**CHAPTER-1**

**INTRODUCTION**

The economy of Bangladesh is mainly based on Agriculture. Livestock plays a crucial role in the agricultural economy. About 36.0 percent of the total animal protein comes from the livestock products in our everyday life. Around 25 percent peoples of the country are directly engaged in livestock sector, and 50 percent peoples are partly associated in livestock production. The contribution of livestock sub-sector to the GDP was 2.95 percent, which was estimated about 17.32 percent to agriculture. The growth of GDP for livestock was 7.23 percent. Bangladesh has 24 million cattle, out of which 6 million are dairy cattle of local and crossbreds **(DLS, 2008).**

The majority of the dairy cattle are in the hands of smallholder dairy producers. The country has one of the highest cattle densities of 145 large ruminants/square kilo meter compared with 90 for India, 30 for Ethiopia, and 20 for Brazil **(Karim, 1997).** The numbers of dairy farms are estimated at about 1.4 million with an average herd size of 1-3 cows **(Hemme, 2008).** Dairying is a part of the mixed farming systems in Bangladesh **(Saadullah, 2001)** and a predominant source of income, nutrition and jobs **(Miyan, 1996; Haque, 2009)**. Dairying is also considered a powerful tool to develop a village micro economy of Bangladesh **(Shamsuddin *et al*., 2007)** to improve rural livelihoods and to alleviate rural poverty.

The feeding regime of cattle is varied according to age, production stage and status, and feed resources. The feeding during the dry period is very important for the performance and general health of the dairy cows during early lactation. Mismanagement of the late gestation diet can have a negative effect on the dry matter intake (DMI) during the beginning of lactation, predisposing the animal to both metabolic and infectious diseases (**Stergaard and Sorensen, 1998; Rukkwamsuk *et al*., 1999**). A balanced supply of micronutrients, such as vitamins A and E, and the trace elements selenium (Se) and zinc (Zn), is also of great importance as deficiency of these micronutrients have been associated with an increased incidence of diseases (**Kellogg, 1990; Hemingway, 1999, Weiss, 2002**). The importance of vitamin E for a well-functioning immune response around parturition has lately been given increasing interest (**Weiss, 1998; NRC, 2001**) and supplementing dairy cows around calving is very important. Physiological equilibrium is maintained mainly by the blood in the body , but many physiological conditions may alter this equilibrium. The importance of hemato-biochemical indices in animal husbandry is well acknowledged. Metabolic disturbance usually by inappropriate feeding without manifestation of clinical symptoms are important in animal husbandry and may cause insufficiently developed breeding cattle (**Radostits** *et al****.,* 2003**). Changes of hematological constituents are important indicators of the physiological or pathological state of the animal (**Ahmed *et al*., 2003**).

The profile of blood metabolites are being used widely to assess general health status, to identify problem and to indicate dietary causes of diseases of low production (**Lee *et al.,* 1978; Jain, 1993**) when thorough history and physical examination fail to yield a diagnosis in difficult cases. Many practitioners turn to blood samples for a complete blood count and chemistry panel, hoping these tests will identify the problem (Mandal, 2010) The blood biochemical profiles are considered important in evaluation the health status of animals. The estimates of biochemical constituents are the prerequisites to diagnose several pathophysiological and metabolic disorders in cattle (**Mc. Dowell, 1992; Chaffe, 1976**).

In Bangladesh, both intensive and household rearing cattle are suffered from various metabolic diseases during different stage of production. But laboratory investigation is merely done to know the status of hematological and biochemical parameters of dairy cattle related to production stage and diseases. Routine examination of blood and serum is necessary for monitoring the health status of cattle and calves. Considering the above backgrounds the present study was undertaken with the following

**Objectives:**

1. To determine the hematology and biochemical values in cows and their calves.
2. To compare the hematology and biochemical values between cows and their calves.

**CHAPTER-2**

**REVIEW OF LITERATURE**

**2.1. Hematology**

 Hematological values of cattle do not significantly differ with seasons (**Aengwanich *et a.l,* 2009**). There is little difference in Biochemical parameters between lactating and non lactating cows (**Jones *et al., 1982***). In most cases, a complete blood count (CBC) is not going to be helpful in determining a specific diagnosis, but it can be helpful in determining the severity of a problem and a prognosis **(Mandal, 2010)**.

**2.1.1. PCV**

The time when anemia is suspected after a physical examination, a packed-cell volume (PCV) is helpful in assessing the severity of the anemia. Although the normal range is 24 percent to 46 percent, the PCV is usually in the upper 20s in adult cattle and slightly higher in calves (Mandal, 2010).

A dehydrated anemic animal that is might have a normal PCV. A red blood cell (RBC) count does not offer any more information than the PCV, but RBC morphology should be evaluated to make sure cell size is not change enough to affect the PCV **(Mandal, 2010)**.

Total protein (TP) levels usually are interpreted with the PCV, and hydration status also must be considered. Anemia and hypoproteinemia suggest acute blood loss in the last few days. If blood loss is chronic, animals have time to adapt and might show only mild clinical signs with a PCV of less than 10 (Mandal, 2010).

Signs of regeneration (reticulocytes, nucleated RBCs, increased MCV) should accompany a low PCV and TP of acute blood loss after 72 hours. With chronic blood loss anemia or hemolytic anemia, the TP is less likely to be low. Hemolytic anemia might be accompanied by icterus on physical examination, and if the hemolysis is intravascular (copper toxicity, *Clostridium novyi*, leptospirosis), hemoglobinuria usually is noted **(Mandal, 2010).**

In subtropical condition hematological parameters of exotic cows at lactating, pregnant ,and non pregnant is PCV % 31.79±1.65 and 29.75±1.80 respectively (**Sattar *et al.*, 2009** ) that normally ranged between PCV % 24.0-46.0 (**Schalm and Jain *et al*., 1975**). In cross breed cows the PCV percentage was 29.04 ± 0.89, 31.8 ± 1.06, 28.5 ± 01.06 in early gestation, mid gestation and late gestation period respectively (**Manzoor *et al.,* 2008**).

**2.1.2. TEC and RBC indices (MCV, MCHC, MCH and Hb %)**

RBC indices (MCV, MCHC and MCH) might be helpful in discovering the type of anemia (regenerative versus non-regenerative) if physical examination and other laboratory tests are inconclusive (Mandal, 2010).

Compared with adult reference intervals, the MCV is lower and RBC count is higher in calves during the 6 month of life. Hemoglobin concentration stays largely within the adult reference interval (**Hege *et al*., 1982**).

Mean MCHC was lower than adult values for 5 weeks, then increased and reached adult values by weeks 10–12. The mean lymphocyte count for calves reached adult reference values at weeks 6–8, and the mean monocyte count increased steadily until 14–16 weeks. For most leukocytes, individual variation was larger during the first 5–8 weeks of life. The mean platelet count for calves was higher than the adult reference interval until weeks 19–21 of age.

In practices, red blood cell, packed cell volume, hemoglobin concentration are helpful in the evaluation of anemia **(Schalm *et al.,* 1975; Davidson *et al.,* 1975**). Anemia is characterized by a reduction in the overall erythrocyte content, number of erythrocytes or hemoglobin concentration ( **Bichard *et al.,* 2006***).*

Increased MCV may be seen in vit B12, folate deficiency and blood parasite infection i.e. *Babesia spp., Theileria spp****.*** (**Schalm *et al.,* 1975; Bichard *et al*., 2006**).

A decrease in Hb concentration was found in non pregnant lactating Holstein-Friesian cows during early lactation(**Esievo, 1979**). On the other hand decreased MCV may be seen in iron deficiency, chronic blood loss blood loss. Falling MCH may give an early clue of impending iron deficiency, since MCH falls before MCV and decreased MCHC occurs in iron deficiency anemia (**Esievo, 1979**).

Values of Hb, ESR, MCHC is significantly lower (p‹0.05) in the non cyclic cows as compared to cyclic or endometritic cows (**Ahmed *et al.,* 2009**).

The MCH and MCHC values are affected by variation in Hb synthesis (**Benjamin, 1978**). The MCHC is a measure of the quantity of Hb in each RBC and also relates to the weight of Hb and volume of cell, the cows having higher Hb concentration showed higher MCH and MCHC values. Higher RBC count with lower Hb concentration may be due to increase in number of erythrocytes with decrease in their size (**Benjamin, 1978**).

