Chapter I: Introduction

Bangladesh, an agro based country which economics depend mostly on agriculture. In 2013-2014 the contribution of agriculture is 3.7%. Livestock plays an important role in nutrition directly through the consumption of animal products by livestock owners and their families and indirectly through the sale of animals and animal products as a source of income (Aengwanich *et al*., 2009). In 2013-2014 livestock contribution in Gross Domestic Product (GDP) was 1.8% (Economic review, 2014). Dairy sector is one of the most important parts in livestock sector in Bangladesh. To meet the demand for milk and meat for the large population of Bangladesh, the numbers of dairy farms rearing high yielding dairy cows are rising gradually. The number of cattle is 23.5 million and milk production is 3.7 million ton (Economic review, 2014). The number of registered farm was 55,174 up to February 2013 and artificial inseminated cattle 1.8 million (Economic review, 2014). To fulfill the extra demand, Bangladesh imports a huge amount of powder milk and dairy products. Under these circumstances, to meet up the deficiency of milk and milk products in a shortest possible time, the government and private organizations are putting efforts to enhance the present milk production status. Besides government, there are many private and cooperative enterprises, like Bangladesh Milk Producers Cooperative Union limited (BMPCUL) known as Milk Vita, Bangladesh Rural Advancement Committee (BRAC), Lal Teer Livestock Limited (LTL), Pran Dairy, Gentech International, and Grameen Motso O Pashusampad Foundation (GMPF) are working for dairy breed development program and providing technical assistance to the farmers.

Poor fertility is of economic importance for dairy enterprises, because it results in higher levels of involuntary replacement and reduced annual milk production. Adequate nutrition before calving and during postpartum period is essential if acceptable estrus and rebreeding performance are to be achieved. The periparturient period is important in terms of its influence on the health and the subsequent performance of dairy cows, since cows develop serious metabolic and physiological changes during these periods (Tanaka *et al*., 2011). In fact, it is well known that during the pregnancy all the metabolic pathways are involved in sustaining the fetus growth (Bell, 2000). The period of

transition between late pregnancy and early lactation presents a huge metabolic challenge to the high-yielding dairy cow and the haemato biochemical profiles are important in evaluating the health status of animals during this transition (Hagawane *et al*., 2009; Bell, 2000). Immediately after the calving, high rates of body condition score losses are associated with a severe negative energy balance status, indicated by alterations in blood metabolite and hormone profiles (Wathes *et al*., 2009).

The changes in biochemical and hematological constituents are important indicators of the physiological or pathological state of the animal (Hassan *et al*., 2012). It is well known that variables such as breed, stage of growth, age, reproduction status and stage of lactation have an influence on many blood parameters (Doornenbal *et al*., 1988).

Therefore the research work on the biochemical indices of cow around periparturient period in commercial dairy farm was performed on following objectives.

Overall objective

To know the nutritional status as well as biochemical profile of dairy cow around periparturient period (before and after parturition) in commercial dairy farm in Chittagong.

Specific objectives

- To determine carbohydrate such as glucose around periparturient period.
- To determine proteins such as total protein and albumin around periparturient period.
- To investigate lipids profile such as cholesterol, triglyceride, Low density lipoprotein (LDL), High density lipoprotein (HDL) around periparturient period.
- To find out mineral level such as calcium, magnesium, phosphorus around periparturient period.

Chapter II: Review of Literature

Dairy cattle are susceptible to increase incidence and severity of disease during the periparturient period (Sordillo *et al*., 2009). The health and metabolism of farm animals have been assessed by measuring serum biochemical parameters. It was noted that human medicine, clinical biochemical analyses have been used extensively in large-scale health investigations (Hewett, 1974). Measurement of the parameter provides practical diagnostic tools for evaluating pathological conditions in live animals or for monitoring the health status of animals (Verheyen *et al*., 2007). Good correlation between the serological abnormalities of herd blood parameters and the existence of clinical problems within the herds has been found. The fertility of farm animals has also been found to be significantly inversely related to levels of serum biochemical parameters such as serum inorganic phosphorus, serum potassium, serum total protein and serum urea nitrogen (Hewett, 1974).

There are several reports exist on serum electrolytes of cattle especially cows during pregnancy and lactation in the literature (Belyea *et al*., 1975). The serum electrolyte profile of the crossbred animals may be different from those of the indigenous cattle as several factors such as herd, age, stage of lactation, feeding, season, gestation and sampling method (Hewett, 1974), and season (Akerejola *et al.*, 1980) and diet (Wilson and Hart, 1932), stage of gestation and lactation (Verheyen *et al*., 2007), breed (Groth *et al*., 1986), disease (Odink *et al*., 1990), presence of haemolysis (Dorner *et al*., 1983) and parity (Verheyen *et al*., 2007) do influence blood parameters.

Pregnancy and lactation are physiological status considered to modify metabolism in animals and induce stress (Iriadam, 2007). Activity of some blood biochemical profile may be used as indicator of physical stress in the parturition and the post partum periods (Tanaka *et al*., 2011; Piccione *et al*., 2010). During the time of immediate postpartum period, the immune system of dairy cow's is challenged severely (Goff, 2006), and the innate and humoral both defense systems are reduced. Diseases and disorder incidences can be high during this time period and have a negative impact on reproductive

performance. Santos et al. (2004) found in a study that inadequate intake of nutrients and inadequate body reserves during early lactation are the major factors affecting reproductive performance of dairy cows. Improving energy balance by increasing energy intake through additional non-fiber carbohydrates or supplemental fat in the diet reduces days to first ovulation and improves conception postpartum (Santos *et al*., 2004). Some strong evidences suggest that management of cows during the prepartum period affects uterine health. Inadequate intake of nutrients prepartum and altered feeding behavior increases the risk of metritis in dairy cows (Santos *et al*., 2004). The pregnancy and lactation phases affect significantly the metabolic profile and so the variation recorded during different physiological phases is expected. The transition from gestation to lactation is a period of great metabolic stress for dairy cows (Rollin *et al*., 2010). In fact, the milk production and its composition are found to profoundly influence the metabolically status of dairy cows (Heck *et al*., 2009).

2.1 The periparturient period

The periparturient period is the most challenging time for the dairy cows. The mid dry period is considered to be a resting stage between two lactations with low nutrient requirements, but as parturition approaches marked changes in hormonal status to accommodate parturition and lacto genesis occur (Bell, 1995). The low requirements of dry cows have given the wrong impression of the critical role of this time. Generally, a reduction in Dry matter intake (DMI) occurs seven to ten days before calving, but the nutrient demand for the growing fetus and initiation of milk production is increasing at the same time (Grummer, 1995). As a consequence there is a gap in nutrient demand with large changes in nutrient metabolism and metabolic disorders may emerge. A high incidence of metabolic diseases, like ketosis, and infectious diseases, e.g. mastitis and endometritis, occurs in early lactation (Rukkwamsuk *et al*., 1999; Stabel *et al*., 2003). For this reason, the transitional period needs to be carefully monitored regarding factors such as management, adequate feed composition, e.g. the balance between energy and protein, adequate micronutrient supplementation, and feeding routines to make the transition from the dry to the lactating stage as smooth as possible. Both over- and under-conditioned dairy cows have a higher incidence of diseases than normally conditioned animals

(Rukkwamsuk *et al*., 1999). Inadequate energy intake during late pregnancy has profound effects on reproductive parameters, such as postpartum interval to first estrus and pregnancy rate (Randel, 1990). Fat animals suffer from a more pronounced and more prolonged depression in DMI after calving, resulting in a deeper negative energy balance than cows in normal body condition (Agenäs *et al*., 2003).

