**Chapter-III**

**Materials and Methods**

**3. 1. Area and Study Population**

Dairy Cows in different stage of lactation with a history of drop in a milk production were included into the study for examination at Biochemistry laboratory, CVASU. Samples were collected from a commercial dairy farm of Chittagong. The total experimental animal was 22 cows, with an average milk production of 17 liter/cow/day. These experimental animals are divided into 2 groups-

**Group 1:** Cows with 1 or 2 lactations (n= 11)

**Group 2:** Cows with 3 or more lactations (n= 11)

**3. 2. Questionnaire Design and Data Collection**

In order to collect relevant information for the study, a standard questionnaire was carefully prepared on the basis of the objectives. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, minimize variation and improve precision of responses (Thrusfield, 2005). The questionnaire was filled up by repeated questioning to the animal owner, personal observation of patient and taking records from register book. On this work, Important animal level data recorded including experimental animals (species), breed, age, sex, body condition of the animal, posture, major diseases, rearing system, washing system, physical status, parity, milk production in different stage of lactation and previous lactation. Clinical examinations findings were noted down accordingly. A complete form of questionnaire is given in the **Annex-I**.

**3. 3. Biochemical Analysis**

Blood for serum analysis was collected into a tube containing clot activator. Within 4 hour after collection serum was obtained after centrifugation at 1700 *g* for 15 min. Aliquots were stored at –20°C until analysis. Serum samples were collected in a sterile vial for biochemical analysis.

All the blood biochemical parameters were estimated using Auto Analyzer in Biochemistry Laboratory, CVASU.

Serum was analyzed for:

* Calcium (**Ca**),
* Phosphorus (**P**),
* Magnesium (**Mg**),
* Total Protein (**TP**),
* Glucose (**Glu**),
* Cholesterol (**Chol**),
* Triglyceride (**Tgl**)

**3.4. Statistical Analysis**

The data obtained were entered and stored in a spread sheet (Microsoft Excel) and transferred into the statistical software STATA 12.1 (STATA Corp., Texas, USA) for statistical analysis. A descriptive analysis was carried out for the obtained data. The data were expressed as mean and standard deviation. Mean values of different blood parameters between group 1 and 2 was tested with t-test.