In subtropical condition hematological parameters of exotic cows at lactating pregnant and non pregnant is Hb (gm/dl) 9.44 ± 0.34 and 9.24 ± 0.35 respectively, TEC (×106/μl) 5.88 ± 0.46 and 5.30 ± 0.58, respectively, MCV (ſt) 55.16 ± 1.72 and 58.17 ± 2.46, respectively, MCH (pg) 16.77 ± 1.14 and 18.78 ± 1.64, respectively, MCHC (gm/dl) 30.09 ± 1.15 and 18.78 ± 1.64, respectively ( **Sattar *et al.*, 2009** ).

The reference range of bovine hematological parameters are TEC (×106/μl) 05.0 - 10.0, MCV (ſt) 40.0 - 60.0, MCH(pg) 11.0 - 17.0, MCHC (gm/dl) 30.0 - 36.0 (**Schalm *et al*., 1975**).

Bovine Hematology reference intervals at 1-14 days old is Hb (gm/dl) 5.7-15.8 , TEC (×106/μl) 4.9-10.9, MCV (ſt) 31.7-49.6 MCH (pg) 10.8-16.5, MCHC (gm/dl) 27.4-39.8, at 2wks -6 months old is Hb (gm/dl) 8.5-14.1, TEC (×106/μl) 6.5-11.9, MCV (ſt) MCH (pg) 26.6-44.3, MCHC (gm/dl) 31.0-32.2, at 6 month -2 years old is Hb (gm/dl) 9.2-15.4 , TEC (×106/μl) 6.1-10.6, MCV (ſt) 31.5-50.9, MCH (pg) 10.7-19.1, MCHC (gm/dl) 31.0-39.0, at 2 year + old is Hb (gm/dl) 8.5-13.2 , TEC (×106/μl) 5-7.7, MCV (ſt) 37.8-56.0, MCH (pg) 14.2-20.1, MCHC (gm/dl) 31.7-40.4 ( **Lumsden *et al* ., 1980**).

In cross breed cows hemato-biochemical parameters at early (up to three month), mid (up to six month) and late (up to nine month) pregnancy wasTEC (×106/μl) 5.09±0.16, 6.33±1.01, 5.22±0.21, respectively, MCV (ſt) 54.68±0.94, 50.53±1.45, 57.28±1.09 respectively, MCH (pg) 32.22±0.41, 32.20±0.74, 31.76±0.44, respectively**,** MCHC (gm/dl) 17.57±0.35, 16.05±0.31, 18.16±0.35, respectively(**Manzoor *et al.,* 2008**).

**2.1.3. Differential Leukocyte Count**

Age has a major effect on several of the parameters (**Jenkins *et al*., 1982; Roussel *et al*., 1982; Doornenbal *et al*., 2006**).

The main cause of neutropenia are occurring with severe infections (**Candyce *et al.,* 2003; Bichard *et al.,* 2006**) such as peritonitis, pyometra and aspiration pneumonia etc. (**Davidson *et al.,* 1998**). Besides, nutritional deficiency that occurs in starvation or anorexia is causes of neutropenia (**Spivak *et al*., 1984**). Lymphopenia are caused in stress, glucocorticoid therapy acute phase of viral infection, septicemia or endotoxemia(**Candyce *et al.,* 2003**). And neutrophilia are caused in physiological changes (fear, excitement and exercise) stress/corticosteroid induced (**Bichard, 2006**) and acute inflammatory response (bacterial infection, necrosis, neoplasia) *(***Davidson *et al.,* 1998;Spivak *et al.,* 1984**). Likewise, lymphocytosis occurred during strong immune stimulation (e.g. chronic stimulation, viremia or immune mediated disease (**Davidson *et al.,* 1998**) and fear or excitement (**Candyce *et al.,* 2003**), whereas monocytopenia is not clinically significant. Causes of monocytosis are chronic inflammation (**Davidson *et al.,* 1998**) such as tuberculosis (**Spivak *et al*., 1984**), tissue necrosis, and stress and glucocorticoid therapy. Possible causes of eosinophilia are parasitic infestation (**Davidson *et al*., 1998; Candyce *et al.,* 2003**)

In subtropical condition hematological parameters of exotic cows at lactating pregnant and non pregnant is neutrophil %28.30±3.20 and 23.30±2.97, respectively, lymphocyte % 59.30 ± 3.11 and 65.20 ± 3.00, respectively, monocyte % 7.20 ± 0.74 and 6.40 ± 0.78, respectively, eosinophil % 4.40 ± 1.25 and 4.30 ± 0.54, respectively, basophil % 0.80 ± 0.33 and 0.80 ± 0.20, respectively **( Sattar *et al.*, 2009** ).

Leukocytosis induced as a result of infection promotes the release of neutrophil from the bone marrow through leukocytosis- inducing factor (LIF) of the plasma; concentration of LIF is increased in bacterial diseases by bacterial products (**Sastri, 1985**). In states of excitement, exercise and strange surroundings there is also leukocytosis (neutrophilia), since adrenaline liberated during these states mobilizes the marginal neutrophil pool cells (**Sastri, 1985**).

**2.2. Serum chemistry**

The majority of biochemical parameters in young animals differ from adults because these are changed with the age and are also influenced by colostrums intake **(Jezek *et al.,* 2006**).

**2.2.1. Total protein and Albumin**

Total protein easily can be evaluated with a refractometer, although a chemistry panel is required to determine albumin and globulin changes specifically. The difference in plasma protein and serum protein is mainly due to fibrinogen. Increases in total protein are due to dehydration or inflammation (Mandal, 2010). Hypoprotenemia most often is caused by lack of adequate protein in the diet or protein loss. Liver is the principal organ of protein synthesis but liver disease usually does not result in a low protein in ruminants. It caused from nutrition problems and chronic parasitism. These animals might be anemic also. Serum protein constitutes a portion of the amino acid pool of the body and as such is believed to be indicative of the nutritional status of the animal (Mandal, 2010).

In shorthorn cattle except for the values at birth, total protein levels were lower (p<0.05) in young animals and higher in mature animals. Albumin levels also lower at birth (p<0.05) and then increase, but fluctuate somewhat. There was no clear effect of age on albumin levels (**Doornenbal *et al*., 1988**). In Jersey cows total protein increased with age over a range of one to six years(**Roussel *et al*., 1982**). In dairy cattle total protein levels were reported to be higher in dry cows, while albumin concentration were lower in lactating non pregnant as compared to lactating pregnant animals **( Peterson *et al*., 1981**).

In animals there is a general modification in serum proteins between early age and very old; thus age is an important consideration in the interpretation of the serum proteins (**Kaneoko *et al*., 1997**). The concentrations of albumin are influenced not only by age but also by nutrition (**Kaneko *et al*., 1997; Knowles *et al.*, 2000**).

In cross breed cows hemato-biochemical parameters at early (up to three month), mid (up to six month) and late (up to nine month) pregnancy was total Protein (gm/dl)6.92±0.33, 8.05±1.17, 7.49±0.22, respectively ( **Manzoor *et al.,* 2008**).

Studied values of serum total protein (gm/dl) at 4-5 years old and 6-10 years old shorthorn cows was2.3 ± 0.3, 2.3 and 39.6 ± 4.4, 38.3 ± 4.6, respectively(**Doornenbal *et al*., 1988**). And shorthorn calves at day 80 total protein (mg/dl) 66.8±3.7, albumin (mg/dl) 40.5±1.9 (**Doornenbal *et al*., 1988**). Bovine serum total protein (gm/lit) and albumin (gm/lit) between 2 wks to 6 month were 48-73 and 25-38, respectively but at 2 years or more serum Protein (gm/lit) and Albumin (gm/lit) 59-81, 29-39, consecutively (**Lumsden *et al.*, 1980**).