2.2 Metabolic diseases of the dairy cow

As research is solidifying the connection between metabolic disease and impaired immune function leading to infectious disease, it seems more prudent than ever to do all that we can to reduce metabolic disease in the dairy cow (Figure 1). To make matters worse, numerous epidemiological studies clearly demonstrate that a cow with one metabolic disorder, such as milk fever, is at much greater risk of developing a second metabolic disorder, such as ketosis, than a cow that did not have any metabolic disease. Lactation imposes tremendous metabolic demands on the dairy cow. The adaptation to these demands at the onset of lactation, coinciding with the birth of the calf, can overwhelm the ability of some cows to cope, leading to breakdowns in metabolism of the cow. The failure to adequately fuel the body during lactation can lead to fatty liver and ketosis. The inability to maintain sufficient concentrations of calcium in the blood to allow normal bodily functions can cause periparturient hypocalcaemia paresis, more commonly known as milk fever. Early lactation is often associated with other common lead to fatty liver and ketosis. The inability to maintain sufficient concentrations of calcium in the blood to allow normal bodily functions can cause periparturient hypocalcaemia paresis, more commonly known as milk fever.

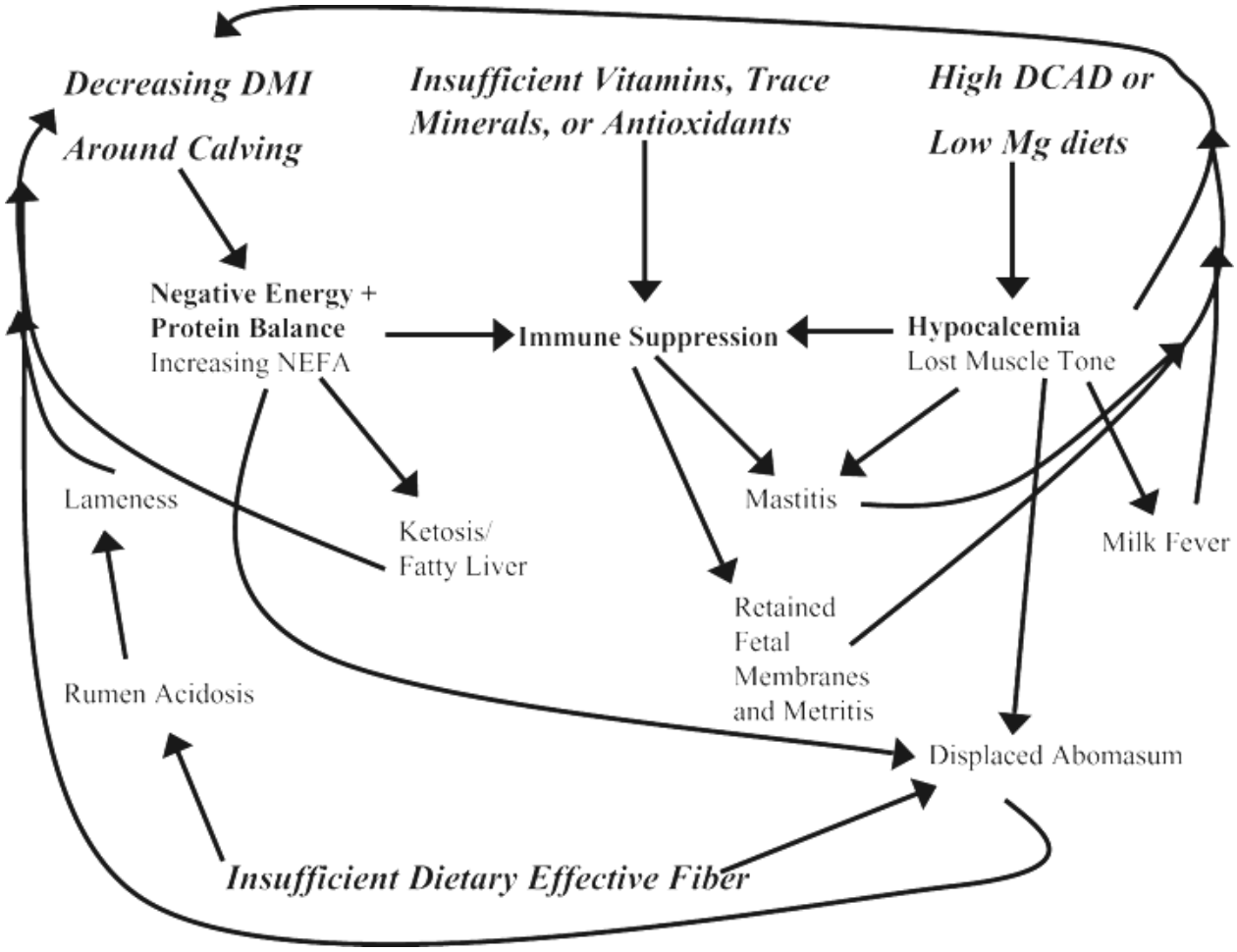


Figure 1: Interrelationships between nutrition and disease in the periparturient dairy cow (Journal of Dairy Science, 2006)

Through genetic selection and improved feeding and management, the milk yield of the modern dairy cows increase 2-3% annually (Hutjens, 1996). As the management becomes more intensive and the number of cows per herd increases, the risk of metabolic disturbances may increase, which can have negative consequences on animal health. This can lead to an increased incidence of metabolic diseases like ketosis, fatty liver and reproductive associated problems (Rukkwamsuk *et al*., 1999). However, the incidence of infectious diseases can also increase due to suppression of immune functions during this

period. Important infectious diseases occurring during the periparturient period are mastitis and endometritis (Gröhn and Rajala-Schultz, 2000).

2.3 Serum biochemical elements

2.3.1 Carbohydrate

2.3.1.1 Glucose

Glucose is a main energy source for definite animal tissues. Glucose is necessary metabolite for growth of tissues, preservation and used as biochemical pathway in the animal bodies. Lactose of milk is produced from blood glucose and is a significant factor in organizing milk yield in dairy animals in mammary cells (Kadwal and Qureshi, 2011). Glucose is involved in stimulating insulin secretion primarily by increasing the concentration of cytoplasmic calcium ions. Apart from mannose, some amino acids and short chain fatty acids (in ruminants) which act directly, beta cell function may be enhanced by cholinergic stimulation, the neuropeptide cholecystokinin, glucagon and gut hormones such as the gastric inhibitory peptide.

Optimal supply of glucose to the liver and mammary gland plays an important role in preserving the health of dairy cows in the early stage of lactation. The first metabolic change in primary ketosis in dairy cows in early lactation is hypoglycemia. It causes serious metabolic changes in the body, manifested through lipid mobilization from body reserves and ketogenesis and lipogenesis in the liver (Vazquez-Anon *et al*., 1994). Glucose is an essential nutrient needed for several tissues, and the high demand in the beginning of lactation often exceeds the amount of glucose available (Holtenius and Holtenius, 1996). A four-fold increase in glucose requirements has been reported in high yielding dairy cows at the beginning of lactation compared to nonlactating cows (Bell and Bauman, 1997). The main substrate for the synthesis of glucose is propionate from microbial fermentation of feed carbohydrates. However, especially in early lactation as a consequence of insufficient DMI and high demand of glucose, the cows are also dependent of endogenous substrates, mainly glucogenic amino acids (glutamine, alanine) from degraded endogenous protein sources, and glycerol from adipose tissue mobilization (Bell and Bauman, 1997).

2.3.2 Protein

Proteins are very important in living beings and they take part in almost all of the life processes. Total proteins of serum are the major blood components responsible for maintaining homeostasis and metabolism. Protein deficiency results in delay onset of puberty, increased days open, decreased dry matter intake (DMI) and lead to energy deficit. Adequate protein intake is necessary for normal fetal growth and development (Gaikwad *et al*., 2007). Protein requirements of the dairy cow are not as clearly defined as those for energy. However, there is a clear difference in crude protein requirements between heifers and mature dairy cows, and between lactating and non-lactating cows. The crude protein requirement for dry and low producing dairy cows is 12-13%, and for high yielding dairy cows it is 15-18% of DMI (NRC, 2001). Heifers need a higher protein intake as they are still growing themselves, but also for the development of the mammary gland. Feeding protein above those recommendations does not confer any advantages (Doepel *et al*., 2002), or may impair DMI and reproduction, as the capacity to detoxify ammonia is reduced by 40% around parturition (Strang *et al*., 1998).

Albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues. It also acts as a plasma carrier by non-specifically binding several hydrophobic steroid hormones and as a transport protein for hemin and fatty acids. Increased proteins and globulin in the blood of cows indicate an activation of immune response following infection of the mammary gland. Serum albumin and immunoglobulins are implicated in udder defense mechanisms (Tsenkova *et al.,* 2001). Almost all proteins in the serum are produced by the liver. Immunoglobulins are the notable exception and they are produced by lymphoid tissue. Serum proteins are relatively short lived with most having half lives of about 10 days. The breakdown of these proteins occurs mostly in the liver with some catabolic activity in the intestine and kidney. Animal plasma normally contains 25-35 gm/L of albumin which constitutes 40 - 60% of the total protein concentration. Fluid accumulations in body cavities and tissue usually result when albumin levels drop below 10 gm/L. However, fluid may accumulate with higher albumin concentrations if hypertension and loss of vessel integrity, etc. are present. Plasma and serum proteins, act as anions in acid-

base balance, take part in coagulation reactions, and serve as carriers for many compounds (Pintea *et al*., 2008).

2.3.3 Lipid

The biological mechanisms that regulate the synthesis and degradation of lipids and lipid transport in plasma are of great significance to animal agriculture. Regulation of lipid synthesis and degradation in meat animals has been studied to some extent. However, the role of plasma lipoproteins in transporting lipid to extra hepatic tissues in meat animals is not well defined (Kris-Etherton and Eteherton, 1982). The lipoprotein fraction transports triglycerids to different organs and tissues. After the loss of triglycerids, LDL are formed and after further metabolism HDL (Basoglu *et al*., 1998). Most often high triglycerides are associated with an increase in LDL cholesterol and a decrease in HDL cholesterol. The serum total cholesterol concentration was minimum following calving and got build up as the lactation progresses (Rowlands *et al*, 1980). 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. Due to improvement of age animal tissues consumption of energy declines with low maintenance requirements and little milk production, the metabolic activities get slower and the serum concentration of triglycerides turn out to be increase in blood. Due to consequence of the extensive adipose tissue mobilization in early lactation there is a manifold rise in plasma concentration of non esterified fatty acid (NEFA). The liver plays an important role in fat metabolism, removing NEFA from the blood. In early lactating cows, about 50% of NEFA are oxidised to ketone bodies or reesterified to triglycerides in the liver (Bell, 1995). However, as ruminants do not competently export triglycerides as part of very low density lipoprotein, significant amounts are stored in the liver (Rukkwamsuk *et al*., 1999). Due to hepatic fat deposition, metabolic disturbances such as fatty liver and ketosis emerge with the production of ketone bodies, which can have negative effects on the immune response.

2.3.4 Minerals

Concerning the electrolytes serum levels, all animals require minerals for growth, reproduction and lactation. Calcium (Ca), phosphorus (P) and magnesium (Mg) are the main mineral elements of body which have many function in dairy cattle especially in high producing ones (Samardzija *et al*., 2011). Milk fever occurs when calcium leaves the blood to support milk production faster than Ca can be put back into the blood from the diet, skeletal Ca stores, and renal conservation of calcium. The disease is characterized by an acute decline in blood Ca concentration to levels that no longer support nerve and muscle function (Goff, 2006). A dietary deficiency or disturbance in metabolism of calcium, phosphorus or vitamin D including imbalance of calcium- phosphorus ratio is the principle cause of osteodystrophies and periparturient hypocalcemia (Smith, 2009). Magnesium deficiency causes lactation tetany in adult dairy cows and hypomagnesaemia tetany of calves. Dairy cows during lactation absorb 1.71 g calcium in turn of each gram phosphorous absorption. Body calcium store during gestation and especially in last two months of pregnancy decreases to a very low level. Each kilogram of milk with 4% fat approximately has 1.22 g calcium (Radostits *et al*., 2000). Amount of phosphorous in dairy cow's feed must be very high because of losing high amount of endogenous phosphorous in feces, absorption of phosphorous from alimentary tract is approximately low, high concentration of phosphorous in milk. In contrast to calcium there was not any mechanism for transfer of bone phosphorous to blood stream (Smith, 2009).

Chapter III: Materials and methods

3.1 Study area

The present investigation was conducted in Paharika Farm Ltd. which is located at Fatikchari in Chittagong district (Figure 2). This farm was selected based on well organized management practices and regular data keeping record. For intensive monitoring, it was easy and the owner of the farm agreed to provide data and sample as the research required.

3.2 Study period

The study was conducted for a period of six months; starting from July 2014 to November 2014.

3.3 Description of study area

Fatikchari is a sub-district in Chittagong district. It is almost hilly area. The farm is situated about five kilometers away from the main highway road of Fatikchari. The farming system of the dairy unit is intensive type and face in system. High yielding varieties of green grasses e. g. Napier, Para, German, Zumboo are available for the cattle in farm area. Advanced pregnant cows are kept in maternal pen separately. The area is well ventilated. Balanced feeding system, deworming and vaccination schedule (Anthrax, Black Quarter, Food and Mouth Disease, Hemorrhagic Septicemia etc.) maintained properly according to the residential doctor of the farm. Cross breed of the Holstein Friesian (HF) are kept for milk production.

3.4 Selection of cow

There are a total of 300 hundred cattle in the farm; among them 30 are male and 270 are female. Among 270 female, 50 are heifers, 90 are dry cow and 130 are milking cow. A total of twenty four cross breed of HF dairy cow were selected for the study. They were same parity and same Body Condition Score (BCS) and will deliver 25-30 days from the start of study.

3.5 Experimental group of dairy cow

Twenty four cows were given unique identification number by proper tagging and monitored for 3 months for three different phases during August to November 2014. The phases were included as one month before expected date of parturition (Stage 1), within 7 days after parturition (Stage 2) and 2 month after parturition (Stage 3).

3.6 Collection of blood sample and separation of serum

Approximately 5ml of blood were aseptically collected between 7am to 10am from jugular vein via vein puncture by proper restraining technique. Collected blood samples were kept in vaccutainer (BD Vaccutainer, USA) without anticoagulant for smooth coagulation. After three hours the coagulated blood samples were centrifuged using centrifuge machine (Hettich ZENTRIFUGEN-EBA 20) for 20 minutes at 3000 rpm. Obtained serum samples were shifted to the eppendrof tube and given unique identification no. Then the samples were kept in cool box for transportation from study area to research laboratory of Department of Physiology, Biochemistry and Pharmacology (DPPB), Chittagong Veterinary and Animal Sciences University (CVASU). After reaching in laboratory the serum samples were further centrifuged using eppendrof centrifuge machine **(**CR-68X, Denmark**)** at 3000 rpm for 15 minutes. The serum was separated and shifted to eppendrof tube using micropipette. The eppendrof tubes were tagged properly. The blood samples were collected 3 times from each tagged cow as one month before parturition, within 7 days after parturition and 2 month after parturition. Therefore, the total obtained blood sample were 72 of which 24 in each group.

3.7 Preservation of serum sample

The obtained serum samples were stored in -20^{o}C until analysis for biochemical test.

3.8 Biochemical assay of serum sample

Proper aseptic measures were done at the time of serum analysis in the laboratory. Serum and all reagents were thawed by keeping in room temperature approximately 30 minutes before the analysis. The serum samples were vortexed for mixing component of serum

uniformly. The serum glucose, Total Protein (TP), Albumin (Alb) , Triglyceride (Tg), Cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Calcium (Ca), Phosphorus (P), Magnesium (Mg) was assayed using automated Biochemical analyzer (Humalyzer-3000, Germany) according to directions provided by the manufacturer of kit. Randox kit were used to determine glucose, TP, Alb, Tg, Cholesterol, Ca; Chroma test kit for P, Mg; Biorex kit for HDL, LDL. A total number of 72 serum samples were analyzed as described in blood sampling protocol).

3.8.1 Carbohydrate assay

Colorimetric spectrophotometric methods were used for determination of glucose Concentrations (Barham and Trinder, 1972).

