 1997). When properly interpreted haematological values give important information concerning clinical status, nutritional balance, deficit condition, treatment monitoring and prognostics.

**CHAPTER 4**

**CONCLUSION**

From the growth trial of this study, the positive and significant relationships were observed in mean weight gain and feed intake. The mean weight gain of prepared biscuits, commercial food and homemade food were 2.11kg/week ±0.13; 2.02kg/week ±0.27 and 1.62kg/week±0.04 respectively. And the feed intake of prepared biscuits and commercial food were 257.11 g/day ±16.45; 258.44 g/day ±12.81 and 250.31g/day ±1.17 respectively. Mean weight gain in prepared biscuits and commercial food was significantly higher than the homemade food though feed intake in all treatment was not differing significantly. Thus indicated similar performance of prepared biscuits and commercial food and clearly both treatment groups did better performance than the homemade food.

At the end of the feeding trial and through the haematological analysis along with body weight gain of the puppies it can be said that the prepared dog biscuit, fulfilling all the nutritional requirements will promote a state of wellbeing with better health and reduce the risk of diseases.

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