 **2.2.2. Glucose**

Glucose is an essential nutrient needed for several tissues, and the high demand in the beginning of lactation often fall down the amount of glucose that should have available **( Ropstad *et al.* 1989**), without have , negative consequences of animal health will occurs ( **Nale, 2003** ). In Shorthorn calves glucose level is higher at birth and then decrease gradually to one years of age. There is no age effect beyond two years of age (**Doornenbal *et al*., 1988**). These results agree with the findings of **(Roussel *et al*., 1982)**, for Jersey cows ranging in age from one to six years.

In cross breed cows glucose (mg/dl) was found 54.06±1.60, 48.87±3.65, 48.21±2.08 at early (up to three month), mid (up to six month) and late (up to nine month) pregnancy, respectively (**Manzoor *et al.,* 2008**) and 3.7 ± 0.7(mg/dl) glucose was found in shorthorn cows (**Doornenbal *et al*., 1988**).

**2.2.3. Calcium and Phosphorus**

Inorganic Phosphorus and Calcium both generally decreased with increasing age beyond one year of age. One of the main functions of these elements is there involvement in skeletal growth in young animals. In older animal there is a decreased need for calcium (Ca) and P for this purpose and this is reflected in lower blood levels (**Doornenbal *et al*., 1988**). Bovine biochemistry reference intervals at 2 wks to 6 month calcium (mmol/lit) 2.35-2.74, but at 2 yrs+ calcium (mmol/lit) 2.10-2.67 **( Lumsden *et al.*, 1980**).

**CHAPTER-3**

**MATERIALS and METHOD**

A study was conducted in Hazi Commercial Dairy Farm (HCDF), Rangunia, Chittagong, for a period of 180 days, during 16th July, 2012 to 16th January 2013.

**3.1 Base line survey**

Before going to final study a baseline survey was done with a pre-set questionnaire to know the demographic information, animal population, general management practices, preventive measures and common diseases of cattle in the Farm for one day.

**Housing System**

Housing pattern is both faces -in and face-out system, each shed is designed for different status of animal for instance milch cow shed, heifer shed, dry cow shed, pregnant cow shed and calf shed with standard space allocation for individual. The floor is made of concrete. Surface of the floor is even and generally no bedding material is used. There is proper drainage facility and quick disposal of animal waste. Farm workers wash the floor two times daily and bath the cows once daily. There is natural air flow system and available light in every shed.

**Feeding System**

There are separate mangers for each of the cattle. Both roughage and concentrates are offered to them. The farmer grows Para and German grass besides the farm area. Available green fodder is supplied from own fodder land and Concentrate from market. Straw can collect from nearby village.

**Feeding schedule**

Daily feeding schedule-Concentrate and straw is supplied at 10 am, green fodder at 12 am again concentrate at 6.30 pm and straw at 7.30 pm.

**Calf Management**

The calves fed colostrums and cow’s milk. Colostrums feeding are practiced by nipple drinker. They supply drinking water from underground water source.

**Production Management**

The farm produces about 510 lit liters in the morning and about 250 liters at afternoon. The average daily yield of the farm is about 760 liters. In this farm machine milking is practiced. Before milking machine wash with PPM (0.1%). They also practice dry cow therapy for drying the cows.

**Breeding management**

Most of the time heat is detected during milking in the morning and AI usually done within 10-14hrs by AI technician. Usually AI is done 2 times per conception for each of the cows for obtaining better result. Sometimes natural service is practiced by the bulls of their own farm. Mostly they use HF and Sahiwal semen. Generally they keep AI sheet as a breeding record which is provided by AI technician. AI technician or Veterinarian diagnoses the pregnancy by rectal palpation.

**Health Therapeutics and Preventive Management**

The infectious diseases are very much limited in this farm. When any symptom of sickness arises the veterinarians of this farm manage that problem. De-warming and vaccination is regularly practiced by the selected veterinarian (Annex-1).

**Table: Vaccination program that follow in the farm:**

|  |  |  |
| --- | --- | --- |
| *Name of the vaccine* | *Frequency of use* | *Groups of animals administered* |
|  FMD Vaccine | Thrice/year | Cows, calves, heifers |
|  BQ Vaccine | Twice/year | Cows, calves, heifers |
| Anthrax vaccine | Once /year | Cows, calves, heifers |

**Table: De-worming program followed in the farm:**

|  |  |  |
| --- | --- | --- |
| *Name of Anthelmentics* | *Frequency of use* | *Dosage* |
| Piper vet® powder(piperazine citrate) | Single dose at 1 month of age | 5-10gm/40kg calf wt. |
| Antiworm bolus® (Levamisole Hydrochlodride +Trichlabendazole) | Every 3months alternate | 1 bolus /60kg body wt |
| LT-vet®(Levamisole Hydrochlodride +Trichlabendazole) | Every 3months alternate | 1 bolus /60kg body wt |

**Percentages of frequency of diseases of animals (last year)**

Common diseases that occur in the farm are mastitis30%, milk fever40%, blot5%, abortion4%, arthritis5%, calf diarrhoea10%, others 6%.

**Table : Commonly used drugs in the farm:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *Antibiotics*  | *NSAIDs* | *Antihistaminics* | *Vitamine andMinerals* | *Hormonal drugs* |
| Renamycin® Oxytetracycline)Pronapen (Streptomycin + Penicillin)S-P vet®(Streptomycin + Penicillin)Acigent® (Gentamycin )Amoxyvet®(Amoxycilli) | Diclovet® (Diclofenac Sodium)Ketovet®(Ketoprofen)  | Astavet® (Antihistamine)Histavet®(Antihistamine)Dellergen®(Antihistamin) | Calcivit plus®(Calcium preparation)Acivit ADE® (Vit A,D and E)Sancal vet®(Calcium Preparation) | Oxcin® ( Oxytocin) |

Hazi Dairy Farm was selected for conducting the study because of its, large population, satisfactory record keeping system, proper feeding and management and also for their kind cooperation.

**3.2 Cross-sectional study**

After the base line survey, a cross-sectional study was conducted to evaluate the haematological and biochemical parameters of cross bred cattle for 180 days. A total of 30 cross bred (HF X Local, Shahiwal X Local,) post parturient cows and their 30 calves were selected randomly from 70 lactating cows recorded in the record book. Following selection of cows and calves the blood samples were collected from those selected animal.

**3.2.1 Sample Collection**

5ml blood sample was collected from jugular vein of cattle and calf aseptically, with 10ml syringe and needle. 3ml was kept in a vial containing anticoagulant (EDTA, 1 mg/ ml) and 2 ml was kept in syringe for smooth coagulation. 2ml anticoagulant mixed blood was centrifuged to collect the plasma. Blood samples were allowed to stand 2 hours at room temperature to allow proper clotting.

**3.2.2 Sample Transportation**

Blood samples were transported to the Biochemistry laboratory, Chittagong Veterinary and Animal Sciences University within keeping in a thermo flask with ice and then fresh blood was examined for TEC, DLC, Hb% and PCV%.

**3.2.3 Serum Sample Preservation**

2 ml of coagulated blood was kept overnight in refrigerator for collecting the serum. The serum samples obtained after centrifugation were stored at 20C in refrigerator for further biochemical test.

**3.2.4. Sample examination**

**3.2.4.1 Hematological Examination**

Different hematological parameters were studied according to the methods described by **Sastri, 1985**. The detail of the parameters studied as follows:For hemoglobin determinationfresh blood mixed with EDTA was kept in the Wintrobe tube and centrifuge at 3000 rpm for 30 minutes. Thus PCV was determined.For hemoglobin determinationN/10 HCl was taken in a graduated hemoglobinometer up to 10 marks and blood was mixed upto 20 cu mm mark**.** Then diluted the acid hematin using water and match the color thus hb% was determined. TEC determined from freshly collected blood using hemocytometer.DLC determined by making of blood smear on the clear glass slide and then drying of the smear. Then blood smear was stained by the Wright’s stain and dried. Prepared smear was examined under microscope (100xs). Mean corpuscular volume, MCV**=**Hematocrit x 10/RBC count.Mean corpuscular hemoglobin,MCH =hemoglobin x 10/ BRC count. Mean corpuscular hemoglobin concentration, MCHC =hemoglobin x 100/hematocrit value.