3.8.1.1 Glucose assay

3.8.1.1.1 Assay principle

The principles outcome of glucose is based on the principle of competitive binding between glucose in the test specimen and GOD-PAP reagent of glucose. The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to a red-violet quinoneimine dye as indicator.

Reaction

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneinine} + 4\ H_2O$$

3.8.1.1.2 Materials and reagents

1. Serum sample
2. Glucose conjugate reagent
3. Precision pipettes: 10 µl, 1.0 ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

3.8.1.1.3 Procedure

The sterile eppendorf tubes were taken. 1000 µl of glucose conjugate reagent was taken each into each eppendorf tube. Then 10 µl of glucose standard was added in with the reagent in eppendorf tube and 10 µl of samples serum were taken in each sample eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Glucose standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with glucose conjugate reagent was examined by biochemical analyzer at 500 nm and the reading were taken. The standard value was used as a compared tool.

3.8.2 Protein assay

Colorimetric spectrophotometric methods were used for determination of albumin and total protein (Doumas *et al*., 1971).

3.8.2.1 Total protein assay

3.8.2.1.1 Assay principle

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

3.8.2.1.2 Materials and reagents

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

3.8.2.1.3 Procedure

This was a photometric colorimetric test for total proteins are called Biuret method. The sterile eppendorf tubes were taken. Then 20 µl of total protein standard was taken in an eppendorf tube and 20 µl of sample serums were taken in each eppendorf tube. 1000 µl of total protein conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes, Total protein standards with conjugate reagents were examined first for determined of the standard value. Finally all eppendorf tubes containing sample serum with TP conjugate reagent was examined by automated Humalyzer at 546 nm and the reading was taken. Standard value was used as a compared tool.

3.8.2.2 Albumin assay

3.8.2.2.1 Assay principle

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

3.8.2.2.2 Materials and reagents

1. Serum sample
2. Albumin conjugate reagent
3. Precision pipettes: 10 µl, 1.0 ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

3.8.2.2.3 Procedure

This was a photometric colorimetric test for albumin is called Bromo Cresol Green method. The sterile eppendorf tubes were taken. Then 10 µl of albumin standards was taken in an eppendorf tube and 10 µl of sample serum were taken in each eppendorf tube. 1000 µl of albumin conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 5 minutes. Albumin standards with

conjugate reagent were examined first for determination of the standard value. Then all eppendorf tubes containing sample serum with albumin conjugate reagent was examined using automated Humalyzer at 578 nm and the reading was taken. The standard value was used as a compared tool.

3.8.3 Lipid profile

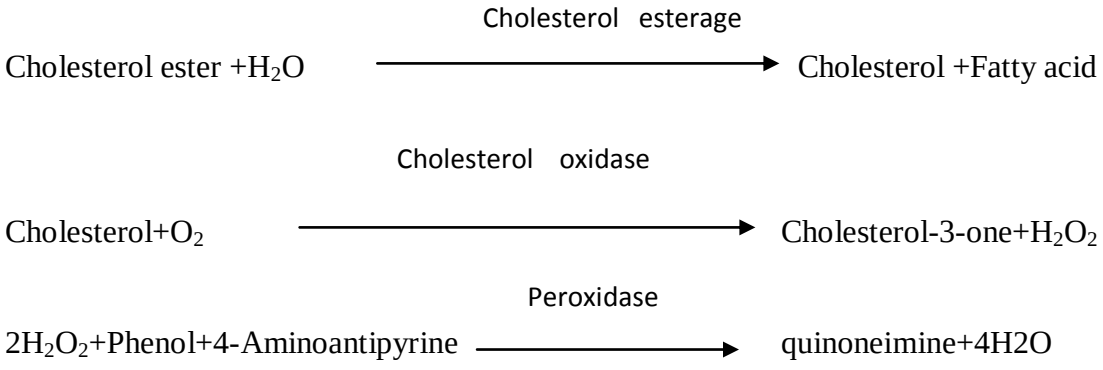
Colorimetric spectrophotometric methods were used for determination of cholesterol concentrations (Roeschlau *et al*., 1974).

3.8.3.1 Cholesterol assay

3.8.3.1.1 Assay principle

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

Reaction



3.8.3.1.2 Materials and reagents

1. Serum sample
2. Cholesterol conjugate reagent
3. Precision pipettes: 10 µl, 1 ml

4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

3.8.3.1.3 Procedure

This was an enzymatic colorimetric test for cholesterol is called CHOD-PAP method. The sterile eppendorf tubes were taken. Then 10 µl of cholesterol standards was taken in an eppendorf tube and 10 µl of sample serums were taken in each eppendorf tube. 1000 µl of cholesterol conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Cholesterol standards with conjugate reagent were examined first for determination of the standard value. Then all eppendorf tubes containing sample serum with cholesterol conjugate reagent was examined by automated humalyzer at wave length 500 nm and the reading was taken. The standard value was used as a compared tool.

3.8.3.2 Triglyceride

3.8.3.2.1 Assay Principle

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

Reaction

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipases}} \text{Glycerol+Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol 3 phosphate} + \text{ADP}$$

$$\text{Glycerol -3- phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Didydroxyacetone+Phosphate+} H_2O_2$$

$$2H_2O + \text{4aminophenazone} + \text{4 Chlorophenolquiniamine} \xrightarrow{\text{POD}} \text{Quiniamine} + HCl + 4H_2O$$

3.8.3.2.2 Materials and reagent

1. Serum sample
2. Tg conjugate reagent
3. Precision pipettes: 10 µl, 1.0 ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves

3.8.3.2.3 Procedure

The sterile eppendorf tubes were taken. Then 1000 µl Tg standards was taken in an eppendorf tube and 10 µl of sample serums were taken in each eppendorf tube The eppendorf tube was then kept in room temperature for 10 minute. Tg standards with conjugate reagent were examined first for determination of the standard value. Then all eppendorf tubes containing sample serum reagent was examined by automated humalyzer at absorbance 500 nm and the reading was taken. The standard value was used as a compared tool.

3.8.3.3 LDL Assay

3.8.3.3.1 Assay Principle

The principles outcome of LDL is based on the principle of competitive bindings between LDL and LDL reagent. Low density lipoproteins are precipitated by the addition of heparin at their isoelectric point (pH-5.04). The HDL and VLDL remain in the supernatant and can be determined by enzymatic methods.

LDL Cholesterol = Total Cholesterol – Cholesterol in the supernatant. The absorbance of this complex is proportional to the LDL concentration in the sample.

3.8.3.3.2 Materials and reagents

1. Serum sample
2. LDL conjugate reagent
3. Precision pipettes: 10 µl, 100 µl, 1 ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

3.8.3.3.3 Procedure

The sterile eppendorf tubes were taken. Then 100 µl of LDL standards was taken in an eppendorf tube and 100 µl of sample serums were taken in each eppendorf tube. 1000 µl of LDL conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then kept in room temperature for 10 minutes and then centrifuged at 4000 rpm for 15 minutes. The LDL concentration of the supernatant was determined within 1 hour after centrifugation. LDL standards with conjugate reagent were examined first for determination of the standard value. Then all eppendorf tubes containing sample serum with LDL conjugate reagent was examined by automated Humalyzer at 500 nm and the reading was taken. The standard value was used as a compared tool.

3.8.3.4 HDL assay

3.8.3.4.1 Assay Principle

Low density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitavily by the addition of phosphotangstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined.

3.8.3.4.2 Materials and reagents

1. Serum sample
2. HDL conjugate reagent
3. Precision pipettes: 500 µl, 1 ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol.

3.8.3.4.3 Procedure

The sterile eppendorf tubes were taken. Then 400 µl of HDL standards was taken in an eppendorf tube and 200 µl of sample serums were taken in each eppendorf tube. 100 µl of distilled water was then added to each eppendorf tube. The eppendorf tubes were kept in room temperature for 10 minutes and then centrifuged at 4000 rpm for 15 minutes. Then 100 µl concentration of the supernatant was taken in another eppendrof tubes and 1000 µl

Cholesterol reagent was added in each tubes and determined within 1 hour after centrifugation. HDL standards with conjugate reagent were examined for determined of the standard value. Then all eppendorf tubes containing sample serum with HDL conjugate reagent was examined by automated Humalyzer at 500 nm and the reading was taken. The standard value was used as a compared tool.