**3.2.4.2 Biochemical Examination**

Total protein, Albumin, Glucose, Serum Calcium, Serum phosphorus were determined by Automated Analyser (Humalyzer 3000® ) **(AOAC 1995)**established in physiology lab of Chittagong Veterinary and Animal Sciences University .

**3.2.4.2.1 Photometric Colorimetric Test for Total Proteins** (***Biuret Method)***

**Reaction Principle:**

 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.

**Contents**

|  |  |
| --- | --- |
| Reagents: | 4×100 ml or 1×1000 ml Color reagent |
|  | Sodium hydroxide | 200mmol/l |
|  | Potassium sodium tartrate | 200mmol/l |
|  | Copper sulfate | 12 mmol/l |
|  | Potassium iodide | 30 mmol/l |
|  | Irritant R 36/38 |  |

|  |  |
| --- | --- |
| Standard | 1×3 ml Standard |
|  | Protein | 8g/dl |
|  | Or | 80g/l |
|  | Sodium azaid | 0.095% |

**Assay**

Wavelength: Hg 546 nm, 520-580 nm

Optical path: 1 cm

Temperature: 20---250C

Measurement: Against reagent blank

Only one reagent blank per series was required.

**Pipetting Scheme**

1000 μl of reagent was taken in previously marked three eppendorf tubes for reagent blank, sample and standard separately by micropipette. 20μl of sample and standard were added in the previously marked eppendorf tube, respectively. Reagent blank eppendorf tube only contain reagent. Sample and standard for total protein were mixed separately in each eppendorf tube and incubated for 10 minutes at 250C. Absorbance of the sample and standard were measured against the reagent blank within 30 min (ΔA) λmax in 546 nm by spectrophotometer of the Humalyzer 3000®.

|  |  |  |
| --- | --- | --- |
| Pipette into cuvettes | Reagent blank | Sample/Standard |
| Sample/Standard | ------ | 20μl |
| Reagent | 1000μl | 1000μl |
| Mixed and incubated for 10 min. at 20---250C. Absorbance of the sample and standard were measured against the reagent blank within 30 min(ΔA) |

**Calculation of the Protein Concentration**

**With standard-**

C=80**×** ΔA sample/ ΔA standard [g/l]

**3.2.4.2.2 Photometric Colorimetric Test for Albumin** (***BCG Method)***

**Reaction Principle:**

 Bromocresol green forms with albumin in citrate buffer a color complex. The absorbance of this complex is proportional to the albumin concentration in the sample.

**Contents**

|  |  |
| --- | --- |
| Reagents: | 4×100 ml or 1×1000 ml Colour reagent |
|  | Citrate buffer (pH 4.2) | 30mmol/l |
|  | Bromocresol green | 260mmol/l |

|  |  |
| --- | --- |
| Standard | 1×3 ml Standard |
|  | Albumin  | 4g/dl |
|  | Or | 40g/l |
|  | Sodium azaid | 0.095% |

**Assay**

Wavelength: Hg 546 nm, 578 nm

Optical path: 1 cm

Temperature: 20---250C

Measurement: Against reagent blank

Only one reagent blank per series was required.

**Pipetting Scheme**

1000 μl of reagent was taken in previously marked three eppendorf tubes for reagent blank, sample and standard separately by micropipette. 10μl of sample and standard were added in the previously marked eppendorf tube, respectively. Reagent blank eppendorf tube only contain reagent. Sample and standard for total protein were mixed separately in each eppendorf tube and incubated for 5 minutes at 250C. Absorbance of the sample and standard were measured against the reagent blank within 30 min (ΔA) λmax in 578 nm by spectrophotometer of the Humalyzer 3000®.

|  |  |  |
| --- | --- | --- |
| Pipette into covettes | Reagent blank | Sample/Standard |
| Sample/Standard | ------ | 10μl |
| Reagent | 1000μl | 1000μl |
| Mixed and incubated for 5 min. at 20---250C. Absorbance of the sample and standard were measured against the reagent blank within 30 min(ΔA) |

**Calculation of the Albumin Concentration**

**With standard**

C=4**×** ΔA sample/ ΔA standard [g/l]

**3.2.4.2.3 Determination of Glucose by Randox method**

**Procedure:**

First hand was washed and put on goggles and gloves. Then all the assembled all the equipment. Then all four test tubes were labeled,-blank, standard, control and patient. After that 0.5ml distilled water was added in each tube. Then 25microlitre of D20 was added in each blank tube. Then placed 25 microlitre of standard solution to the standard tube. Pipette 25 microlitre of the control to the control tube. Finally 25 microlitre of the patient serum to the patient test tube and 5.0 ml of glucose working solution to each tube was placed. Mix each of tube and incubate room temperature for 45 minutes. Then read the absorbance (a) for each tube at 425 to 475nm against the blank within 30 minuits.

**Calculation:**

## Glucose (mg/dl = A (patient or control) x (standard value)

##  A (standard)

**3.2.4.2.4 Determination of Calcium by Colorimetric method:**

**Principle:**

The calcium is precipitated as calcium phosphate from an alkalinized trichloroacetic acid serum filtrate and estimated as phosphate by the Benedict and Theis (1) molybdic oxide colorimetric method, slightly modified.

**Procedure:**

 Place 2 cc. of blood serum in a small flask and add 4 cc. of distilled water and 4 cc. of 20 per cent trichloroacetic acid. Mix thoroughly; allow standing 10 minutes, and filtering through a double acid-washed calcium-free filter paper. Transfer 5 cc. of the trichloroacetic acid filtrate to a 15 cc. conical centrifuge tube which has been thoroughly cleaned by immersion in bichromate sulfuric acid “cleaning solution” for several hours. Place 1 drop of 1 per cent phenolphthalein in the tube and add, drop at a time, 20 per cent calcium-free sodium hydroxide until a definite pink color is obtained. Add 1 cc. of 1 per cent trisodium phosphate, twirl the tube until thoroughly mixed, cork, and set aside for 1 hour. After 1 hour’s standing, centrifuge for 3 minutes. Decant carefully the supernatant fluid from the calcium phosphate precipitate. Place the inverted tube upon a pad of filter paper to drain for 2 or 3 minutes, and then wipe away adherent solution from the mouth of the tube with a clean cloth or paper. Wash twice with 5 cc. portions of 50 per cent alcohol made faintly alkaline to phenolphthalein with a few drops of calcium-free alkali. In washing, the mat of calcium phosphate in the bottom of the tube must be thoroughly broken up with a glass stirring rod, and the process of centrifuging, decanting, and draining the tube should be carried out as described above. Dissolve the washed precipitate in 5 cc. of 5 per cent sulfuric acid by volume (5 cc. concentrated H2S04 per 100 cc. of water), and decant into a Rothberg-Evans sugar tube, or a graduated test-tube; wash the centrifuge tube twice with approximately 3 cc. and 2 cc. portions of the 5 per cent sulfuric acid, adding the washings to the graduated tube. In a similarly graduated tube place 10 cc. of standard phosphate solution containing 0.05 mg. of phosphorus, and add 0.5 cc. of concentrated sulfuric acid. Now add to each tube 1 cc. of 5 per cent sodium molybdate and 1 cc. of hydroquinone bisulfite reagent. Place the tubes in a boiling water bath for 10 minutes. Remove, cool, dilute the standard to 15 cc. and the unknown to a volume giving a color that will approximately match the standard (15 cc. in normal bloods), and compare in a calorimeter in the usual manner.

## Calculation:

## Concentration= Asample/Astandard x Standard conc

## (mmol/l) (mmol/l)

##  3.2.4.2.5 Principle and Procedure of estimation of Phosphorus

## Principle:

## Inorganic phosphates reacts with amonium molybdate in the presence of sulfuric acid to form a phospholybdic complex which is measured at 340 nm.

## Procedure:

Firstly working reagent , samples and controls were separated from other instruments and placed in to reaction temperature. Then adjust the photometer to 0 absorbance with the reagent blank. Then pipette placed in to a cuvette. After that mix, insert and start the alarm clock, then incubate for 5 minuites at selectet temperature. Finally read the absorbsnce(A) of the samples and the standard at 340nm against the reagent blank.