3.8.4 Mineral assay

All serum samples were analyzed for serum total Ca, Mg and P concentrations by automated Humalyzer. Total serum concentration of phosphorus was determined using the heteropoly acid-blue method (Boltz and Lueck, 1958).

3.8.4.1 Calcium (Ca) assay

3.8.4.1.1 Assay Principle

The principle outcome of calcium is based on the principle of competitive bindings between Ca and Ca reagent which is a Colorimetric method that is O-Cresolphthalein complexone, without depolarization. Calcium ion forms a violet complex with O-Cresolphthalein complexone in an alkaline medium. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.

3.8.4.1.2 Materials and reagents

1. Serum sample
2. Ca conjugate reagent
3. Precision pipettes: 25 µl, 500 µl
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

3.8.4.1.3 Procedure

The sterile eppendorf tubes were taken. Then 25 µl of Ca standards was taken in an eppendorf tube and 25 µl of sample serums were taken in each eppendorf tube. 500 µl R1 and 500 µl R2 of Ca conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 5 minutes. Ca standards with conjugate

reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with Ca conjugate reagent was examined by automated Humalyzer at 570 nm and the reading was taken. The standard value was used as a compared tool.

3.8.4.2 Magnesium (Mg) assay

3.8.4.2.1 Assay Principle

The principles outcome of Magnesium (Mg) is based on the principle of competitive bindings between Mg and Mg reagent. Magnesium combines with Calmagite in an alkaline medium to form a red coloured complex. Interference of calcium and proteins is eliminated by the addition of specific chelating agents and detergents. Intensity of the colour formed is directly proportional to the amount of magnesium present in the sample.

Reaction:

Calmagite + Mgnesium $\xrightarrow{P^H\ 11.5}$ Calmagite-Magnesium complex

3.8.4.2.2 Materials and reagents

1. Serum sample
2. Mg conjugate reagent
3. Precision pipettes: 10 µl, 1.0 ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

3.8.4.2.3 Procedure

The sterile eppendorf tubes were taken. Then 10 µl of Mg standards was taken in an eppendorf tube and 10 µl of sample serums were taken in each eppendorf tube. 1000 µl of Mg conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 5 minutes. Mg standards with conjugate reagent were examined first for determination of the standard value. Then all eppendorf tubes containing sample serum with Mg conjugate reagent was examined by automated

Humalyzer at 520 nm and the reading was taken. The standard value was used as a compared tool.

3.8.4.3 Phosphorus (P) assay

3.8.4.3.1 Assay Principle

The principles outcome of Phosphorus is based on the principle of competitive bindings between Phosphorus and Phosphorus reagent which is a Photometric UV Test for the determination of Phosphorus. Phosphorus reacts with molybdate in strong acidic medium to form a complex. The absorbance of this complex in the near UV is directly proportional to the phosphate concentration.

Reaction

$$PO_4^{3-} + H^+ + (NH_4)_6Mo_7O_{24} \xrightarrow{Pi} \text{Phosphomolybdic complex}$$

3.8.4.3.2 Materials and reagents

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

3.8.4.3.3 Procedure

The sterile eppendorf tubes were taken. Then 10 µl of Phosphorus standards was taken in an eppendorf tube and 10 µl of sample serums were taken in each eppendorf tube. 1000 µl of Phosphorus conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 5 minutes. Phosphorus standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with Phosphorus conjugate reagent was examined by automated Humalyzer at 340 nm and the reading was taken. The standard value was used as a compared tool.

3.9 Statistical analysis

All results are expressed as means ± Standard deviation (SD). The data of biochemical analysis were first stored in MS-Excel 2007 and then exported into STATA 11 (Stata Corporation, USA). One way ANOVA was used to compare the biochemical values among three groups of cow whilst t-test was used to compare between the last two groups. A probability level of $p<0.05$ was considered to be significant.

Chapter IV: Results

4.1 Carbohydrate and protein level in dairy cow before and after parturition

The blood biochemical profile (carbohydrate and protein level) of dairy cow before and after parturition is shown in Table 1. The high level of serum glucose (65.8±13.5 mg/dl) was found in Stage 1 (one month before parturition) than Stage 2 (49.8±8.6 mg/dl, within 7 days after parturition) and Stage 3 (59.1±9.6 mg/dl, two month after parturition). On the other hand, higher level of TP was found in Stage 3 (79.9±5.9 g/L) than Stage 1 and Stage 2. The Albumin level significantly decreased ($p<0.001$) in Stage 1 (Table 1).

Table 1: Comparative assessment of carbohydrate and protein level in three different physiological conditions of studied dairy cow.

Parameter	Reference value**	Stage-1	Stage-2	Stage-3	*p*
Glucose (mg/dl)	45-75	65.8 ± 13.5	49.8 ± 8.6	59.1 ± 9.6	0.068
Total protein (g/L)	67.4-74.6	61.9 ± 6.4	63.5 ± 8.2	79.9 ± 5.9	0.271
Albumin (g/L)	30.3-35.5	18.1 ± 1.2	38.7 ± 12.7	25.4 ± 3.4	<0.001*

Legend: Stage 1: Before one month of parturition
Stage 2: Within seven days after parturition
Stage 3: After two month of parturition
Results were expressed as mean ± SD ((n=24 in each group).
*Significantly different among the group

4.2 Lipid profile in dairy cattle before and after parturition

Table 2 depicts the value of serum lipid profile of the three different stages of dairy cow. A significant increase ($p<0.001$) in all the lipid profiles (Cholesterol, Triglyceride, LDL, HDL) was found in dairy cow after parturition (Stage 2 and Stage 3) than before parturition (Stage 1).

Table 2: Comparative assessment of lipid profile level in three different physiological conditions of studied dairy cow

Parameter	Reference value**	Stage 1	Stage 2	Stage 3	*p*
Cholesterol (mg/dl)	203-230	121.3 ±23.8	188.2 ± 48.9	238.4 ± 45.9	0.003*
Triglyceride (mg/dl)	0-14	47.7 ± 20.3	49.5 ± 13.2	85.03 ± 8.8	<0.001*
LDL (mg/dl)	32-46	37.9 ± 30.8	81.9 ± 54.2	111.8 ±52.9	0.019*
HDL(mg/dl)	93-101	92.9 ± 27.2	116.2 ± 42.8	143.6 ± 60.9	<0.001*

Legend: Stage 1: Before one month of parturition
Stage 2: Within seven days after parturition
Stage 3: After two month of parturition
Results were expressed as mean ± SD ((n=24 in each group).
*Significantly different among the group

4.3 Mineral profile in dairy cattle before and after parturition

The mineral level i.e., Ca, Mg, P was estimated in dairy cow at periparturient period. It was found that a significantly higher level ($p<0.05$) of Ca, Mg and P in dairy cow of Stage 1 (before one month of parturition). However, the lowest value of Ca and Mg were observed as 6.5±1 mg/dl and 2.1±0.3 respectively, in Stage 2 (Table 3).

Table 3: Comparative assessment of minerals profile level in three different physiological conditions in studied dairy cow

Parameter	Reference value**	Stage 1	Stage 2	Stage 3	*p*
Ca (mg/dl)	9.7-12.4	9.3 ± 1.8	6.5 ± 1.0	7.2 ± 0.9	0.006*
Mg (mg/dl)	1.8-2.3	2.6 ± 0.6	2.1 ± 0.3	2.2 ± 0.3	0.003*
P (mg/dl)	5.6-6.5	9.3 ±1.8	6.2 ± 2.6	6.1 ± 1.5	0.027*

Legend: Stage 1: Before one month of parturition
Stage 2: Within seven days after parturition
Stage 3: After two month of parturition
Results were expressed as mean ± SD ((n=24 in each group).
*Significantly different among the group
**Kaneko *et al*., 1997

4.4 Overall comparative nutritional assessment in serum biochemical level between two stages of dairy cow after parturition

Table 4 represents the comparative nutritional assessment in serum biochemical level between two stages of dairy cattle after parturition. Glucose and TP were significantly higher ($p<0.01$) in cow of after two months parturition (59.1 ± 9.6 mg/dl and 79.9 ± 5.9 g/L respectively) while albumin was found highest in cow immediately after parturition (38.7 ± 12.7 g/L) which was also significant.