**The color is stable for 1 houre.**

**Calculation:**

##  Asample /Astandard x Cstandard = mg/dl Phosphorus

**3.3: Data entry and Analysis:**

All recorded data entered into the MS Excel-2007and sorted out. These data were brought to the STATA 11.0 for descriptive statistical analysis (mean, SD, median). The level of significance was determined at P<0.05. Al

**CHAPTER-4**

**RESULTS**

**4. Cross sectional study**

**4.1.1 Hematology**

There is significant age related variation for most hematological and biochemical parameters.

**Table 1 Different Hematological Parameters of Cows and their Calves in Hazi Commercial Dairy Farm**

|  |  |
| --- | --- |
| Hematological parameters  | Type of animals |
|  **Cow (N-30)** |  **Calf (N-30)** | P-Value |
| **Mean± SD** | **Min-Max** | **Mean± SD** | **Min-Max** |
| Total Erythrocyte Count ( ×106/μl) | 5.63 ± 0.73 | 4.6-7.0 | 6.37 ± 0.66  | 5.3-7.8 | 0.000\*\* |
| Packed Cell Volume (%) | 27.97 ± 3.41 | 22-38 | 28.60± 4.04 | 21-37 | 0.515 |
| Hemoglobin (gm/dl)  | 7.86 ± 0.679 | 6.5-9.2 | 8.28 ± 0.84 | 7.1-9.2 | 0.038\*\* |
| MCV (ſt) | 49.99±5.16 | 37.68-60.37 | 44.62±3.71 | 36.92-55.74 | 0.000\*\* |
| MCH (pg) | 14.09±1.47 | 11.83-16.96 | 15.15±8.38 | 11.08-47.06 | 0.499 |
| MCHC % | 28.08±2.49 | 21.14-32.31 | 29.21±2.56 | 23.82-35.71 | 0.087 |

**\*\*= P< 0.05 (significant in 95% confidence interval).**

Table showed the hematological parameters of cows and their calves. Among the different hematological parameters Total Erythrocyte Count (TEC), Haemoglobin (Hb) and Packed Cell Volume (PCV) were differed significantly between cows and calves. The average TEC in cow and calf was 5.63(106/μl) and 6.37(106/μl) respectively. The hemoglobin was significantly higher in calf (8.28 gm/dl) than cow (7.86 gm/dl).The higher level of Hb in calf due to increased rate of haemopoisis (Al-Busadah 2007and Alsaad 2009). Present study also shows that PCV in cow and calf is 27.97 ± 3.4% and 28.60 ± 4.047% respectively. It indicates calf has somewhat higher PCV percentage but variation is insignificant. MCV from the above table shows that value was significantly higher in cows (49.99 ± 5.16 ft) than the calves (44.62 ± 3.71). On the other hand MCH and MCHC both were higher in calves 15.15 ± 8.38 pg and 29.21 ± 2.56 %, respectively than the cows but these differences were not significantly differed.

**Table 02: Differential Leukocyte Count of the Cow and calf in Hazi Commercial Diary Farm**

|  |  |
| --- | --- |
| Hematological parameters | Type of animals |
| **Cow(N-30)** | **Calf(N-30)** | P-Value |
| **Mean± SD** | **Min-Max** | **Mean± SD** | **Min-Max** |  |
| Lumphocyte % | 61.46±4.52 | 49-68 | 64.26±5.91 | 55-72 | 0.044\*\* |
| Monocyte % | 6.87±2.37 | 3-17 | 9.26±3.39 | 2-21 | 0.002\*\* |
| Neutrophil % | 21.10±5.29 | 12-36 | 19.63±4.75 | 6-27 | 0.263 |
| Eosinophil % | 10.07±3.75 | 2-18 | 6.26±2.93 | 2-14 | 0.000\*\* |
| Basophil % | 0.73±0.86 | 0-4 | 0.26±0.62 | 0-2 | 0.178 |

**\*\*= P<.05 (significant in 95% confidence interval)**

The table 3 depicted the proportion of different leukocytes (lymphocyte, monocyte, neutrophil, eosinophil and basophil) in cow and calf in Hazi Commercial Dairy farm. The proportion of Lymphocyte, monocyte and eosinophil were significantly differed between cow and calf. On the other hand neutrophil and basophil were not significantly differed between these animals

**4.1.2 Serum Biochemistry**

**Table 03: Biochemical parameters in cows and their calves in Hazi commercial Dairy Farm.**

|  |  |  |
| --- | --- | --- |
|  Serum Biochemical parameters | Type of animals | P-Value |
| **Cow (N-30)** | **Calf (N-30)** |
| **Mean± SD** | **Min-Max** | **Mean± SD** | **Min-Max** |
| Total protein (mg/dl) | 7.70 ± 1.17 | 6.12-11.65 | 6.60 ± 0.81 | 4.98- 7.73 | 0.000 \*\* |
| Albumin (mg/dl) | 3.54 ± 0.61 | 2.67-3.88 | 3.41 ± 0.57 | 1.9- 4.3 | 0.403 |
| Globulin (mg/dl) | 4.00 ± 0.53 | 2.81-5.02 | 3.12± 0.63 | 2.02-5.06 | 0.000\*\* |
| Albumin/ Globulin | 0.89 ± 0.19 | 0.57-1.38 | 1.14 ±0.34 | 0.5-1.88 | 0.001\* |
| Cholesterol (mg/dl)  | 21.20 ± 3.50 | 17.7-24.7 | 15.65 ± 2.8 | 12.85-18.45 | 0.000\*\* |
| Calcium(mg/dl) | 9.29 ± 2.06 | 7.30-16.90 | 10.45 ± 2.67 | 7.30- 16.90 | 0.066 |
| Phosphorus(mg/dl) | 1.86 ± 0.30 | 1.58-2.90 | 2.10 ± 0.50 | 1.58-2.90 | 0.032 |
| Glucose(mg/dl) | 43.81 ± 12.17 | 20.0-68.70 | 57.68 ± 2.67 | 31.7-90.8 | 0.000\*\* |

**\*\*= P<.05 (significant in 95% confidence interval).**

Table 3 showed the different serum biochemical parameters of cow and calf in Hazi Commercial Dairy Farm. The total protein, globulin, cholesterol and glucose level in serum were significantly varied in cow and calf (p<0.000).

**Total protein:**

The total protein level was higher in cow (7.70 mg/dl) that could be related with inflammatory processes i.e. mastitis, metritis or have had intake of improved dietary concentrates. Total protein contents are an indicative of nutritive status of an animal reflecting food intake and metabolism. The higher level of globulin also observed in cow which coincided with higher serum total proteins level (Kulkarni, B.A, *et al*) (1983).

**Albumin and Globulin:**

Albumin and globulin both are higher in cow (3.54 ± 0.61 mg/dl) and (4.00 ± 0.53) respectively than calf, but globulin level is significantly higher in cow (4.00mg/dl) than calf (3.12mg/dl) due to reflecting food intake and metabolism (Kulkarni *et al*, 1983).

**Blood Glucose**

The blood glucose level is regarded as one of the indicators of energy status in ruminants. The blood glucose level was lower in cow (43.81 mg/dl) as compared to calf (57.68mg/dl). The lowered means of blood glucose concentrations in cow might be due to the lactating stage. During lactation glucose is needed in mammary glands for synthesis of lactose (Hagawane *et al*, 1997)

**Cholesterol:**

The higher level of cholesterol was found in cow (21.20 mg/dl). The ascending trend of cholesterol concentration in cow might be due to the lactation progress (Rowland ,G.J, et.al, 1980). On the other hand the calf is in growing stage where there is no chance of excess production of cholesterol. The higher level of cholesterol with advancement of lactation was a physiological adjustment to meet the lactation requirements. The hormonal level of estrogen along with thyroxin played a vital role in reducing the cholesterol levels during pregnancy.

**Calcium:**

 There was a drop in calcium level in cow (9.29mg/dl) which associated with impaired absorption of food metabolites from the gastrointestinal precursors, excessive loss through urine, colostrums as it was much more drained in the colostrums during excessive milking and due to insufficient mobilization from the skeleton. However, the calcium level was higher in calf since s/he consumes milk.

**Phosphorus:**

There was an increase in phosphorus level in calf (2.10 mg/dl) as compared to cow (1.86 mg/dl). Moderate depressed phosphorus in cow’s serum due to the used in the synthesis of colostrums and milk for cow.

**CHAPTER-5**

**DISCUSSION**

**5.1. Hematology**

**5.1.1. PCV**

The results of the present study show that PCV is higher in calf. Although the normal range is 24 percent to 46 percent, the PCV is usually in the upper 20s in adult cattle and slightly higher in calves (Mandal, 2010) This may be due to higher RBC count in calf.

**5.1.2. TEC**

Here we found that TEC is higher in calves than the cows. RBC count is higher in calves during the 6 month of life (Hege *et al*., 1982). In female Holstein Cattle RBC was higher in calf (at 1-14 days old TEC (×106/μl) was 4.9-10.9, at 2wks -6 months old TEC (×106/μl) was 6.5-11.9) than the adult ( at 2 year + old TEC (×106/μl) was 5-7.7) ( Lumsden *et al* ., 1980).

**5.1.3. Hb%**

Our study reveals that Hb concentration in calf (8.28± gm/dl) is higher than cow. It may be due to more erythropoisis in calf than the cows. In female Holstein Cattle Hb% was higher in calves ( at 1-14 days old is Hb (gm/dl) 5.7-15.8 , at 2wks -6 months old Hb (gm/dl) 8.5-14.1) than the adult cows ( at 2 year + old is Hb (gm/dl) 8.5-13.2 ) ( Lumsden *et al* ., 1980).

**5.1.4. MCV, MCH, and MCHC**

Here present study shows that MCV is significantly lower in calves (44.62±3.71 ft) than postparturian cows.Compared with adult reference intervals, the MCV is lower in calves during the 6 month of life (Hege *et al*., 1982). Present study also shows that MCH % and MCHC % slightly insignificantly higher in calves. MCH and MCHC values are affected by variation in Hb synthesis (Benjamin, 1978). The MCHC is a measure of the quantity of Hb in each RBC and also relates to the weight of Hb and volume of cell, the calves having higher Hb concentration showed higher MCH and MCHC values. Higher RBC count with lower Hb concentration may be due to increase in number of erythrocytes with decrease in their size (Benjamin, 1978).

**5.1.5. Differential Count**

Lymphocyte and Monocyte is significantly higher in calves. A difference in neutrophil count in calf is somewhat lower than cow. Though it is insignificant but it may be due to nutritional deficiency that occurs in starvation or anorexia that cause neutropenia (Spivak *et al*., 1984). Eosinophil is higher in cows and it is significant. Possible causes of eosinophilia in cows are parasitic infestation (Davidson *et al*., 1998; Candyce *et al.,* 2003). Differences in Basophil % are not significant.

**5.2. Serum biochemistry**

**5.2.1. Total protein**

In our study total protein level was significantly higher in post parturient cows (7.70 ± 1.17 mg/dl) than milk feeding calves (6.60 ± 0.81mg/dl). In shorthorn cattle total protein levels were lower in young animals (p‹ 0.05) and higher in mature animals (Doornenbal *et al*., 1988). In Jersey cows, total protein values were increased with age over a range of one to six years (Roussel *et al*., 1982). So age is an important consideration in the interpretation of serum proteins (Kaneoko *et al*., 1997).

**5.2.2. Albumin**

Albumin is somewhat higher in cow than calf but variation is insignificant. Albumin levels lower at birth (p<0.05) and then increase, but fluctuate somewhat. There was no clear effect of age on albumin levels (Doornenbal *et al*., 1988).

**5.2.3. Cholesterol**

The higher level of cholesterol was found in cow (21.20 mg/dl). The ascending trend of cholesterol concentration in cow might be due to the lactation progress (Kaneoko *et al*., 1997). On the other hand the calf is in growing stage where there is no chance of excess production of cholesterol. The higher level of cholesterol with advancement of lactation was a physiological adjustment to meet the lactation requirements. The hormonal level of estrogen along with thyroxin played a vital role in reducing the cholesterol levels during pregnancy.

**5.2.4. Glucose**

Glucose level found in cow and calf were 43.81±12.17mg/dl (2.56±mmol/lit) and 57.68±2.67 mg/dl (3.20± mmol/lit) respectively and it significantly differ. In Shorthorn calves glucose level is higher at birth and then decrease gradually to one years of age (Doornenbal *et al*., 1988). In Jersey cow’s glucose level is higher in calves than cows (Roussel *et al*., 1982). This indicate milk feeding calf have higher glucose percentages than lactating cow. This higher percentages in milk feeding calf may be due to their monogastric nature and their milk ingestion regularly (Doornenbal *et al*., 1988).

**5.2.5. Calcium**

Calcium level found in cow and calf were 9.29±2.06 mg/dl (2.33±mmol/lit) and 10.45±2.67604 mg/dl (2.61± mmol/lit) respectively. This indicate milk feeding calf have slightly higher calcium percentages than lactating cow. In Shorthorn cattle calcium and Inorganic Phosphorus is higher in milk feeding calves and calcium and Inorganic Phosphorus both generally decreased with increasing age beyond one year of age (Doornenbal *et al*., 1988). Lower percentages in lactating cows may be due to calcium losses during milk production. In young animal there is involvement of skeletal growth but in older animal there is a decreased need for calcium (Ca) and Pi for this purpose and this is why lower calcium level in blood levels of cows than calves (Doornenbal *et al*., 1988).

**5.2.6. Phosphorus**

Phosphorus level found in cow and calf were1.86 ± 0.30 and 2.10 ± 0.50(mg/dl) respectively. It indicate that milk feeding calf have slightly higher phosphorus level than post parturient cow due to higher content of phosphorus in milk like Calcium. . In Shorthorn cattle calcium and Inorganic Phosphorus is higher in milk feeding calves and calcium and Inorganic Phosphorus both generally decreased with increasing age beyond one year of age (Doornenbal *et al*., 1988).

**CHAPTER-6**

**CONCLUSION**

The result found that there is some haemato-biochemical difference between cow’s and their calves but this value somewhat differ from the exotic cattle. Discrepancies in values for various hematological parameters between our findings and previous studies may be explained by differences in sampling interval, methods used, numbers of cows sampled, and/or degree of metabolic disturbances. Moreover, genetic differences between cows **(**Mallard *et al*., 1998) and subtropical conditions of the present study might have played a role for the differences with other studies. Finally hematological and biochemical values were an efficient tool for evaluation of physiological status, metabolic disorders, management problems of the farm which have great relation to health status of the animal.