On the other hand, a significant increase ($p<0.01$) in all the lipid profile (Cholesterol, Triglyceride, LDL, HDL) was found in cow of after two months of parturition.

Besides that, though Ca level was found significantly higher (7.2±0.9 mg/dl) in cow of after two months of parturition but no significant variation was observed in Mg and P level when compared between two stages of dairy cow after parturition.

Table 4: Overall comparative nutritional assessment in serum biochemical level between two stages of dairy cattle after parturition

Parameter	Within seven days after parturition Mean ± SD (95% CI)	Two months after parturition Mean ± SD (95% CI)	*p*
Carbohydrate			
Glucose (mg/dl)	49.8 ± 8.6 (46.2-53.5)	59.1 ± 9.6 (55.1-63.2)	0.007*
Protein			
Total protein (g/L)	63.5 ± 8.2 (60.04-66.9)	79.9 ± 5.9 (77.4-82.4)	<0.001*
Albumin (g/L)	38.7 ± 12.7 (33.3-44.03)	25.4 ± 3.4 (23.9-26.8)	<0.001*
Lipid profile			
Triglyceride (mg/dl)	49.5 ± 13.2(45.9-55.04)	85.03 ± 8.8 (81.3-88.7)	<0.001*
Cholesterol (mg/dl)	188.2 ± 48.9 (167.6-208.9)	238.4 ± 45.9 (249.02-257.8)	<0.001*
LDL (mg/dl)	81.9 ± 54.2 (59.1-104.8)	111.8 ±52.9 (59.4-134.1)	0.07
HDL(mg/dl)	116.2 ± 42.8 (98.1-134.3)	143.6 ± 60.9 (117.9-169.4)	0.07
Mineral profile			
Ca (mg/dl)	6.5 ± 1.01 (6.1-6.9)	7.2 ± 0.9 (6.7-7.5)	0.04*
Mg (mg/dl)	2.1 ± 0.3 (1.9-2.2)	2.2 ± 0.3 (2-2.2)	0.67
P (mg/dl)	6.2 ± 2.6 (5.1-7.2)	6.1 ± 1.5 (5.5-6.8)	0.98

*Significantly different between the groups

Chapter V: Discussion

5.1 Carbohydrate

5.1.1 Glucose

The highest level of glucose was found before parturition, which is 65.8±13.5 mg/dl. But when we have compared the glucose level of two stages after parturition then we found significant difference between after parturition and two month after parturition. It may be due to degree of hypoglycemia in periparturient cows which were more marked before than after calving (West, 1990). In contrast, serum glucose concentration was found significantly higher in periparturient cows than in early and late lactation. Glucose was observed to increase in the first week before parturition and returned to the normal level in the fourth week after pregnancy (Al-Mujalli, 2008).

5.2 Protein

5.2.1 Total Protein

No significant variation ($p = 0.271$) of protein level was found in the three stages of samples whereas highest TP was found after two months of parturition (79.9±5.9 g/L). In a previous study it was found that the total serum proteins levels were significantly affected by the physiological period and increased during lactation if compared to late gestation (Piccione *et al*., 2012). The variations reflect the maternal requirements of proteins need for milking and providing immunoglobulin (Mohri *et al*., 2007; Roubies *et al*., 2006; Bell *et al*., 2000). The TP values were found in Holstein Friesian cows as 55.2 ± 4.5 g/L, 48.3 ± 8.8 g/L, 63.8 ± 4.6 g/L in late gestation, post partum and 15th Week lactation respectively (Picciione *et al* ., 2012). The higher concentrate-to-forage ratio provided during the lactation is generally associated with lower levels of fibre and higher levels of starch in the diet, which gives rise to an increased production of propionic acid in the rumen and an increased microbial protein supply (Heck *et al*., 2009). TP decreasing trend in advanced pregnancy was reported in non descriptive cows (Mehta *et al*., 1989), and Jersy and HF cross cows (Ghosh *et al*., 1991).

5.2.2 Albumin

In case of albumin the value was observed as 18.1±1.2 g/L, 38.7±12.7 g/L, 25.4±3.4 g/L in the consecutive stage and p value <0.001. In our study the level of albumin was increased after parturition (within 7 days) and was decreased after two month of parturition significantly. In previous findings it was observed that albumin was low in the first week before parturition and returned to the normal level at the end of the fourth week after parturition (Abdul-Aziz and Al-Mujalli, 2008). Another study found that serum albumin values decreased non significantly from day 90 to the day 275 in heifers. The levels in cows on the day 275 were not significantly lower than that observed day 90. Though individually the levels of albumin did not vary significantly in heifers and cows at different stages of gestation, a significant decline was observed (Padodara *et al*., 2012). Tainturier et al. (1984) found the albumin level in serum remained unchanged during pregnancy. Some authors (Little, 1974 and Manston *et al*., 1975) had noted average decrease in serum albumin concentration in dairy cows of about 10% at or close to calving.

5.3 Lipid profile

5.3.1 Cholesterol and Triglycerides

Significant variation in cholesterol level was found after two months of parturition which is highest then earlier two stages. Same way there was also significant variation found in case of triglycerides which were highest in two month after parturition and the value was 85.0±8.7 mg/dl. In a study it was observed that total cholesterol and triglycerides, resulted significantly affected by the physiological status, and showed substantial increases during the mild lactation (Piccione *et al*., 2012). It may be, during the puerperal period, there is an increase in the demands for regulatory mechanism, responsible for all the processes involved with milking (Krajnicakova *et al*., 2003). For this reason, characteristic changes in lipid metabolism were found during pregnancy and lactation in most mammals (Roche *et al*., 2009). Endocrine profiles change and lipolysis and lipogenesis are regulated to increase lipid reserve during pregnancy, and, as a result, these reserves are utilized following parturition and the initiation of lactation (Roche *et al*., 2009; Nazifi *et al*., 2002). Same results were found by other researchers, demonstrating

that concentrations of total lipid and triglycerides increased at parturition, despite the kind of fed administered (Douglas *et al*., 2004). On the other hand, some researchers suggested that in dairy cows immunological conditions after calving are related to serum total cholesterol values during the dry period.

5.3.2 LDL and HDL

In our study we found higher level of HDL as 143.6±60.9 mg/dl after two month of parturition. Serum HDL level was low in before one month parturition and increased after consecutive stages significantly. In case of LDL we found highest level of LDL 111.8±52.9 mg/dl in the same stage of HDL and LDL level was low in before one month parturition and increased after consecutive stages significantly. We found both serum LDL and HDL were increasing after parturition. But no significant variation found in LDL and HDL between the two stages after parturition. In cattle HDL is the major fraction comprising more than 80% of the lipoproteins (Holtenius, 1988). In a experiment it was found that the cholesterol and HDL levels were also significantly higher in cows in late lactation. LDL concentrations in periparturient cows were significantly lower than in late lactation (Basoglu *et al*., 1998).

5.4 Minerals profile

5.4.1 Calcium, Phosphorus and Magnesium

Ca level was observed 9.3±1.8 mg/dl before parturition, 6.5±1.0 mg/dl after parturition and after two month of parturition 7.2 ± 0.9 mg/dl. We observed serum Ca level decrease after parturition significantly. It may due to the dry period condition of the animals. Due to production of colostrums and increase of milk production Ca level may decrease after parturition. In a previous study Ca levels were found 7.7±0.4 mg/dl, 5.7±1.0 mg/dl, 7.5±0.6 mg/dl, in late gestation, post partum, 2^{nd} week lactation respectively (Piccione *et al*., 2012) which is almost similar to our study. Significant variation of phosphorus value was observed in our study. In case of magnesium it was significantly higher in one month before parturition and slightly decreases in the consecutive stages. Piccione et al. (2012) found in their study 5.7±0.7 mg/dl, 4.9±0.8 mg/dl, 4.5±0.2 in late gestation, post partum,

2^{nd} week lactation mg/dl, 15^{th} week lactation respectively. In case of the magnesium we observed the level of serum magnesium decrease after parturition significantly. In other study it was found 2.1±0.2 mg/dl, 1.7±0.9 mg/dl, 2.1±0.2 mg/dl, 2.07±0.08 mg/dl in late gestation, post partum, 2^{nd} week lactation, 5^{th} week lactation respectively.