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**Appendix**

**Table 1: Hematological values in Cow and calf.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Cow** | **Calf** | **Cow** | **Calf** | **Cow** | **Calf** | **Cow** | **Calf** | **Cow** | **Calf** | **Cow** | **Calf** |
| ***COW I D*** | ***Calf I D*** | ***PCV%*** | ***PCV%*** | ***Hb(gm%)*** | ***Hb (gm %)*** | ***TEC(×106/mm)*** | ***TEC(×106/mm)*** | ***MCV*** | ***MCV*** | ***MCH*** | ***MCH*** | ***MCHC%*** | ***MCHC%*** |
| ***(µm3/fl)*** | ***(µm3/)*** | ***(pg)*** | ***(pg)*** |
| C1 | K1 | 29 | 24 | 8.9 | 7.8 | 6.8 | 6.5 | 42.65 | 36.92 | 13.09 | 12 | 30.69 | 32.5 |
| C2 | K2 | 28 | 27 | 8 | 8.3 | 6.2 | 6.7 | 45.16 | 40.3 | 12.9 | 12.39 | 28.57 | 30.74 |
| C3 | K3 | 27 | 28 | 7.4 | 8.6 | 5.9 | 6.3 | 45.76 | 44.44 | 12.54 | 44.44 | 27.41 | 30.71 |
| C4 | K4 | 24 | 32 | 7.8 | 9.2 | 4.9 | 6.8 | 48.98 | 44.06 | 15.92 | 47.06 | 32.5 | 28.75 |
| C5 | K5 | 26 | 31 | 8.4 | 7.8 | 6.9 | 6.9 | 37.68 | 44.93 | 12.17 | 11.3 | 32.31 | 25.16 |
| C6 | K6 | 38 | 26 | 9.2 | 7.2 | 7 | 6.5 | 54.29 | 40 | 13.14 | 11.08 | 24.21 | 27.69 |
| C7 | K7 | 28 | 30 | 7.6 | 8 | 5.2 | 6.8 | 53.84 | 44.12 | 14.62 | 11.76 | 21.14 | 26.67 |
| C8 | K8 | 32 | 22 | 9 | 6.8 | 5.3 | 5.6 | 60.37 | 39.29 | 16.98 | 12.14 | 28.13 | 30.91 |
| C9 | K9 | 28 | 24 | 7.5 | 7.5 | 6 | 5.9 | 46.66 | 40.68 | 12.5 | 12.71 | 26.79 | 31.25 |
| C10 | K10 | 27 | 28 | 7.2 | 7.9 | 5.2 | 6.4 | 51.92 | 43.75 | 13.85 | 12.34 | 26.67 | 28.21 |
| C11 | K11 | 30 | 37 | 7.5 | 10.6 | 5.3 | 7.8 | 56.6 | 47.44 | 14.15 | 13.59 | 25 | 28.65 |
| C12 | K12 | 33 | 29 | 8.8 | 8.1 | 5.8 | 6.4 | 56.9 | 45.31 | 15.17 | 12.66 | 26.66 | 27.93 |
| C13 | K13 | 27 | 26 | 7.1 | 7.8 | 6 | 6 | 45 | 43.33 | 11.83 | 13 | 26.3 | 29.99 |
| C14 | K14 | 26 | 30 | 7.5 | 8.8 | 5.6 | 6.9 | 46.43 | 43.48 | 13.39 | 12.75 | 28.85 | 29.33 |
| C15 | K15 | 29 | 31 | 8.1 | 7.8 | 6.2 | 7 | 46.77 | 44.29 | 13.06 | 11.14 | 27.93 | 25.48 |
| C16 | K16 | 27 | 25 | 8 | 8.2 | 5 | 5.8 | 54 | 43.1 | 16 | 14.14 | 29.63 | 32.8 |
| C17 | K17 | 25 | 23 | 7.8 | 7.7 | 4.6 | 5.3 | 54.35 | 43.4 | 16.96 | 14.53 | 31.2 | 33.48 |
| C18 | K18 | 28 | 27 | 8.3 | 8.3 | 6.1 | 5.6 | 45.9 | 48.21 | 13.6 | 14.82 | 29.64 | 30.74 |
| C19 | K19 | 23 | 34 | 6.6 | 8.1 | 4.8 | 6.1 | 47.92 | 55.74 | 13.75 | 13.28 | 28.7 | 23.82 |
| C20 | K20 | 27 | 30 | 8.1 | 9 | 4.8 | 6.8 | 56.25 | 44.12 | 16.88 | 13.24 | 30 | 29.99 |
| C21 | K21 | 35 | 26 | 8.6 | 7.9 | 7 | 5.7 | 50 | 45.61 | 12.29 | 13.86 | 24.57 | 30.38 |
| C22 | K22 | 28 | 29 | 7.3 | 8.4 | 5.2 | 5.9 | 53.85 | 49.15 | 14.04 | 14.23 | 26.07 | 28.97 |
| C23 | K23 | 26 | 21 | 7.8 | 7.5 | 5.2 | 5.3 | 50 | 39.62 | 15 | 14.15 | 30 | 35.71 |
| C24 | K24 | 24 | 27 | 7.1 | 8.2 | 4.6 | 5.5 | 52.17 | 49.09 | 15.43 | 14.91 | 29.58 | 30.37 |
| C25 | K25 | 27 | 35 | 8.2 | 9.3 | 5.4 | 7.3 | 50 | 47.95 | 15.19 | 12.74 | 30.37 | 26.57 |
| C26 | K26 | 25 | 30 | 7.2 | 8 | 5.7 | 6.6 | 43.86 | 45.45 | 12.63 | 12.12 | 28.8 | 26.67 |
| C27 | K27 | 30 | 31 | 8.4 | 8.6 | 6.2 | 6.4 | 48.39 | 48.44 | 13.55 | 13.44 | 28 | 27.74 |
| C28 | K28 | 29 | 33 | 7.6 | 9.5 | 5 | 7 | 58 | 43.42 | 15.2 | 12.5 | 26.21 | 28.79 |
| C29 | K29 | 22 | 26 | 6.5 | 7.3 | 4.7 | 5.7 | 46.81 | 45.61 | 13.83 | 12.81 | 29.55 | 28.08 |
| C30 | K30 | 31 | 36 | 8.3 | 10.2 | 6.3 | 7.6 | 49.21 | 47.37 | 13.17 | 13.42 | 26.77 | 28.33 |

**Table 2: Hematological values in Cow and calf.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***COW I D*** | ***Calf I D*** | **Cow** | **Calf** | **Cow** | **Calf** | **Cow** | **Calf** | **Cow** | **Calf** | **Cow** | **Calf** |
| **L%** | **L%** | **M%** | **M%** | **N%** | **N%** | **E%** | **E%** | **B%** | **B%** |
| C1 | K1 | 54 | 63 | 4 | 7 | 22 | 15 | 16 | 14 | 4 | 1 |
| C2 | K2 | 55 | 72 | 6 | 8 | 27 | 17 | 11 | 2 | 1 | 1 |
| C3 | K3 | 58 | 62 | 7 | 10 | 33 | 22 | 2 | 6 | 0 | 0 |
| C4 | K4 | 53 | 64 | 9 | 12 | 30 | 20 | 8 | 2 | 0 | 0 |
| C5 | K5 | 49 | 68 | 3 | 10 | 36 | 18 | 12 | 3 | 0 | 1 |
| C6 | K6 | 65 | 83 | 17 | 2 | 14 | 6 | 4 | 5 | 0 | 0 |
| C7 | K7 | 64 | 66 | 6 | 14 | 14 | 18 | 16 | 2 | 0 | 0 |
| C8 | K8 | 66 | 69 | 9 | 8 | 18 | 20 | 6 | 3 | 1 | 0 |
| C9 | K9 | 67 | 57 | 6 | 21 | 12 | 15 | 14 | 7 | 1 | 0 |
| C10 | K10 | 62 | 63 | 7 | 8 | 20 | 20 | 11 | 8 | 0 | 1 |
| C11 | K11 | 59 | 67 | 6 | 10 | 22 | 18 | 12 | 5 | 1 | 0 |
| C12 | K12 | 67 | 71 | 5 | 7 | 18 | 13 | 9 | 9 | 1 | 0 |
| C13 | K13 | 60 | 60 | 8 | 8 | 25 | 21 | 8 | 10 | 0 | 1 |
| C14 | K14 | 61 | 58 | 6 | 10 | 21 | 25 | 12 | 7 | 0 | 0 |
| C15 | K15 | 66 | 63 | 7 | 9 | 19 | 17 | 8 | 10 | 0 | 1 |
| C16 | K16 | 58 | 68 | 6 | 8 | 17 | 17 | 18 | 7 | 1 | 0 |
| C17 | K17 | 62 | 63 | 5 | 10 | 16 | 14 | 16 | 11 | 1 | 2 |
| C18 | K18 | 68 | 72 | 6 | 6 | 22 | 17 | 9 | 4 | 1 | 1 |
| C19 | K19 | 63 | 58 | 9 | 11 | 20 | 24 | 6 | 7 | 2 | 0 |
| C20 | K20 | 65 | 56 | 7 | 14 | 21 | 25 | 7 | 5 | 0 | 0 |
| C21 | K21 | 61 | 65 | 8 | 8 | 23 | 23 | 7 | 6 | 1 | 0 |
| C22 | K22 | 59 | 67 | 6 | 7 | 21 | 16 | 12 | 9 | 2 | 1 |
| C23 | K23 | 62 | 62 | 7 | 8 | 19 | 24 | 11 | 6 | 1 | 0 |
| C24 | K24 | 66 | 69 | 5 | 9 | 17 | 16 | 12 | 8 | 0 | 0 |
| C25 | K25 | 65 | 63 | 8 | 6 | 22 | 24 | 5 | 7 | 0 | 0 |
| C26 | K26 | 64 | 66 | 6 | 7 | 18 | 23 | 11 | 3 | 1 | 1 |
| C27 | K27 | 64 | 57 | 7 | 11 | 17 | 25 | 11 | 5 | 1 | 2 |
| C28 | K28 | 60 | 60 | 8 | 14 | 22 | 22 | 9 | 4 | 1 | 0 |
| C29 | K29 | 61 | 55 | 6 | 8 | 24 | 27 | 8 | 9 | 1 | 0 |
| C30 | K30 | 60 | 61 | 6 | 7 | 23 | 27 | 11 | 4 | 0 | 1 |

**Table 3: Biochemical values in cow and calf.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Cow** | **Calf** | **Cow** | **Calf** | **Cow** | **Calf** | **Cow Calf** |  **Cow** | **Calf** |
| ***COW*** ***I D*** | ***Calf*** ***I D*** | **Total Protein** | **Total Protein** | **Albumin** | **Albumin** | **Glucose** | **Glucose** | **Phosphorus** | **Calcium** | **Calcium** |
| **gm/dl** | **gm/dl** | **gm/dl** | **gm/dl** | ***(mg/dl)*** | **(mg/dl)** | ***(mg/dl)*** | ***(mg/dl)*** | ***(mg/dl)*** | **(mg/dl)** |
| C1 | K1 | 7.51 | 7.21 | 3.83 | 3.93 | 45.2 | 9.4 | 1.90 | 2.00 | 8.2 | 9.4 |
| C2 | K2 | 6.97 | 5.95 | 3.24 | 3.41 | 54.8 | 7.6 | 1.67 | 2.90 | 7.3 | 7.6 |
| C3 | K3 | 7.81 | 6.38 | 2.97 | 3.38 | 36.7 | 6.8 | 1.58 | 1.58 | 8.2 | 6.8 |
| C4 | K4 | 7.78 | 6.67 | 3.23 | 3.25 | 41.5 | 11.2 | 2.56 | 2.15 | 10.2 | 11.2 |
| C5 | K5 | 7.23 | 6.73 | 3.57 | 2.5 | 47.9 | 8.8 | 1.99 | 1.65 | 9 | 8.8 |
| C6 | K6 | 8.53 | 5.65 | 3.61 | 1.9 | 64.2 | 14.4 | 2.90 | 2.20 | 10.1 | 14.4 |
| C7 | K7 | 11.65 | 8.31 | 5.39 | 3.25 | 63.6 | 10.8 | 2.60 | 2.15 | 14.2 | 10.8 |
| C8 | K8 | 11.5 | 8.88 | 5.5 | 2.69 | 68.7 | 13.1 | 1.90 | 2.00 | 8.8 | 13.1 |
| C9 | K9 | 7.91 | 7.52 | 3.58 | 4.3 | 46.8 | 18.6 | 2.25 | 2.10 | 16.9 | 18.6 |
| C10 | K10 | 6.81 | 6.72 | 3.5 | 3.92 | 20 | 6.3 | 2.65 | 1.90 | 7.7 | 6.3 |
| C11 | K11 | 8.4 | 6.37 | 3.79 | 3.78 | 37.6 | 7.3 | 1.80 | 1.58 | 7.4 | 7.3 |
| C12 | K12 | 6.42 | 6.19 | 2.67 | 3.58 | 38.2 | 10.2 | 1.75 | 1.80 | 8.1 | 10.2 |
| C13 | K13 | 7.39 | 5.83 | 2.94 | 3.81 | 39.9 | 9.5 | 2.30 | 2.10 | 9.3 | 9.5 |
| C14 | K14 | 7.79 | 6.31 | 3.47 | 3.21 | 38.8 | 12.2 | 2.20 | 2.60 | 11.2 | 12.2 |
| C15 | K15 | 7.75 | 6.87 | 3.59 | 2.93 | 52.3 | 8.6 | 1.55 | 2.10 | 7.4 | 8.6 |
| C16 | K16 | 7.62 | 6.35 | 3.33 | 3.11 | 41.2 | 10.1 | 2.25 | 1.85 | 7.8 | 10.1 |
| C17 | K17 | 7.37 | 7.2 | 3.58 | 4.1 | 60.3 | 8.3 | 1.95 | 2.25 | 8.9 | 8.3 |
| C18 | K18 | 6.81 | 6.13 | 2.87 | 3.32 | 29.2 | 11.7 | 1.65 | 2.95 | 7.9 | 11.7 |
| C19 | K19 | 7.6 | 7.42 | 3.27 | 4.2 | 37.7 | 11.4 | 1.59 | 1.76 | 9.2 | 11.4 |
| C20 | K20 | 7.81 | 5.8 | 3.71 | 3.9 | 25.9 | 10.7 | 2.60 | 2.75 | 10.2 | 10.7 |
| C21 | K21 | 7.42 | 6.31 | 3.63 | 2.58 | 41.3 | 14.4 | 2.39 | 1.65 | 9.6 | 14.4 |
| C22 | K22 | 6.96 | 7.73 | 3.59 | 3.8 | 50.2 | 9.8 | 2.45 | 1.60 | 8.4 | 9.8 |
| C23 | K23 | 6.12 | 6.58 | 3.31 | 3.98 | 30.6 | 11.2 | 2.60 | 1.90 | 7.8 | 11.2 |
| C24 | K24 | 7.93 | 6.1 | 2.91 | 3.13 | 31.8 | 8.2 | 1.76 | 1.50 | 8.3 | 8.2 |
| C25 | K25 | 7.39 | 5.92 | 3.76 | 3.73 | 27.9 | 7.8 | 1.85 | 2.80 | 9 | 7.8 |
| C26 | K26 | 7.56 | 6.48 | 3.88 | 3.12 | 36.7 | 10.3 | 1.80 | 2.30 | 8.8 | 10.3 |
| C27 | K27 | 7.1 | 6.79 | 3.48 | 3.91 | 51 | 7.6 | 1.70 | 1.95 | 10.1 | 7.6 |
| C28 | K28 | 7.5 | 5.81 | 3.59 | 3.69 | 47.3 | 12.9 | 2.55 | 2.10 | 9.2 | 12.9 |
| C29 | K29 | 7.35 | 4.98 | 3.73 | 2.58 | 45 | 13.2 | 2.10 | 2.80 | 7.8 | 13.2 |
| C30 | K30 | 6.98 | 6.79 | 2.7 | 3.35 | 62.1 | 11.1 | 2.00 | 1.90 | 11.8 | 11.1 |

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**Chittagong Veterinary and Animal Sciences University**

**Format of case recording sheet :**

**Case Registration No.: Date of Registration:**

1. **Name of the owner:** …………………………………………………………………...

**Occupation:** ……………….……… **Village/Ward:** …………………………………

**Upazilla:** …………………………………… **District:** …………………………….....

1. **Patients Data:**

**Species:** Cattle **Breed:** HF × Local /Shahiwal × Local

**Age:** ………………… **Sex:** M/F**Parity**:…………… **Weight:** ……………

**Body Condition Score (BCS):** 1(Cachectic)/ 2(Poor)/ 3(Fair)/ 4(Good)/ 5 (Over weight/Fat)

1. **Clinical History:**

**Onset:** Sudden/Gradual **Duration of illness:** ……. hrs/days

**Weakness:** Yes/No.

**Pre-disposing Factors:**………………………………………………..............................................................................................................................................................................................

1. **Clinical Examination:**

**Temperature:** ………. **°**F **Hair Coat:** Shiny/ Rough and Stray/ lesions/ other.

**Visible mucous membrane:** Pale/ Pink/ Icteric/ Cyanotic/ other (……………)

**Dehydration:** Normal/ mild/ moderate/ Severe **Abdomen size:** Normal/ Distended

**Feces:** Visible worm/ Bloody/ Blackish/ Greenish/ Milky white/ mucous/ foul odors/ others

**Major Clinical Sign:** ………………………………………………………

**General attitude:** Alert/ Dull/ Depressed/ Other. **Mouth lesion:** Y/N.

1. **Referred for:** Hematology.
2. **Samples/ specimen:** Blood.
3. **Diagnosis:**

**Presumptive:** ……………………. **Confirmatory:** ………………………….

**Signature**