The passage of calcium across the placenta is unidirectional; back transfer of this element is very limited, so, the mobilization from bone and the increased absorption from the gastrointestinal tract are required to re-establish homeostasis (Liesegang, 2008; Szenci *et al*., 1994). Also it is true that the requirement of calcium and phosphorus depends also on the physiological status and on the animal's productivity (Brezezinska and Krawczyk, 2009). Milk phosphorus and calcium output is directly related to milk yield, as milk phosphorus concentration is constant (Valk *et al*., 2002). In fact, increasing the milk production, more phosphorus from the ingested amount is transferred to milk and less is excreted with faeces (Valk *et al*., 2002). In our study, phosphorus, magnesium serum levels were decreased after parturition.

One of the study revealed that serum level of Ca decreased significantly at calving compared with one month before and after calving. P also decreased significantly at calving, but remained depressed compared with one month before calving. On the other hand, Mg remained fairly constant over the time (Meglia, 2004).

Chapter VI: Conclusion

The present study found different level of carbohydrate, protein, lipid profile, mineral level around periparturient period in cow in studied dairy farm. The analyzed parameters indicate that, there was alteration of serum biochemical levels in study population in three different stages of periparturient period. For comprehensive evaluation of the values of different parameters of the different stages of parturient event further study should be carried out.

Chapter VII: Limitations

1. Only a single farm was selected.
2. Sample size was small.
3. Medication and feeding system was not considered.
4. The biochemical values were not compared to other local dairy breed of cattle.
5. Almost same parity cows were selected.
6. Limitation of budget.

Chapter VIII: Recommendations

Intensive intervention study should be carried out to know the nutritional status and serum biochemical level in different stages of parturition of local dairy cattle and others commercial dairy farms in the country. There should need to control monitoring group of study population. It should be better if considering feeding, medication, management system.

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Appendices

Table 5: Serum biochemical level of cows before parturition

Cow ID	Glucose (gm/dl)	TP(g/L)	Tg (mg/dl)	Chol (mg/dl)	Alb (g/L)	HDL (mg/dl)	LDL (mg/dl)	Ca (mg/dl)	Mg (mg/dl)	P (mg/dl)
181	84.1	56.7	37	120.5	17.4	89.9	38	9.8	3.9	7.1
235	80.3	65.3	36.3	152.4	16	26.7	132.96	8.4	1.7	11.2
214	67.4	50	39	104.5	17.8	94.6	17.7	9.2	1.6	8.6
142	65.2	64.3	52.6	168.5	19	101.4	77.62	9.7	3.9	9
173	66.8	68.4	55.1	122.6	16.9	118.4	15.22	10.4	2.7	5.1
86	57.3	57.8	26.8	103.8	17.2	98.4	10.76	8.7	3.3	8.9
81	84.1	68.4	68.2	120.4	19.3	33	101.04	8.1	2.6	4.2
243	56.8	55.8	49.7	130.2	18	124.4	15.74	7.6	2.2	5.7
135	65.5	68.3	57.5	86	16.7	85.64	11.86	8.2	3.1	6.3
128	68.3	57.4	21.4	122.5	17.8	84.7	42.08	12.8	2.3	9
178	64.9	67.3	26.8	68.8	19.2	64	10.16	9.6	2.7	9.2
244	57.9	56.4	80.9	140.3	18.9	126.5	29.98	9.2	2.6	6.9
293	40.9	68.3	58.4	160	15.6	124.6	47.08	7.5	2.1	4.5
151	56.3	74.3	36.8	125.5	19.3	116.2	16.66	9.8	2.1	6.7
239	93.7	58.5	91.4	146.2	19	135.5	28.98	10.3	2.9	6.4
236	78.5	60.3	70.4	104	19.3	99.5	18.58	8.9	2.1	7.6
10	67.4	57.3	37	124.3	18.5	69	62.7	9	2.3	9
228	56.8	67.4	21.4	124.5	17.3	86.4	42.38	12.8	2.5	9
169	40.5	68.4	81.9	82.1	16.7	92.5	5.98	6.5	2.1	9.4
170	50.2	65.4	49.7	144.1	19.3	101.5	52.54	12.9	2.7	9.5
204	65.8	50.7	18.3	108.4	19.8	60.2	51.86	11.1	2.3	9.9
1	53.5	65.3	36.4	122.4	20.2	98.5	31.18	8.7	2.6	7.8
2	80.4	57.3	34.2	115.6	17.5	101.3	21.14	7.2	2.7	7.1
3	76	58.3	58.3	112.8	18.3	96.8	27.66	7.4	2.4	6.7

Table 6: Serum biochemical level of cows within 7 days after parturition

Cow ID	Glucose (gm/dl)	TP(g/L)	Tg(mg/dl)	Chol (mg/dl)	Alb (g/L)	HDL (mg/dl)	LDL (mg/dl)	Ca (mg/dl)	Mg (mg/dl)	P (mg/dl)
181	43.9	67.4	27	227.8	37	125.4	107.8	7.3	2.5	9.2
235	42.7	56.8	35.4	170.7	36	85.1	92.68	7.2	2.3	9.6
214	38.6	57.4	42.8	330	63	96.8	241.76	6	2.3	6.8
142	56.2	53	39.9	224.7	44	128.7	103.98	5.7	2.1	3.8
173	45.8	68.4	34.4	164.1	45	71.4	99.58	7.4	1.9	3.9
86	53.7	68.5	54.1	150.8	49	55.1	106.52	7.4	1.7	4.5
81	57.3	58.3	43.8	236	31	164.3	80.46	8	1.9	4.4
243	44.3	59.4	45.9	176.1	24.6	104.1	81.18	5.4	2.1	5.2
135	45.8	58	48.3	153.8	40	146.4	17.06	8	2.4	5.5
128	46.8	65.4	66.9	183.9	38	78.5	118.78	6.5	1.7	1
178	55.5	57	65.1	177	37.6	105.2	84.82	5.6	1.9	5.4
244	48.6	54.8	53.8	256.9	43	244.7	22.96	6.1	2.3	2
293	57.8	66	81.1	228	19	83.3	160.92	6.1	2.5	6.4
151	44.5	67.4	66.1	194.9	49	179.1	29.02	7.1	2.5	6
239	51.5	68.5	51.4	177.2	66	135.6	51.88	7.5	2.1	6.4
236	69.3	65.4	57.2	251.1	56	121.9	140.64	5.9	1.5	5.7
10	46.6	78	54.6	173.4	45	111.4	72.92	6.3	1.8	8.8
228	56.7	89	53.8	171	47	62.5	119.26	5.1	1.9	13.2
169	39.9	56.9	48.8	154.2	25.3	67.3	96.66	7.4	2.4	8.2
170	34.5	58	21.2	168.9	27.5	135	38.14	6.1	1.7	8
204	44.7	67	44.1	181.7	32.4	151.6	38.92	5.9	2.2	5.4
1	67.5	57.4	56.3	110.1	19.8	90.4	30.96	4.8	1.8	6.1
2	56.6	58.3	50.5	126.5	24.7	110.5	26.1	5.1	2.5	5.9
3	47.3	67.5	45.3	128.9	28.4	134.4	3.56	8.3	2.4	6.2

Table 7: Serum biochemical level of cows 2 months after parturition

Cow ID	Glucose (gm/dl)	TP(g/L)	Tg(mg/dl)	Chol (mg/dl)	Alb (g/L)	HDL (mg/dl)	LDL (mg/dl)	Ca (mg/dl)	Mg (mg/dl)	P (mg/dl)
181	65.9	89.1	91.8	203.1	21.1	123	98.46	8	1.9	6.5
235	60.4	73	72	147.8	22.3	88.1	74.1	9	2.3	7.5
214	72.6	70.4	87.4	227.3	29.3	121	123.78	6.6	2.2	6.3
142	66.7	84.2	78.4	274.4	27.8	193.3	96.78	9.6	2	5.4
173	60.6	89.4	75.2	245.6	28.5	87	173.64	7.3	2.3	3.6
86	72	79.2	99.2	254.8	28.6	183.8	90.84	8.2	2.3	5.9
81	62	81.2	73	283.1	21.1	257.2	40.5	6.4	2.1	5
243	54.8	75.9	98	238	25.5	176.2	81.4	7.2	2	5.9
135	60.4	68.7	80	304.9	25	281.9	39	7.4	1.9	5.6
128	65.8	87.4	91.2	311.6	24.3	245.3	84.54	6.5	1.7	4.8
178	47.3	81.5	93.4	118.1	23.4	86.4	50.38	6.8	2.3	7.3
244	49	83.5	93.4	302.1	25.6	161	159.78	5.7	2.7	6.8
293	60.6	76.2	90.2	219.6	24.2	158.2	79.44	6.8	2.8	7
151	62.4	78.4	73.3	246.2	29	75.6	185.26	7.4	2	4.5
239	40.5	81.5	86.5	245.7	25.3	26.3	236.7	6.7	2.2	7.5
236	44.3	84.7	70	256	24.2	122.3	147.7	8.4	2.6	6.9
10	67.9	91.3	83	229.9	26.5	122.7	123.8	7.6	2.4	6.6
228	50.2	78.4	81.2	251.9	25.1	177	91.14	6.4	2	5.3
169	78.3	73.9	84.2	235.7	34.3	127.3	125.24	6.8	1.8	6.5
170	58.3	75.4	90	268.6	27.4	108.2	178.4	6.7	2.2	5.1
204	48.9	81	76.4	256	27.4	103.7	167.58	5.8	2.4	11.2
1	54.5	80.3	98.2	198.4	20.5	113.6	104.44	7.4	1.7	4.8
2	51.4	78.6	88.3	211.6	23.8	120.8	108.46	6.5	1.5	5.3
3	64.3	74.3	86.2	190.8	19.5	187.4	20.64	5.8	2.1	6

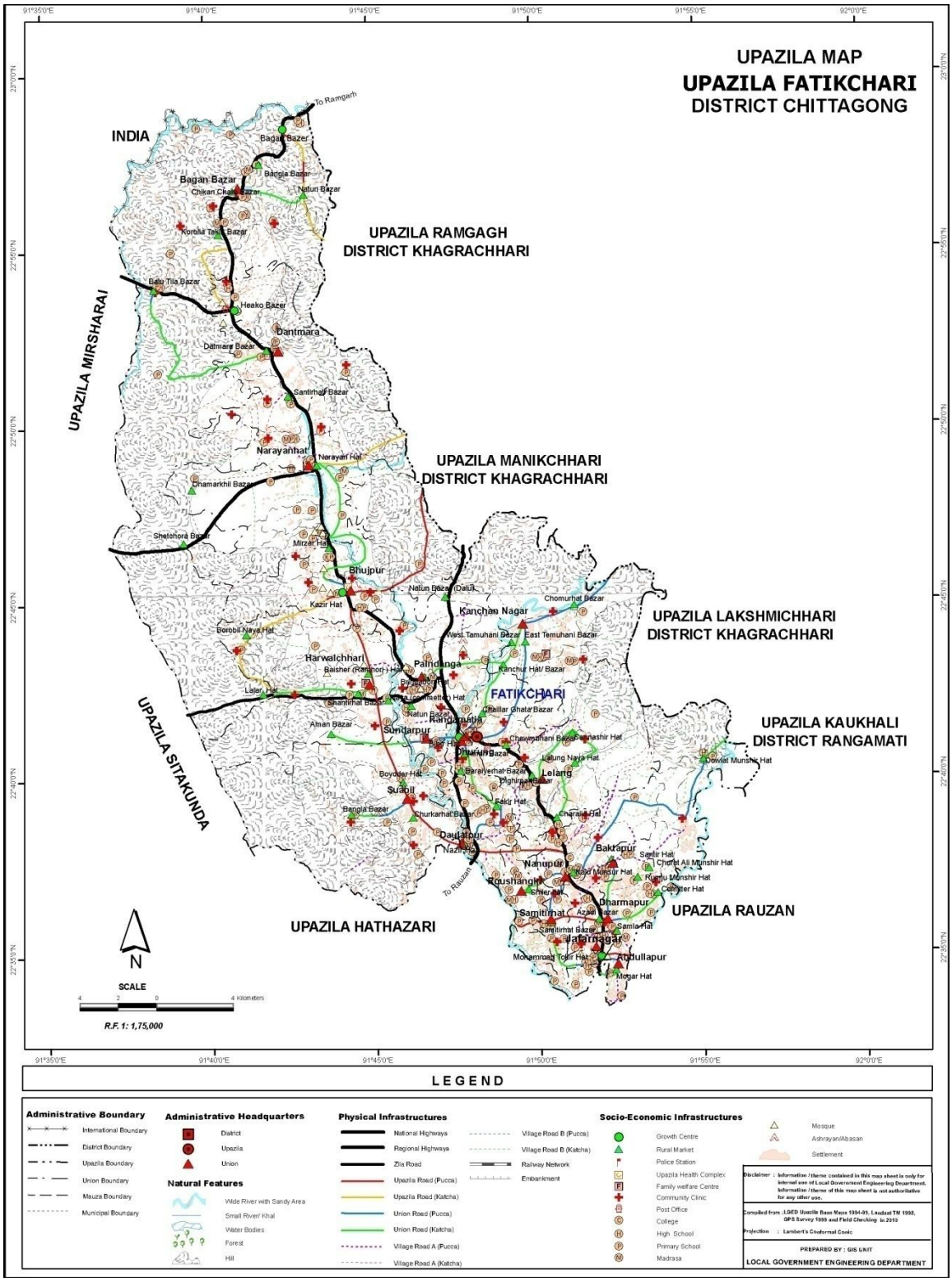

Figure 2: Map of Fatikchari (Source: LGRD Map)

Figure: 3- Activities performed during the study; A- Collection of blood, B- Separation of Serum, C- Reagent preparation for biochemical assay, D- Assay of biochemical level.

Questionnaire

Assessment of Nutritional status of peri-parturient dairy cows

Department of Physiology, Biochemistry and Pharmacology
Faculty of Veterinary Medicine
Chittagong Veterinary and Animal Sciences University
Conducted by: Mohammad Yousuf
MS Fellow (Biochemistry)
Session: 2013-2014

Sl No. 1. Name of the farm: 2.Name of Owner:

3. Address:
4. Cattle no:
a. Milking cow: b. Dry cow: c. Heifer: d. Bull: e. Calf
Type of ration:(In dry period/ After parturition /two month after parturition):

Type	Amount (kg)/animal
Concentrate	
Roughage	

1. ID:
2. Age:
3. Genotype (Cross):
4. Body weight:
5. Age:
6. 1st heat after last calving:
7. Last date of Insemination:
8. Date of Calving:
9. Gestation period:
10. No. of insemination per calving:
11. Calf wt:
12. Parity:
13. Milk yield/d:
14. Type of Disorders: Pre.Perturition Post perturition
15. Remarks(Medication,etc):

Brief Biography

DR. Mohammad Yousuf passed the Secondary School Certificate Examination in 2000 and then Higher Secondary Certificate Examination in 2002. He obtained Doctor of Veterinary Medicine in 2010 from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. After completing his DVM course successfully he served as Technical Officer (Livestock) in GRAUS, Ruma, Bandarban for two years and then he joined as Additional Veterinary Surgeon in Food and Agriculture Organization for 8 months. Now he is candidate for the degree of MS in Biochemistry under the Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University.